WELCOME TO INVIVOGEN'S LATEST CATALOG

InvivoGen has created this brand new catalog to provide you with the information that you need on our principal product lines:

Cell Culture and Transfection Reagents: Powerful reagents to prevent microbial contamination in cell culture, plus a wide variety of selection antibiotics and reporter (secreted embryonic alkaline phosphatase (SEAP) or Lucia luciferase) detection reagents, and a transfection reagent;

Mammalian Expression Vectors: A broad array of cloning vectors, that include plasmidic and lentiviral vectors, and CpG-free plasmids, a choice of inducible reporter plasmids, plus an expanding library of human and murine genes, and a wide variety of cellular promoters;

Reporter Cells: A growing collection of functionally validated and mycoplasma-free human and murine cell lines, such as HEK293-, Jurkat-, RAW 264.7- and THP-1-derived cells, that report on one or more pattern recognition receptor (PRR), cytokine, or transcription factor activities using SEAP and/or Lucia luciferase, plus a recently launched line of knockout cells that lack major innate immune sensors or signaling proteins;

PRR Ligands: An expanding collection of agonists and control ligands for all the major PRRs, such as Toll-like receptors (TLRs), NOD-like receptors (NLRs) and cytosolic DNA sensors (CDSs), including ligands of natural and synthetic origin, all screened and validated in-house;

Inhibitors: A wide selection of functionally validated compounds to directly or indirectly block the principal immune (TLR, NLR, CDS) pathways and related signaling pathways;

Antibodies and Recombinant Proteins: A large variety of biologically validated monoclonal and polyclonal antibodies against major human and murine innate immune targets, as well as isotype families of clinically relevant antibodies, plus a selection of recombinant cytokines;

Vaccination: Pre-clinical grade vaccine adjuvants that comprise alum, emulsions, such as CFA and saponin, and PRR ligands, plus various ovalbumin-derived products.

About InvivoGen

InvivoGen strives to provide life scientists worldwide with innovative, high quality and reliable reagents. As specialists in Mammalian Cell Engineering and Innate Immunity, we are passionate about designing, developing, and bringing to market new tools and services for cutting-edge research. At our three regional headquarters (Toulouse, San Diego and Hong Kong) and through our dedicated multilingual customer support staff, we can provide researchers around the globe with the reagents and product knowledge that they demand.

Serving the bioresearch community

Our specialized R&D teams work together seamlessly to design, produce and validate novel products for research in areas as diverse as gene therapy, molecular immunology and vaccination. Whatever research challenges you are facing, count on InvivoGen to provide you with the tools and solutions that you need to make the scientific breakthroughs of tomorrow. Our high-quality reagents are biologically validated and subjected to rigorous quality control to ensure proper activity and lack of contaminants such as mycoplasma and LPS.

Want to learn more about InvivoGen and our products?

Visit our website:

www.invivogen.com

ORDERING INFORMATION

ORDER BY TELEPHONE

To Place an Order

Include the following information:
1. Institution or customer account number
2. Shipping address
3. Billing address
4. Purchase order number
5. Name and telephone number of end user
6. Name and telephone number of purchasing agent
7. Quantity, catalog code, and the description of the product(s)
8. For Visa, MasterCard or American Express orders, please provide card number, expiration date, verification number and name on the card.

*If you are placing an order from a country within the European Community please include your VAT registration number so the proper VAT treatment can be applied.

ORDER BY FAX

Shipping Information

All products are shipped via 2-3 day express air, unless specified otherwise. Orders for temperature sensitive products are packaged with 8 lbs of dry ice and are shipped overnight express. Shipping and handling charges are pre-paid by InvivoGen and added to the invoice. All domestic shipments are shipped via InvivoGen's designated carrier. All orders shipped via alternate carrier are subject to a handling fee to be added to the invoice. Shipping days are Monday through Friday except for items that must ship on dry ice. Items shipping on dry ice are shipped Monday and Tuesday.

ORDER BY E-MAIL OR ONLINE

Online Ordering

Orders may be placed online at invivogen.com. Simply register online to set up an account and add the products you wish to purchase to your cart (international customers may need to order through a local distributor). If you already know the catalog codes for the products you wish to order you may enter them directly using our Quick Order option. Orders can also be placed via e-mail to sales@invivogen.com for orders in the US, sales@invivogen.fr for orders in Europe and sales.hk@invivogen.com for orders in Hong Kong. All orders online will receive e-mail confirmation when orders are shipped.

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1 CELL CULTURE & TRANSFECTION

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MICROBIAL CONTAMINATION

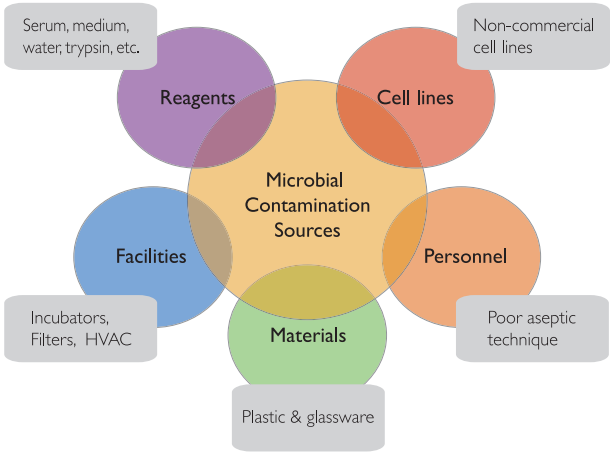
Microbial contamination of cell cultures is easily the most common problem encountered in cell culture laboratories, sometimes with very serious consequences. The use of infected cell lines can lead to unreliable experiments and unsafe biologicals and biopharmaceutical drugs, and is costly in time and materials. Microbial contamination falls into two groups: those that can be easily detected (e.g. bacteria, yeast and fungi) and those that are more difficult to detect (e.g. mycoplasma). While it is impossible to eliminate contamination entirely, it is possible to reduce its frequency and seriousness by gaining a thorough understanding of it sources and by following good aseptic technique.

Mycoplasma Contamination

Mycoplasmas are the smallest and simplest self-replicating organisms. They lack a rigid cell wall and grow mostly associated with the mammalian cell membranes. In most cases, there are no signs of mycoplasma contamination. They cannot be detected by visual inspection and do not cause consistent perceptible changes in a cell culture, such as rapid pH change and medium turbidity. Thus, mycoplasmas commonly remain undetected in the cell cultures for long periods. Mycoplasmas can cause disastrous effects on eukaryotic cells, as they can alter every cellular parameter, from proliferation to virus susceptibility and production, leading to unreliable experimental results and potentially unsafe biological products[1, 2]. This is a serious problem, as 5 to 35% of cell-lines worldwide are infected with mycoplasmas[2-4].

Bacterial Contamination

Although bacterial contamination can be detected using a light microscope, it can easily be mistaken for cellular debris, especially when the baterial contamination is in the early stages of infection. Signs of bacterial contamination include signs of mobility and a sudden decrease in pH with the culture media changing to a yellowish color. Bacteria are a large and ubiquitous group of unicellular microorganisms. They are typically a few micrometers in diameter, and can have a variety of shapes, ranging from spheres to rods and spirals. Because of their ubiquity, size, and fast growth rates, bacteria are the most commonly encountered biological contaminants in cell culture[5, 6].



Fungal Contamination

With yeast, molds and fungi, the pH of the culture remains stable in the initial stages of contamination, then rapidly increases as the culture becomes more heavily infected and gets turbid. By microscopy fungi usually appear as long thin filaments, while yeast are round or oval bodies that can form chains or clusters[7]. In the advanced stages of contamination, fuzzy patches can be easily seen in the culture[8]. Fungal contamination presents difficulties for eradication, as it can spread via spore mobility in air. Spores of many fungal species can survive in extremely harsh and inhospitable environments in their dormant stage, only to become activated when they encounter suitable growth conditions. Cell cultures can often be cured of fungal contamination when detected early and treated with certain antibiotics.

Endotoxin Contamination

Endotoxin, also known as lipopolysaccharide (LPS), is the major cell wall component of Gram-negative bacteria. Endotoxin is a potent stimulator of the humoral and cellular response *in vivo*. *In vitro*, endotoxins can introduce a bias in experiments involving cells sensitive to endotoxins[9, 10]. Thus, monitoring the presence of endotoxins in cell culture reagents is crucial. Sources of endotoxins include media, sera, water, buffers and other cell culture reagents, such as trypsin. Other biologically active organic contaminants that can induce significant experimental variabilityinclude flagellin and lipoproteins. Care needs to be taken with solutions that are sterile but may still contain bacterial components, such as endotoxins that could interfere with cell cultures.

1. Drexler H. & Uphoff C., 2002. Mycoplasma contamination of cell cultures: Incidence, sources, effects, detection, elimination, prevention. Cytotechnology. 39:75-90. **2. Rottem S. & Barile M., 1993.** Beware of mycoplasma. Trends in biotechnology. 11:143-50. **3. McGarrity G. *et al.*, 1988.** Annual report to international research program in comparative mycoplasmology. International Organization of mycoplasmology. **4. Young L. *et al.*, 2010.** Detection of Mycoplasma in cell cultures. Nature Protocols 5, 929-934. **5 Ryan J., 2008.** Understanding and managing cell culture contamination. Corning Life Sciences, Technical Literature. **6. Lincoln C. & Gabridge M. 1998.** Cell culture contamination: Sources, consequences, prevention, and elimination. Methods in cell biology. 57:49-65. **7. Mather J. & Roberts E., 1998.** Contamination: How to avoid it, recognize it, and get rid of it. In: Introduction to cell and tissue culture: theory and technique. Chapt. 7. p117-9. **8. Nandi S., 2009.** Animal Cell culture and virology. Chapt. 17 p. 99-9. **9. Gould M. *et al.*, 1984.** Endotoxin in vertebrate cell culture: Its measurement and significance. Uses and standardization of vertebrate cell lines. Tissue Culture Association, Gaithersburg, MD. 125-36. **10. Weber M. *et al.*, 1995.** Effects of lipopolysaccharide on transfection efficiency in eukaryotic cells. BioTechniques 19:930-9.

BE VIGILANT TO KEEP YOUR CELLS SAFE

InvivoGen offers a wide range of highly effective antimicrobial agents to help you detect, eliminate and prevent microbial contamination.

DETECTION

PlasmoTest™ - Detection of mycoplasma contamination
HEK-Blue™ LPS Detection Kit 2- Detection of endotoxins

ELIMINATION

Plasmocin™ Treatment - Treatment for mycoplasma contamination
Plasmocure™ - Alternative treatment for mycoplasma contamination
Fungin™ - Elimination and prevention of yeast and fungal infections
Normocure™ - Elimination of bacterial infections

PREVENTION

Plasmocin™ Prophylactic - Prevention of mycoplasmas
Normocin™ - Prevention of mycoplasmal, bacterial and fungal infections
Primocin™ - Prevention of mycoplasmal, bacterial and fungal infections in primary cells

1

MYCOPLASMA DETECTION

PlasmoTest™

PlasmoTest™ provides a simple, rapid and reliable assay for the visual detection of mycoplasma contamination in cell cultures. This colorimetric assay is the first to utilize cells to signal the presence of mycoplasmas. PlasmoTest™ is provided as a kit, that contains the Mycoplasma sensor cells and all the reagents needed to perform the assay, including positive and negative controls. PlasmoTest™ allows testing of up to 250 samples.

Description

PlasmoTest™ comprises the HEK-Blue™-2 cells and the PlasmoTest™ Reagent Kit, which includes the HEK-Blue™ Detection medium (see below). HEK-Blue™-2 cells are the mycoplasma sensor cells. When grown in HEK-Blue™ Detection medium, they detect the presence of mycoplasmas leading to a color change of the medium. These mycoplasma sensor cells recognize mycoplasmas through Toll-Like Receptor 2 (TLR2), a pathogen recognition receptor. In the presence of mycoplasmas, TLR2 initiates a signaling cascade leading to the activation of NF-κB and AP-1. These transcription factors induce the secretion of SEAP (secreted embryonic alkaline phosphatase) in the supernatant which is readily detected by the purple/blue coloration of the HEK-Blue™ Detection medium.

HEK-Blue™-2 cells

The Mycoplasma sensor cells, are engineered HEK293 cells. These cells stably express TLR2 and multiple genes from the TLR2 pathway and coexpress the SEAP reporter gene, placed under the control of a promoter inducible by the transcription factors NF-κB and AP-1.

PlasmoTest™ Reagent Kit

The PlasmoTest™ Reagent Kit contains enough reagents to test up to 250 samples. These reagents are the positive and negative controls, the HEK-Blue™ water (sterile endotoxin-free water) and the following:

- **HEK-Blue™ Detection** is a medium specifically designed for the detection of SEAP. It contains a color substrate that produces a purple/blue color following its hydrolysis by SEAP (see p. 16).
- **HEK-Blue™ Selection** is a solution that combines several selective antibiotics. These antibiotics guarantee the persistent expression of the various transgenes introduced in HEK-Blue™-2 cells.
- **Normocin™** is included in the kit to protect HEK-Blue™-2 cells from any potential microbial contamination, whether caused by mycoplasmas, bacteria or fungi.

Contents

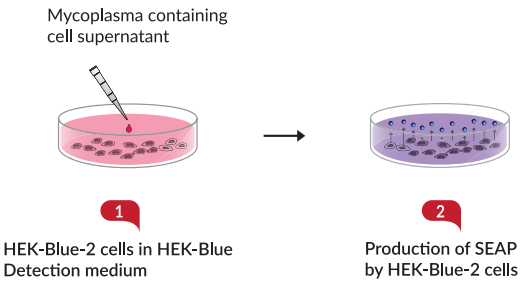
PlasmoTest™ comprises the HEK-Blue™-2 cells (3-7 x 10^6 cells) and the following components:

- HEK-Blue™ Selection (250X - 2 ml)
- HEK-Blue™ Detection (1 pouch to prepare 50 ml)
- HEK-Blue™ water (60 ml)
- Normocin™ (500X - 1 ml)
- Positive control & negative control (1 tube each)

HEK-Blue™-2 cells are shipped on dry ice. All other products are shipped at room temperature.

Buy PlasmoTest™ once, and then reorder only the PlasmoTest Reagent Kit or the reagents separately to perform further assays.

Principle



Principle of PlasmoTest™ - A small volume (20 µl) of the cell culture supernatant is added to the HEK-Blue™-2 cells in HEK-Blue™ Detection medium. Mycoplasmas present in the supernatant are sensed by TLR2 leading to the activation of NF-κB and the production of SEAP in the supernatant. SEAP catalyzes the hydrolysis of a chromogenic substrate contained in HEK-Blue™ Detection medium, leading to a purple/blue color that can be read by a spectrophotometer at 620-655 nm.

PRODUCT	QUANTITY	CAT. CODE
PlasmoTest™	1 kit (250 samples)	rep-pt1
PlasmoTest™ Reagent Kit	Refill kit (500 samples)	rep-ptrk
HEK-Blue™ Detection	5 pouches	hb-det2
HEK-Blue™ Selection	10 x 1 ml	hb-sel
Normocin™	500 mg	ant-nr-1
PlasmoTest™ Controls	200 tests	pt-ctr2

RECENT CITATIONS

Baumann AM. *et al.*, 2015. 9-O-Acetylation of sialic acids is catalysed by CASD1 via a covalent acetyl-enzyme intermediate. Nat Commun. 6:7673.

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McCaffrey J. *et al.*, 2016. Transcending epithelial and intracellular biological barriers; a prototype DNA delivery device. J Control Release. 226:238-47.

Park SH. *et al.*, 2016. SIRT2-Mediated Deacetylation and Tetramerization of Pyruvate Kinase Directs Glycolysis and Tumor Growth. Cancer Res. 76(13):3802-12.

ENDOTOXIN DETECTION

HEK-Blue™ LPS Detection Kit 2

Lipopolysaccharide (LPS), also known as endotoxin, is the major cell wall component of Gram-negative bacteria. LPS is a potent stimulator of the vertebrate innate immune system and can cause fever, septic shock and eventually death. In vitro, it can introduce a bias in experiments involving cells sensitive to LPS. Thus, monitoring the presence of LPS in biological reagents is crucial. Current methods for the detection of endotoxins rely on the Limulus Amebocyte Lysate (LAL), an extract of blood cells from an horseshoe crab, that reacts with endotoxin. A major drawback of the LAL test is overcoming assay inhibition. InvivoGen provides the HEK-Blue™ LPS Detection Kit 2, a simple, rapid and reliable assay to detect the presence of endotoxin in virtually all biological samples, including particulate compounds, such as vaccine adjuvants, and inhibitors of the LAL test. The HEK-Blue™ LPS Detection Kit 2 is a cell-based colorimetric assay for the detection of biologically active endotoxin that offers a sustainable alternative to the LAL test.

- **Versatile -** Measure endotoxin level in virtually all biological reagents
- **Highly sensitive -** Detect as little as 0.01 EU/ml
- **Economical -** Up to 500 samples can be tested with the kit

Description

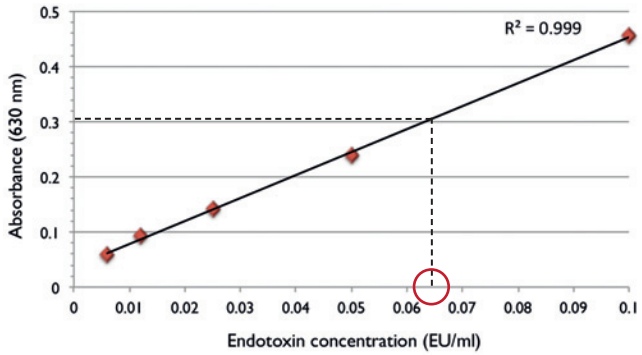
The HEK-Blue™ LPS Detection Kit 2 is anassay intended for the detection and quantification of biologically active LPS for research purposes. It is based on the activation of Toll-like receptor (TLR) 4, the mammalian endotoxin sensor. TLR4 recognizes structurally different LPS from Gram-negative bacteria. Proprietary cells engineered to become extremely sensitive to LPS, called HEK-Blue™-4 cells, are the main feature of this endotoxin detection kit. These cells stably express human TLR4 and an NF-κB-inducible secreted embryonic alkaline phosphatase (SEAP) reporter gene. The presence of minute quantities of LPS, starting as low as 0.01 EU/ml, are detected by the HEK-Blue™-4 cells, leading to the activation of NF-κB. Using QUANTI-Blue™, a SEAP detection medium that produces a purple/blue color, NF-κB activation can be observed with the naked eye or measured at 620-655 nm. Since the absorbance is in direct proportion to the amount of endotoxin present, the concentration of endotoxin can be calculated from a standard curve obtained using serial dilutions of the HEK-Blue™ Endotoxin Standard (a preparation of E. coli 055:B5 LPS standardized against FDA approved control standard endotoxin (CSE)).

Contents

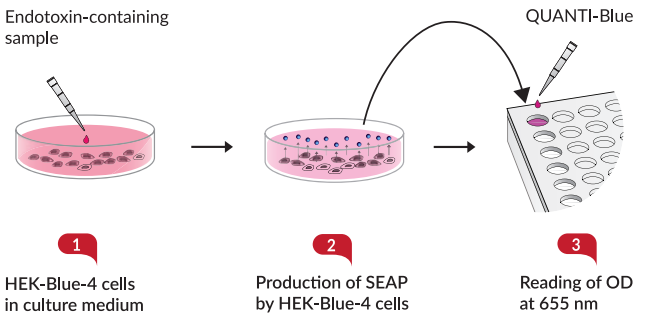
The HEK-Blue™ LPS Detection Kit 2 contains the following components:

- 1 vial of HEK-Blue™-4 cells (3-7 x 10^6 cells)
- 8 tubes of 250X HEK-Blue™ Selection (1 ml each)
- 4 tubes of 500X Normocin™ (1 ml each)
- 1 pouch of QUANTI-Blue™ (100 ml)
- 2 tubes of HEK-Blue™ Endotoxin Standard (50 EU each)
- 1 bottle of endotoxin-free water (50 ml)

Calculation of Endotoxin Concentration (Graphic Method)



Principle



Principle of the HEK-Blue™ LPS Detection Kit 2 - A small volume (20 µl) of the sample or a serial dilution of the HEK-Blue™ Endotoxin Standard is added to the HEK-Blue™-4 cells. Endotoxins present in the sample or standard are sensed by TLR4 leading to the activation of NF-κB and the production of SEAP in the supernatant. When a small volume (20 µl) of the supernatant is combined with QUANTI-Blue™, which contains a SEAP chromogenic substrate, a purple/blue color appears. SEAP is quantitated by measuring the absorbance at 620-655 nm and extrapolating against a standard curve (see graphic).

PRODUCT	QUANTITY	CAT. CODE
HEK-Blue™ LPS Detection Kit 2	1 kit	rep-lps2
HEK-Blue™ Selection	10 x 1 ml	hb-sel
Normocin™	10 x 1 ml	ant-nr-1
QUANTI-Blue™	5 pouches	rep-qb1
HEK-Blue™ Endotoxin Standard	10 x 50 EU	rep-hbes-10

RECENT CITATIONS

Ko SH. *et al.*, 2016. Bacteroides fragilis Enterotoxin Upregulates Heme Oxygenase-1 in Intestinal Epithelial Cells via a Mitogen-Activated Protein Kinases- and NF-κB-Dependent Pathway, Leading to Modulation of Apoptosis. Infect Immun. [Ahead of print]

Spindelboeck W. *et al.*, 2016. Triple Therapy with First Generation Protease Inhibitors for Hepatitis C Markedly Impairs Function of Neutrophil Granulocytes. PLoS One. 11(3):e0150299.

ANTIMICROBIAL REAGENTS

Plasmocin™ - Mycoplasma elimination & prevention

Description

Plasmocin™ is a well-established antimycoplasma reagent. It contains two bactericidal components strongly active against mycoplasmas that allow their elimination in only 2 weeks. The first component acts on the protein synthesis machinery while the second acts on DNA replication. These two specific and separate targets are found only in mycoplasmas and many other bacteria and are completely absent in eukaryotic cells.

In contrast to other anti-mycoplasma compounds, Plasmocin™ is active on both free mycoplasmas and intracellular forms. This advantage is conferred by one component of Plasmocin™ which is actively transported into mammalian cells. It ensures that following treatment with Plasmocin™ a cell culture is not reinfected by mycoplasmas released from intracellular compartments of infected cells.

Many cell lines infected by mycoplasmas have been successfully treated with Plasmocin™, including embryonic stem cells, hybridomas and retrovirus packaging cells.

Plasmocin™ Treatment (ant-mpt)

To eliminate mycoplasmas use Plasmocin™ treatment at 25 µg/ml for two weeks in the infected culture.

Plasmocin™ Prophylactic (ant-mpp)

To prevent mycoplasma contamination, use Plasmocin™ prophylactic at 2.5-5 µg/ml on a regular basis in cell culture.

Contents

Plasmocin™ is provided as a yellow solution either at a concentration of 25 mg/ml (Plasmocin™ Treatment) or 2.5 mg/ml (Plasmocin™ Prophylactic). One ml Plasmocin™ Treatment is enough to treat 1 L medium. Plasmocin™ is shipped at room temperature. Store at -20°C. Plasmocin™ is stable for 6 months at 4°C and for 2 years at -20°C.

PRODUCT	QUANTITY	CAT. CODE
Plasmocin™ Treatment	50 mg (2 x 1 ml)	ant-mpt
Plasmocin™ Prophylactic	25 mg (10 x 1 ml)	ant-mpp

Plasmocure™

Mycoplasma elimination

Description

In very rare cases, mycoplasmas resistant to Plasmocin™ have been reported. To eradicate these mycoplasmas, InvivoGen has developed a new antimycoplasma agent called Plasmocure™. Plasmocure™ combines two antibiotics that act through different mechanisms of action than those in Plasmocin™. A two week treatment with Plasmocure™ was found sufficient to completely eliminate the mycoplasmas. A moderate toxicity can be observed during the course of the treatment but full recovery of the cell line is expected once mycoplasmas are eliminated.

Plasmocure™ is a sterile ready-to-use solution. Simply add to mycoplasma contaminated cell cultures for 2 weeks at the recommended concentration of 50 µg/ml.

Contents

Plasmocure™ is provided as a colorless solution at a concentration of 100 mg/ml. One ml Plasmocure™ is enough to treat 2 L medium. Plasmocure™ is shipped at room temperature. Store at -20°C. Plasmocure™ is stable for 6 months at 4°C and for 2 years at -20°C.

PRODUCT	QUANTITY	CAT. CODE
Plasmocure™	100 mg (1 x 1 ml)	ant-pc

Normocure™

Bacteria elimination

Description

Normocure™ is broad-spectrum antibacterial agent highly effective against Gram$^-$ and Gram$^+$ bacteria. Normocure™ contains three bactericidal components belonging to different antibiotic families. They act by inhibiting the protein synthesis or disrupting membrane integrity. Their targets are different and completely absent in eukaryotic cells.

Cell cultures contaminated with bacteria from the environment, such as *Staphylococcus* species and *Achromobacter* species, can be efficiently cured by Normocure™ treatment.

Normocure™ is a sterile solution that can be added directly to the cell culture medium at the recommended concentration of 100 µg/ml. After 3 passages every 3-4 days, the bacterial contamination is totally eliminated.

Contents

Normocure™ is provided as a ready-to-use red solution at a concentration of 50 mg/ml. One tube of 1 ml Normocure™ treats 500 ml of cell culture medium. Normocure™ is shipped at room temperature and should be stored at -20°C. Normocure™ is stable for 6 months at 4°C and for 2 years at -20°C.

PRODUCT	QUANTITY	CAT. CODE
Normocure™	100 mg (2 x 1 ml)	ant-noc

Normocin™

Bacteria prevention

Description

Normocin™ is an innovative formulation of three antibiotics active against mycoplasmas, bacteria and fungi. Normocin™ contains two compounds that act on mycoplasmas and both Gram+ and Gram- bacteria by blocking DNA and protein synthesis. The third compound eradicates yeasts and fungi by disrupting ionic exchange through the cell membrane.

The active concentration of Normocin™ (100 µg/ml) displays no toxicity to the cell line being treated. Normocin™ is used in combination with Pen/Strep solutions to broaden the anti-bacterial spectrum.

Contents

Normocin™ is provided as a sterile red solution at a concentration of 50 mg/ml. Normocin™ is available in 1 ml vials or 20 ml bottles. One ml Normocin™ is enough to treat 500 ml medium. Product is shipped at room temperature and should be stored at -20°C. Normocin™ is stable for 2 years at 4°C or -20°C.

PRODUCT	QUANTITY	CAT. CODE
Normocin™	500 mg (10 x 1 ml)	ant-nr-1
	1 g (1 x 20 ml)	ant-nr-2

Primocin™

Bacteria prevention

Description

Primocin™ is an antibiotic formulation specifically designed to protect primary cell lines from cell culture contaminations. Primocin™ is active against both Gram+ and Gram- bacteria, mycoplasmas and fungi. There is no need to add Pen/Strep. Primocin™ is non-toxic to primary cells. It acts on targets found only in micro-organisms.

Primocin™ is a sterile water soluble solution that can be added directly to the cell culture medium. The solution is at 50 mg/ml and the recommended working concentration is 100 µg/ml.

Contents

Primocin™ is provided as a sterile light yellow solution at a concentration of 50 mg/ml. Primocin™ is available in 1 ml vials or 20 ml bottles. One ml Primocin™ is enough to treat 500 ml medium. Primocin™ is shipped at room temperature and upon receipt should be stored at -20°C. Primocin™ is stable for 3 months at 4°C and for 18 months at -20°C.

PRODUCT	QUANTITY	CAT. CODE
Primocin™	500 mg (10 x 1 ml)	ant-pm-1
	1 g (1 x 20 ml)	ant-pm-2

Fungin™

Fungi elimination

Description

Fungin™ is a soluble form of Pimaricin, a polyene introduced in the 1950's as an anti-fungal agent. This antimycotic compound kills yeasts, molds and fungi by disrupting ionic exchange through the cell membrane.

Fungin™ is a highly stable compound. It is shipped at room temperature and remains active after 6 days at 37°C. Fungin™ is water soluble unlike Amphotericin B which needs to be dissolved in toxic deoxycholate. Fungin™ exhibits no cytotoxicity to cells being treated, and no deleterious effects on cell metabolism.

Fungin™ is cell culture tested, and may be added to media containing commonly used antibacterial agents.

Contents

Fungin™ is provided as a pale yellow solution at a concentration of 10 mg/ml. One 1.5 ml vial is able to treat 300 ml to 1.5 liters of medium depending on the severity of contamination. Fungin™ is shipped at room temperature and should be stored at -20°C. Fungin™ is stable for 2 years at 4°C or -20°C.

PRODUCT	QUANTITY	CAT. CODE
Fungin™	75 mg (5 x 1.5 ml)	ant-fn-1
	200 mg (1 x 20 ml)	ant-fn-2

RECENT CITATIONS

Plasmocin™ - Principe DR. *et al.*, 2016. TGFβ Signaling in the Pancreatic Tumor Microenvironment Promotes Fibrosis and Immune Evasion to Facilitate Tumorigenesis. Cancer Res. 76(9):2525-39.

Hartmann P. *et al.*, 2016. Endothelial Dicer promotes atherosclerosis and vascular inflammation by miRNA-103-mediated suppression of KLF4. Nat Commun. 7:10521.

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Plasmocure™ - Thiel WH. *et al.*, 2016. Smooth Muscle Cell-targeted RNA Aptamer Inhibits Neointimal Formation. Mol Ther. 24(4):779-87.

Lee H. *et al.*, 2016. Drug Repositioning for Cancer Therapy Based on Large-Scale Drug-Induced Transcriptional Signatures. PLoS One. 11(3):e0150460.

Primocin™ - Ferrer-Mayorga G *et al.*, 2016. Vitamin D receptor expression and associated gene signature in tumour stromal fibroblasts predict clinical outcome in colorectal cancer. Gut. [Ahead of print].

Cao J. & Poss KD., 2016. Explant culture of adult zebrafish hearts for epicardial regeneration studies. Nat Protoc. 11(5):872-81.

Normocin™ - Shukla S. *et al.*, 2016. Inhibition of telomerase RNA decay rescues telomerase deficiency caused by dyskerin or PARN defects. Nat Struct Mol Biol. 23(4):286-92.

Hamdorf M. *et al.*, 2015. miR-128 represses L1 retro-transposition by binding directly to L1 RNA. Nat Struct Mol Biol. 22(10):824-31.

Fungin™ - Kriek JM. *et al.*, 2016. Female genital tract inflammation, HIV co-infection and persistent mucosal Human Papillomavirus (HPV) infections. Virology. 493:247-54.

Sanders B. *et al.*, 2016. Improved geometry of decellularized tissue engineered heart valves to prevent leaflet retraction. Ann Biomed Eng. 44(4):1061-71.

SELECTION ANTIBIOTICS

InvivoGen is a leader in the production of selection antibiotics. We manufacture the largest choice of antibiotics for the selection of stable mammalian cell lines. Our state-of-the-art facilities allow us to produce large quantities of high quality antibiotics at competitive prices. InvivoGen's selective antibiotics are ready-to-use, cell culture-tested solutions, and available in quantities from small to bulk.

High Quality
InvivoGen's antibiotics meet rigorous standards to ensure rapid, reliable and reproducible results. They have passed stringent quality control, including verification of potency, purity and stability using microbiological and chromatographic methods.

Ready-to-use Cell Culture Tested Solutions
No weighing needed - Our antibiotics are available as filter-sterilized solutions for customer convenience and validated for cell culture usage.

Endotoxin Tested
InvivoGen's selective antibiotics contain no detectable levels of endotoxin at working concentrations. This eliminates the risk of activating cells that express TLR4 (the receptor for endotoxins), such as immune cells, which can lead to biased results.

Selection in Both Mammalian Cells and *E. coli*
Matches up to the antibiotic resistance genes carried by InvivoGen plasmids, built for selection in both mammalian and bacterial cells.

Antibiotic Resistance Genes
All InvivoGen's antibiotics are paired with resistance genes that are active in both *E. coli* and mammalian cells. They are available in their wild-type form in many plasmids provided by InvivoGen, or as new synthetic alleles devoid of CpGs.

Also Available in Fast-Media®
All our selective antibiotics are available in Fast-Media®, our ready-made *E. coli* selection media, that can be prepared in just 5 minutes without autoclaving. The antibiotics are at the appropriate concentration in pre-mixed LB or TB media for selection of *E. coli* transformants (see p. 37).

SELECTIVE ANTIBIOTIC	SELECTION TARGET	WORKING CONCENTRATION	RESISTANCE GENE	FORMULATION
Blasticidin	Mammalian cells Bacteria	1 - 10 µg/ml 25 - 100 µg/ml	*Bsr*	100 mg, 500 mg (solution) 1 g (powder)
G418 Sulfate	Mammalian cells	400 - 1000 µg/ml	*Neo*	1 g, 5 g (solution)
Hygromycin B Gold	Mammalian cells Bacteria	50 - 200 µg/ml 50 - 100 µg/ml	*Hph*	1 g, 5 g (solution)
Phleomycin	Fungi, yeasts	10 - 150 µg/ml	*Sh ble*	100 mg, 500 mg (solution) 250 mg, 500 mg, 1 g (powder)
Puromycin	Mammalian cells Bacteria	1 - 10 µg/ml 100 - 125 µg/ml	*Pac*	100 mg, 500 mg (solution)
Zeocin™	Mammalian cells Bacteria	50 - 300 µg/ml 25 µg/ml	*Sh ble*	1 g, 5 g (solution) 1 g, 5 g (powder)

Blasticidin

Description

Blasticidin is a peptidyl nucleoside antibiotic isolated from *Streptomyces griseochromogenes*. It specifically inhibits protein synthesis in both prokaryotes and eukaryotes by interfering with the peptide bound formation in the ribosomal machinery. Resistance to blasticidin is conferred by the blasticidin resistance gene from *Bacillus cereus* (*bsr*) which encodes a deaminase. Typically, bacteria are sensitive to blasticidin concentrations of 25-100 µg/ml, and mammalian cells to 1-10 µg/ml.

Contents

Blasticidin is provided as a colorless solution at 10 mg/ml. Blasticidin is shipped at room temperature and should be stored at -20°C. Blasticidin is stable for 2 years when stored at -20°C.

PRODUCT	QUANTITY	CAT. CODE
Blasticidin	100 mg (10 x 1 ml)	ant-bl-1
	500 mg (50 x 1 ml)	ant-bl-5
	500 mg (1 x 50 ml)	ant-bl-5b
	1 g (powder)	ant-bl-10p

G418 Sulfate

Description

G418 is an aminoglycoside antibiotic similar in structure to gentamicin B1, produced by *Micromonospora rhodorangea*. G418 blocks polypeptide synthesis by inhibiting the elongation step in both prokaryotic and eukaryotic cells. Resistance to G418 is conferred by the *neo* gene from Tn5 encoding an aminoglycoside 3'-phosphotransferase, APH 3' II. Selection in mammalian cells is usually achieved in three to seven days with concentrations ranging from 400 to 1000 µg/ml. Cells that are dividing are affected sooner than those that are not.

Contents

G418 is provided as a colorless solution at 100 mg/ml. G418 is shipped at room temperature and should be stored at -20°C. G418 is stable for 2 years when stored at -20°C.

PRODUCT	QUANTITY	CAT. CODE
G418 Sulfate	1 g (10 x 1 ml)	ant-gn-1
	5 g (1 x 50 ml)	ant-gn-5

Hygromycin B Gold

Description

Hygromycin B is an aminoglycoside antibiotic produced by *Streptomyces hygroscopicus*. It inhibits protein synthesis by interfering with translocation and causing mistranslation at the 70S ribosome. Hygromycin B is effective on most bacteria, fungi and higher eukaryotes. Resistance to hygromycin is conferred by the *hph* gene from *E. coli*. Hygromycin B is normally used at a concentration of 50-200 µg/ml in mammalian cells and 100 µg/ml in bacteria.
Hygromycin B Gold, previously named HygroGold™, is a high purity (≥90%) preparation of hygromycin B.

Contents

Hygromycin B Gold is provided as a 100 mg/ml yellow solution. It is also available as a powder. Hygromycin B Gold products are shipped at room temperature. Store at -20°C. Hygromycin B Gold solution and powder are stable for 2 years when stored at -20°C.

PRODUCT	QUANTITY	CAT. CODE
Hygromycin B Gold	1 g (10 x 1 ml)	ant-hg-1
	5 g (1 x 50 ml)	ant-hg-5

RECENT CITATIONS

Blasticidin - Pillay S. *et al.*, 2016. An essential receptor for adeno-associated virus infection. Nature. 530(7588):108-12.

Walls AC. *et al.*, 2016. Cryo-electron microscopy structure of a coronavirus spike glycoprotein trimer. Nature. 531(7592):114-7.

G418 - Gerin I. *et al.*, 2016. ISPD produces CDP-ribitol used by FKTN and FKRP to transfer ribitol phosphate onto α-dystroglycan. Nat Commun. 7:11534.

Marques CA. *et al.*, 2016. Diverged composition and regulation of the Trypanosoma brucei origin recognition complex that mediates DNA replication initiation. Nucleic Acids Res. 44(10):4763-84.

Hygromycin B Gold - Rambout X. *et al.*, 2016. The transcription factor ERG recruits CCR4-NOT to control mRNA decay and mitotic progression. Nat Struct Mol Biol. 23(7):663-72.

Gekas C. *et al.*, 2016. β-Catenin is required for T-cell leukemia initiation and MYC transcription downstream of Notch1. Leukemia. [Ahead of print].

RELATED PRODUCTS

PRODUCT	INFO
HEK-Blue™ CLR Selection	Visit website
HEK-Blue™ Selection	Visit website

Phleomycin

Description

Phleomycin is a glycopeptide antibiotic of the bleomycin family, isolated from a mutant strain of *Streptomyces verticillus*. It binds and intercalates DNA thus destroying the integrity of the double helix. Phleomycin is active against most bacteria, filamentous fungi, yeast, plant and animal cells. Use of phleomycin is recommended for cells poorly sensitive to Zeocin™ (see below), i.e. filamentous fungi and some yeasts. Phleomycin resistance is conferred by the *Sh ble* gene from *Streptoalloteichus hindustanus* which encodes a protein that binds to phleomycin, inhibiting its DNA cleavage activity. Typically, phleomycin is used at a concentration of 10 µg/ml for yeasts and 25-150 µg/ml for filamentous fungi.

Contents

Phleomycin is provided as a blue solution at 20 mg/ml or as a powder. Phleomycin is shipped at room temperature. Store the solution at -20°C and the powder at 4°C. Phleomycin is stable for 2 years when properly stored.

PRODUCT	QUANTITY	CAT. CODE
Phleomycin	100 mg (5 x 1 ml)	ant-ph-1
	500 mg (25 x 1 ml)	ant-ph-5
	500 mg (25 ml)	ant-ph-5b
	250 mg (powder)	ant-ph-2p
	500 mg (powder)	ant-ph-5p
	1 g (powder)	ant-ph-10p

Puromycin

Description

Puromycin is an aminonucleoside antibiotic produced by *Streptomyces alboniger*. It specifically inhibits peptidyl transfer on both prokaryotic and eukaryotic ribosomes. This antibiotic inhibits the growth of Gram positive bacteria and various animal and insect cells. Puromycin can also be used in some particular conditions for the selection of *E. coli* transformants. Resistance to puromycin is conferred by the *Pac* gene encoding a puromycin N-acetyl-transferase (PAC) that was found in a *Streptomyces* producer strain. Animal cells are generally sensitive to concentrations from 1 to 10 µg/ml.

Contents

Puromycin hydrochloride is provided as a colorless solution at 10 mg/ml. Puromycin is shipped at room temperature and should be stored at -20°C. Puromycin is stable for at least 1 year when stored at -20°C.

PRODUCT	QUANTITY	CAT. CODE
Puromycin	100 mg (10 x 1 ml)	ant-pr-1
	500 mg (50 x 1 ml)	ant-pr-5
	500 mg (1 x 50 ml)	ant-pr-5b

Zeocin™

Description

Zeocin™ is a formulation of phleomycin D1, a copper-chelated glycopeptide antibiotic produced by *Streptomyces CL990*. Zeocin™ causes cell death by intercalating into DNA and cleaving it. This antibiotic is effective on most aerobic cells and is therefore useful for selection in bacteria, eukaryotic microorganisms, plant and animal cells. Resistance to Zeocin™ is conferred by the *Sh ble* gene product which inactivates Zeocin™ by binding to the antibiotic. Zeocin™ is used at a concentration of 50-300 µg/ml for selection in mammalian cells and 25 µg/ml for bacterial selection.

Contents

Zeocin™ is provided as a blue solution at 100 mg/ml. Zeocin™ is shipped at room temperature and upon receipt should be stored at -20°C. Zeocin™ is stable for at least 1 year at -20°C.

PRODUCT	QUANTITY	CAT. CODE
Zeocin™	1 g (10 x 1 ml)	ant-zn-1
	5 g (50 x 1 ml)	ant-zn-5
	5 g (1 x 50 ml)	ant-zn-5b
	1 g (powder)	ant-zn-1p
	5 g (powder)	ant-zn-5p

RECENT CITATIONS

Phleomycin - Marques CA. *et al.*, 2016. Diverged composition and regulation of the Trypanosoma brucei origin recognition complex that mediates DNA replication initiation. Nucleic Acids Res. 44(10):4763-84.

Schulz D. *et al.*, 2016. Base J and H3.V Regulate Transcriptional Termination in Trypanosoma brucei. PLoS Genet. 12(1):e1005762.

Puromycin - Pillay S. *et al.*, 2016. An essential receptor for adeno-associated virus infection. Nature. 530(7588):108-12.

Shostak A. *et al.*, 2016. MYC/MIZ1-dependent gene repression inversely coordinates the circadian clock with cell cycle and proliferation. Nat Commun. 7:11807.

Zeocin™ - Martell JD. *et al.*, 2016. A split horseradish peroxidase for the detection of intercellular protein-protein interactions and sensitive visualization of synapses. Nat Biotechnol. [Ahead of print].

Shostak A. *et al.*, 2016. MYC/MIZ1-dependent gene repression inversely coordinates the circadian clock with cell cycle and proliferation. Nat Commun. 7:11807.

RELATED PRODUCTS

PRODUCT	INFO
Fast-Media®	See page 37

TRANSFECTION REAGENT - LyoVec™

LyoVec™ is the first lyophilized cationic lipid-based transfection reagent. LyoVec™ belongs to the family of phosphonolipids originally described by Floch et al., as efficient transfection reagents[1, 2]. Their positive charge enables them to bind to DNA, and their phospholipid structure promotes fusion with cellular membranes for DNA delivery. The major constituent of LyoVec™ is the phosphonolipid DTCPTA, which is coupled with DiPPE, a neutral lipid that helps destabilizing membrane bilayers, therefore increasing the in vitro transfection efficiency of LyoVec™.

Rapid and Simple-to-use
Hands-on time: No more than 15 min
No preparation required the day prior transfection

Optimized Procedure
Works similarly at various lipid/DNA ratios
No optimization of transfection conditions
Soluble in water up to 5X

Maximum Transfection Efficiency
Transfects with high efficiencies a broad spectrum of mammalian cells, including primary cells and non-adherent cells
Effective for transient and stable transfections

Minimal Cytotoxicity
Works in the presence of serum
No need to wash cells after transfection

Increased Stability
Stable for over one year when lyophilized
Stable for up to six months when rehydrated

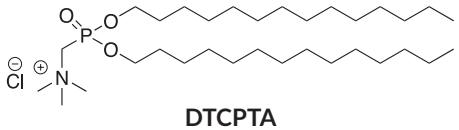
Long shelf-life of pDNA/LyoVec Complexes
In contrast with other cationic lipids, plasmid DNA (pDNA)/ LyoVec™ complexes remain fully active for transfection for at least two months at 4°C. Thus, preparation of large volumes of complexes can be made and reused repeatedly, saving you time.

Contents
LyoVec™ is provided in different formats:
- **lyec-1:** 5 vials (sufficient to perform 200 reactions)
- **lyec-2:** 10 vials (sufficient to perform 400 reactions)
- **lyec-12:** 4 vials (sufficient to perform 160 reactions) and 1 vial of control LyoVec™-GFP/SEAP complex.
- **lyec-22:** 9 vials (sufficient to perform 360 reactions) and 1 vial of control LyoVec™-GFP/SEAP complex.

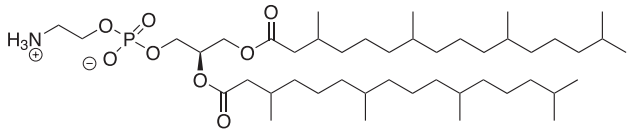
LyoVec™ is provided as a lyophilized powder, sterile and packaged in sealed 2 ml glass vials. After reconstitution, each vial yields 2 ml of transfection reagent allowing 40 transfections of 1-4 x 10^5 cells/well in a 12-well plate. Upon receipt, store at 4°C. LyoVec™ is stable for up to 1 year when properly stored.

PRODUCT	QUANTITY	CAT. CODE
LyoVec™	10 ml (200 reactions)	lyec-1
	20 ml (400 reactions)	lyec-2
	8 ml (160 reactions)	lyec-12
	18 ml (360 reactions)	lyec-22

Structure



DTCPTA
Di-tetradecylphosphoryl-N,N,N-trimethylmethanaminium chloride



DiPPE
1,2-Diphytanoyl-sn-Glycero-3-Phosphoethanolamine

1. Floch *et al.*, 1997. Cationic phosphonolipids as non viral vectors for DNA transfection in hematopoietic cell lines and CD34+ cells. Blood Cells, Molec.& Diseases 23: 69-87. **2. Guillaume-Gable *et al.*, 1998.** Cationic phosphonolipids as nonviral gene transfer agents in the lung of mice. Hum. Gene Ther. 9: 2309-2319.

Procedure

1• Add 2 ml of deionized sterile water per vial. Homogenize by vortexing gently for 30 seconds. Place the vial at 4°C for at least 30 minutes prior to initial transfection.

2• In a sterile microfuge tube at room temperature, add 1 µg plasmid DNA (pDNA) to 100 µl of LyoVec™. Mix gently.

3• Incubate at room temperature for at least 15 minutes to allow the formation of the complex.

4• Add 100 µl pDNA/LyoVec™ complexes to 1 ml cell suspension prepared in serum-containing medium. Swirl to distribute the complexes uniformly.

5• Incubate at 37°C in 5% CO2 for 24-72 hours before testing transgene expression or applying antibiotic selection.

RECENT CITATIONS

Starokadomskyy P. *et al.*, 2016. DNA polymerase-α regulates the activation of type I interferons through cytosolic RNA:DNA synthesis. Nat Immunol. 17(5):495-504.

Crill EK. *et al.*, 2015. RIG-I is required for VSV-induced cytokine production by murine glia and acts in combination with DAI to initiate responses to HSV-1. Glia. 63(12):2168-80.

REPORTER DETECTION REAGENTS

SEAP DETECTION

Secreted embryonic alkaline phosphatase (SEAP) is a reporter widely used to study promoter activity or gene expression. It is a truncated form of human placental alkaline phosphatase (PLAP) by deletion of the GPI anchor. Unlike endogenous alkaline phosphatases, PLAP is extremely heat stable and resistant to the inhibitor L-homoarginine. SEAP is secreted into the cell culture supernatant and therefore offers many advantages over intracellular reporters. It allows the determination of reporter activity without disturbing the cells, does not require the preparation of cell lysates and can be used for kinetic studies. For the rapid and convenient detection of SEAP in cell supernatants, InvivoGen has developed QUANTI-Blue™ and HEK-Blue™ Detection, a SEAP detection reagent and a SEAP detection culture medium, respectively.

QUANTI-Blue™

Description
QUANTI-Blue™ is a detection reagent developed to determine the levels of SEAP in biological samples. QUANTI-Blue™ offers many advantages over the conventional SEAP Reporter Assay Kit based on the pNPP substrate, including ease of use, short hands-on-time and visual readout. The same cell cultures can be repeatedly sampled for kinetic studies or further experimentation. SEAP activity can be detected as early as 15 min after incubation of the samples in QUANTI-Blue™.

Applications
Detection of SEAP in biological samples
Kinetic studies of SEAP

Readout Method
Naked eye (purple/blue color)
Spectrophotometry (620 - 655 nm)

Quality Control
Each lot is extensively tested to ensure lot-to-lot reproducibility using biochemical techniques and HEK-Blue™ TLR cells.

Contents
QUANTI-Blue™ is provided in a 5- or 10-pouch unit. Each pouch allows the preparation of 100 ml of detection medium. Store at room temperature. Pouches are stable 12 months at 4°C. After preparation, product is stable for 2 weeks at 4°C and for 2 months at -20°C.

PRODUCT	QUANTITY	CAT. CODE
QUANTI-Blue™	5 pouches (5 x 100 ml)	rep-qb1
	10 pouches (10 x 100 ml)	rep-qb2

HEK-Blue™ Detection

Description
HEK-Blue™ Detection is a cell culture medium that detects SEAP as the reporter protein is secreted by the cells. HEK-Blue™ Detection contains all the nutrients necessary for cell growth and a specific SEAP colorimetric substrate. The hydrolysis of the substrate by SEAP produces a purple/blue color that can be easily detected with the naked eye or measured with a spectrophotometer.

Applications
Real-time detection of SEAP produced by cells
Applicable to high-throughput screening

Readout Method
Naked eye (purple/blue color)
Spectrophotometry (620 - 655 nm)

Quality Control
Each lot is extensively tested to ensure lot-to-lot reproducibility using biochemical techniques and HEK-Blue TLR cells.

Contents
HEK-Blue™ Detection is provided in a 5- or 10-pouch unit. Each pouch allows the preparation of 50 ml of detection medium. Store at room temperature. Pouches are stable 12 months at 4°C. After preparation, product is stable for 2 weeks at 4°C and for 2 months at -20°C.

PRODUCT	QUANTITY	CAT. CODE
HEK-Blue™ Detection	5 pouches (5 x 50 ml)	hb-det2
	10 pouches (10 x 50 ml)	hb-det3

RELATED PRODUCTS

PRODUCT	INFO
SEAP Reporter Assay Kit	Visit website
Recombinant SEAP Protein	See page 137
SEAP Reporter Gene (pSELECT-zeo-SEAP)	Visit website

LUCIFERASE DETECTION

InvivoGen has developed Lucia luciferase, a completely novel and optimized luciferase with strong bioluminescent activity. It is expressed by a synthetic gene designed on natural secreted luciferase genes from marine copepods. Lucia luciferase generates a 1000-fold higher bioluminescent signal compared to the commonly used Firefly and Renilla luciferases. Similarly to Renilla luciferase, Lucia luciferase utilizes coelenterazine, an ATP-independent substrate. Lucia luciferase has several advantages over currently available luciferases and reporter systems:

- Lucia luciferase is efficiently secreted into the cell culture supernatant. No cell lysis required.

- Reporter activity is determined quickly and easily in real-time without disturbing cells in one simple endpoint assay.

- Stably expressed Lucia luciferase is ideal for use in cell based assays and high-throughput applications using QUANTI-Luc™.

For more information on Lucia luciferase, visit: www.invivogen.com/review-lucia

QUANTI-Luc™

Description

QUANTI-Luc™ is a lyophilized assay reagent containing all the components required to quantitatively measure the activity of Lucia luciferase and other coelenterazine-utilizing luciferases. QUANTI-Luc™ is optimized for use with Lucia luciferase reporter cell lines for fast and efficient real-time measurements directly from the cell culture media. QUANTI-Luc™ contains the coelenterazine substrate for the luciferase reaction, which produces a light signal that is quantified using a luminometer and expressed as relative light units (RLU). The signal produced correlates to the amount of luciferase protein expressed, indicating promoter activity in the reporter assay.

One step reagent

No additional reagents required! QUANTI-Luc™ contains the coelenterazine substrate with stabilizers and all the necessary components for the luciferase assay. It comes lyophilized and just requires addition of water to prepare the assay reagent.

Substrate stability

When reconstituted the substrate is stable for up to a month in contrast to other commercially available coelenterazine-based assay buffers. Suitable for multiple application use.

Low cost and versatile

Use for low or high throughput applications at lower cost compared to commercially available reagents. Not shipped on dry ice, easy to store and to use. Light emission can be measured using a luminometer without the need for an automated injector.

Contents

QUANTI-Luc™ is provided in a 2- or 5-pouch unit. Each pouch makes 25 ml of reagent allowing the preparation of 500 wells of a 96-well plate. Product is shipped at room temparature. Store at -20°C for up to 12 months. After preparation, product is stable for 1 week at 4°C and for 1 month at -20°C.

PRODUCT	QUANTITY	CAT. CODE
QUANTI-Luc™	2 pouches (2 x 25 ml)	rep-qlc1
	5 pouches (5 x 25 ml)	rep-qlc2

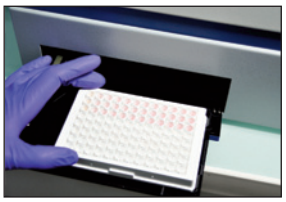
RELATED PRODUCT

PRODUCT	INFO
Recombinant Lucia Luciferase Protein	See page 137

Procedure

1• Prepare QUANTI-Luc™ by adding 25 ml water to the contents of one pouch.

2• Transfer aliquots of cell culture medium to opaque 96-microwell plate.



3• Add 50 µl QUANTI-Luc™ to each well. Measure luminosity in endpoint mode when using Lucia luciferase or in kinetic mode depending on the coelenterazine-luciferase used.

RECENT CITATIONS

QUANTI-Luc™ - Mitchell E. *et al.*, 2016. Behavioural traits propagate across generations via segregated iterative-somatic and gametic epigenetic mechanisms. Nat Commun. 7:11492

Dey B. *et al.*, 2015. A bacterial cyclic dinucleotide activates the cytosolic surveillance pathway and mediates innate resistance to tuberculosis. Nat Med. 21(4):401-6.

QUANTI-Blue™ - van der Plas MJ. *et al.*, 2016. Pseudomonas aeruginosa elastase cleaves a C-terminal peptide from human thrombin that inhibits host inflammatory responses. Nat Commun. 7:11567.

Karmakar M. *et al.*, 2016. Neutrophil P2X7 receptors mediate NLRP3 inflammasome-dependent IL-1β secretion in response to ATP. Nat Commun. 7:10555.

HEK-Blue™ Detection - Chen L. *et al.*, 2016. Outer membrane vesicles displaying engineered glycotopes elicit protective antibodies. PNAS 113(26):E3609-18.

O'Brien M. *et al.*, 2016. CD4 Receptor is a Key Determinant of Divergent HIV-1 Sensing by Plasmacytoid Dendritic Cells. PLoS Pathog. 12(4):e1005553.

2 MAMMALIAN EXPRESSION VECTORS

Cloning Vectors

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CLONING VECTORS

InvivoGen offers a large collection of cloning vectors designed for many different applications. These plasmids allow high levels of expression of one or two genes or shRNAs, including native or tagged genes, in vitro *and/or* in vivo. *They are available with a choice of selectable markers that can be used in both* E. coli *and mammalian cells.*

PLASMIDS	APPLICATION	SELECTION	FEATURES	PAGE
pCpGfree	Sustained *in vivo* gene expression or promoter CpG methylation studies	- Zeocin™	- CpG-free plasmid backbone - With MCS or reporter (mSEAP, Lucia)	28
pFUSE-CHIg & pFUSE2-CLIg	Generation of recombinant antibodies of all isotypes	- Blasticidin (pFUSE2-CLIg) - Zeocin™ (pFUSE-CHIg)	- Constant regions of heavy and light chains - Kappa and lambda light chains - α, δ, ε, γ and μ heavy chains - Two transcription units - Untagged or Lucia-tagged heavy chains	126-128
pFUSE-Fc & pFUSEN-Fc	Generation of Fc-fusion proteins in C- or N-terminal	- Zeocin™	- IgG Fc regions of human, mouse, rabbit or rat origin - Fc regions with or without introns - Native or engineered Fc regions - Untagged or Lucia-tagged Fc regions	20-22
pMONO	Expression of one gene of interest	- Blasticidin - Hygromycin B - Kanamycin/G418 - Zeocin™	- One transcription unit - Strong and constitutive promoters	24
pNiFty	Study of the NF-κB or IFN signaling pathway	- Ampicillin - Zeocin™	- NF-κB-inducible (ELAM) or IFN-inducible (ISG56K) promoter - SEAP or firefly luciferase reporter gene	29
pSELECT	Expression of one gene of interest	- Blasticidin - Hygromycin B - Kanamycin/G418 - Puromycin - Zeocin™ - GFP-Zeocin™	- Two transcription units - Strong and constitutive promoters	24
pSELECT-Tag	Expression of a tagged gene	- Blasticidin - Zeocin™	- GFP, HA, His or Lucia luciferase tag - Two transcription units - Strong and constitutive promoters	25
pVITRO	Expression of two genes of interest *in vitro*	- Blasticidin - Hygromycin B - Kanamycin/G418 - Puromycin	- Two transcription units - Choice of strong and constitutive promoters - With two MCS or choice of two reporters (GFP/SEAP, Lucia/SEAP)	26
pVIVO	Expression of two genes of interest *in vivo*	- Hygromycin B (*E. coli*)	- Two transcription units - Choice of inducible or constitutive promoters - With 2 MCS or choice of 2 reporters (GFP/SEAP, Lucia/SEAP)	27

Fc-FUSION PROTEINS - pFUSE-Fc Vectors

pFUSE-Fc is a family of plasmids developed to facilitate the construction of Fc-Fusion proteins by fusing a sequence encoding a given protein to the Fc region of an immunoglobulin. The Fc-fusion proteins are called immunoadhesins when the Fc region is combined with the functional domain of a binding protein, such as a receptor, ligand or cell-adhesion molecule. The Fc region comprises the CH2 and CH3 domains of the IgG heavy chain and the hinge region. The hinge serves as a flexible spacer between the two parts of the Fc-Fusion protein, allowing each part of the molecule to function independently.

- Large choice of Fc regions from different species: human, mouse, rat and rabbit
- Native Fc regions or engineered Fc regions with altered effector functions
- C-terminal or N-terminal Fc-fusions

Description

pFUSE-Fc vectors feature the following elements:
- composite EF-1α/HTLV promoter for high level expression of the fc-fusion protein
- multiple cloning site (MCS) located upstream or downstream of the Fc region to facilitate the cloning of the functional domain of a given protein
- large choice of Fc regions to generate fc-fusion proteins with different characteristics (species, isotypes, altered effector functions)
- IL-2 signal sequence (optional) for the secretion of Fc-Fusion proteins derived from non-secretory proteins

pFUSE-Fc vectors are selectable with Zeocin™ in *E. coli* and mammalian cells. They can be used for transient or stable transfection.

A Choice of pFUSE Plasmids

pFUSE-Fc plasmids are designed for the construction of C-terminal Fc-fusions (protein-Fc fragment). These plasmids come with a large choice of Fc regions (see p. 22). Some Fc regions have been engineered to modify their effector functions. pFUSE-Fc plasmids are available with or without the IL-2 signal sequence (IL2ss).

pINFUSE-Fc plasmids feature genomic Fc regions. The presence of introns is known to enhance the level of gene expression, as splicing is known to promote rapid and efficient mRNA export. pINFUSE-Fc plasmids are available with the most commonly used Fc fragments, with or without IL2ss.

pFUSEN-Fc plasmids allow the fusion of an Fc domain to the N-terminus of a protein of interest (Fc fragment-protein). They contain a secretion cassette comprising, in its 5' to 3' direction, the IL2ss, an immunoglobulin Fc domain and cloning sites to insert the protein of interest. These plasmids are available with a selection of Fc regions (see p. 23), some of which have been engineered to alter their effector functions.

pFUSE-Lucia-Fc & pFUSEN-Lucia-Fc plasmids express Lucia luciferase, a secreted luciferase that offers advantageous characteristics when associated with Fc-fusion proteins. It possesses superior carrier ability for excellent secretion of the chimeric protein. It provides a simple means to screen for recombinant clones and it minimally affects the activity of the protein of interest.

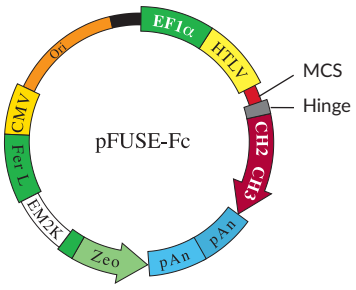
pFUSE-SEAP-Fc plasmids are control plasmids that express the secreted embryonic alkaline phosphatase (SEAP) reporter gene fused at its 3' end to an Fc fragment.

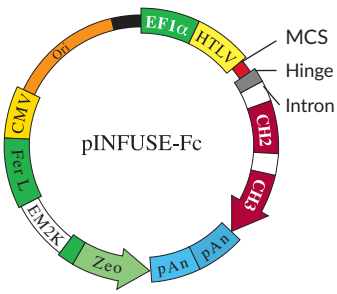
Contents

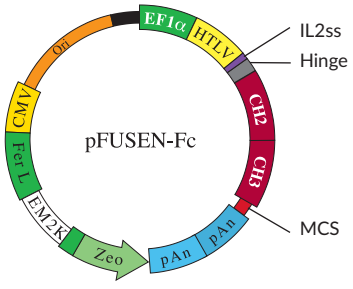
All pFUSE-Fc plasmids are provided as 20 µg of lyophilized DNA. Product is shipped at room temperature and should be stored at -20°C. Plasmid is stable for up to one year when properly stored.

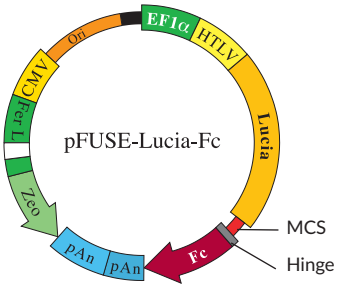
Immunoglobulin Effector Activities and Affinities

Isotype	Species	ADCC	CDC	Protein A binding	Protein G binding
hIgG1	Human	+++	+++	++++	++++
hIgG2	Human	+/-	+	++++	++++
hIgG3	Human	+++	++++	-	++++
hIgG4	Human	+/-	-	++++	++++
mIgG1	Mouse	-	+/-	+/-	++++
mIgG2a	Mouse	+++	+++	++++	++++
mIgG2b	Mouse	+++	+++	+++	+++
mIgG3	Mouse	+++	+	++	+++
rIgG	Rabbit	N/A	+++	++++	+++
rtIgG2b	Rat	++	++	-	++









Engineering Fc regions for altered properties

The Fc region of an immunoglobulin mediates its serum half-life and effector functions, such as complement-dependent cytotoxicity (CDC), antibody-dependent cellular phagocytosis (ADCC) and antibody-dependent cell phagocytosis (ADCP). Engineering the Fc region of a therapeutic monoclonal antibody or Fc fusion protein allows the generation of molecules that are better suited to the pharmacological activity required of them[1].

Engineered Fc regions for increased half-life

One approach to improve the efficacy of a therapeutic antibody is to increase its serum persistence, thereby allowing higher circulating levels, less frequent administration and reduced doses. The half-life of an IgG depends on its pH-dependent binding to the neonatal receptor (FcRn). FcRn, which is expressed on the surface of endothelial cells, binds the IgG in a pH-dependent manner and protects it from degradation. Some antibodies that selectively bind the FcRn at pH 6.0, but not pH 7.4, exhibit a higher half-life in a variety of animal models. Several mutations located at the interface between the CH2 and CH3 domains, such as T250Q/M428L[2] and M252Y/S254T/T256E + H433K/N434F[3], have been shown to increase the binding affinity to FcRn and the half-life of IgG1 *in vivo*. However, there is not always a direct relationship between increased FcRn binding and improved half-life[4].

Engineered Fc regions for altered effector function

Depending on the therapeutic antibody or Fc fusion protein application, it may be desired to either reduce or increase the effector function. For antibodies that target cell-surface molecules, especially those on immune cells, abrogating effector functions is required. Conversely, for antibodies intended for oncology use, increasing effector functions may improve the therapeutic activity.

The four human IgG isotypes bind the activating Fcγ receptors (FcγRI, FcγRIIa, FcγRIIIa), the inhibitory FcγRIIb receptor, and the first component of complement (C1q) with different affinities, yielding very different effector functions[5]. Binding of IgG to the FcgRs or C1q depends on residues located in the hinge region and the CH2 domain. Two regions of the CH2 domain are critical for FcγRs and C1q binding, and have unique sequences in IgG2 and IgG4. Substitutions into human IgG1 of IgG2 residues at positions 233-236 and IgG4 residues at positions 327, 330 and 331 were shown to greatly reduce ADCC and CDC[6, 7]. Furthermore, Idusogie et al. demonstrated that alanine substitution at different positions, including K322, significantly reduced complement activation[8]. Similarly, mutations in the CH2 domain of murine IgG2a were shown to reduce the binding to FcγRI, and C1q[9].

Numerous mutations have been made in the CH2 domain of human IgG1 and their effect on ADCC and CDC tested in vitro[7-10]. Notably, alanine substitution at position 333 was reported to increase both ADCC and CDC[7, 9]. Lazar *et al*. described a triple mutant (S239D/I332E/A330L) with a higher affinity for FcγRIIIa and a lower affinity for FcγRIIb that resulted in enhanced ADCC[11]. The same mutations were used to generate an antibody with increased ADCC[12]. Richards et al. studied a slightly different triple mutant (S239D/I332E/G236A) with improved FcγRIIIa affinity and FcγRIIa/FcγRIIb ratio that mediates enhanced phagocytosis of target cells by macrophages[13].

Due to their lack of effector functions, IgG4 antibodies represent the preferred IgG subclass for receptor blocking without cell depletion. IgG4 molecules can exchange half-molecules in a dynamic process termed Fab-arm exchange. This phenomenon can occur between therapeutic antibodies and endogenous IgG4. The S228P mutation has been shown to prevent this recombination process, allowing the design of less unpredictable therapeutic IgG4 antibodies[14, 15].

1. Park HI. *et al.*, 2016. The Highly Evolvable Antibody Fc Domain. Trends Biotechnol. [Ahead of print]. Review. **2. Hinton PR. *et al.*, 2004.** Engineered human IgG antibodies with longer serum half-lives in primates. J Biol Chem. 279(8):6213-6. **3. Vaccaro C. *et al.*, 2005.** Engineering the Fc region of immunoglobulin G to modulate in vivo antibody levels. Nat Biotechnol. 23(10):1283-8. **4. Datta-Mannan A. *et al.*, 2007.** Humanized IgG1 Variants with Differential Binding Properties to the Neonatal Fc Receptor: Relationship to Pharmacokinetics in Mice and Primates. Drug Metab. Dispos. 35: 86 - 94. **5. Bruhns P. *et al.*, 2009.** Specificity and affinity of human Fcgamma receptors and their polymorphic variants for human IgG subclasses. Blood. 113(16):3716-25. **6. Armour KL. *et al.*, 1999.** Recombinant human IgG molecules lacking Fcgamma receptor I binding and monocyte triggering activities. Eur J Immunol. 29(8):2613-24. **7. Shields RL. *et al.*., 2001.** High resolution mapping of the binding site on human IgG1 for Fc gamma RI, Fc gamma RII, Fc gamma RIII, and FcRn and design of IgG1 variants with improved binding to the Fc gamma R. J Biol Chem. 276(9):6591-604. **8. Idusogie EE. *et al.*, 2000.** Mapping of the C1q binding site on rituxan, a chimeric antibody with a human IgG1 Fc. J Immunol. 164(8):4178-84. **9. Steurer W. *et al.*, 1995.** Ex vivo coating of islet cell allografts with murine CTLA4/Fc promotes graft tolerance. J Immunol. 155(3):1165-74. **10. Idusogie EE. *et al.*, 2001.** Engineered antibodies with increased activity to recruit complement. J Immunol. 166(4):2571-5. **11. Lazar GA. *et al.*, 2006.** Engineered antibody Fc variants with enhanced effector function. PNAS 103(11): 4005–4010. **12. Ryan MC. *et al.*, 2007.** Antibody targeting of B-cell maturation antigen on malignant plasma cells. Mol. Cancer Ther., 6: 3009 - 3018. **13. Richards JO,. *et al.*, 2008.** Optimization of antibody binding to FcgammaRIIa enhances macrophage phagocytosis of tumor cells. Mol Cancer Ther. 7(8):2517-27. **14. Labrijn AF. *et al.*, 2009.** Therapeutic IgG4 antibodies engage in Fab-arm exchange with endogenous human IgG4 in vivo. Nat Biotechnol. 27(8):767-71. **15. Scapin G. *et al.*, 2015.** Structure of full-length human anti-PD1 therapeutic IgG4 antibody pembrolizumab. Nat Struct Mol Biol. 22(12):953-8. **16. Oganesyan V., *et al.* 2009.** Structural characterization of a human Fc fragment engineered for extended serum half-life. Molec Immunol 46: 1750-1755. **17 Yeung Y.A. *et al.*, 2009.** Engineering Human IgG1 Affinity to Human Neonatal Fc Receptor: Impact of Affinity Improvement on Pharmacokinetics in Primates. J. Immunol. 182: 7663-7671. **18. Qiao S-W, *et al.*, 2008.** Dependence of antibody-mediated presentation of antigen on FcRn. PNAS 105(27): 9337-9342.

Features of Engineered Fc

Engineered Fc	Isotype	Mutations	FcR/C1q Binding	Effector Function	Ref.
hIgG1e1-Fc	Human IgG1	T250Q/M428L	Increased binding to FcRn	Increased half-life	2
hIgG1e2-Fc	Human IgG1	M252Y/S254T/T256E + H433K/N434F	Increased binding to FcRn	Increased half-life	3
hIgG1e3-Fc	Human IgG1	E233P/L234V/L235A/ΔG236 + A327G/A330S/P331S	Reduced binding to FcγRI	Reduced ADCC and CDC	6, 7
hIgG1e4-Fc	Human IgG1	E333A	Increased binding to FcγRIIIa	Increased ADCC and CDC	8, 10
hIgG1e5-Fc	Human IgG1	S239D/I332E/A330L	Increased binding to FcγRIIIa	Increased ADCC	11, 12
hIgG1e6-Fc	Human IgG1	P257I/Q311I	Increased binding to FcRn	Unchanged half-life	4
hIgG1e7-Fc	Human IgG1	K326W/E333S	Increased binding to C1q	Increased CDC	9
hIgG1e9-Fc	Human IgG1	S239D/I332E/G236A	Increased FcγRIIa/FcγRIIb ratio	Increased macrophage phagocytosis	13
hIgG1e11-Fc	Human IgG1	M252Y/S254T/T256E	Increased binding to FcRn	Increased half-life	16
hIgG1e12-Fc	Human IgG1	M428L/N434A	Increased binding to FcRn	Increased half-life	17
hIgG1e13-Fc	Human IgG1	I253A	Reduced binding to FcRn	Enhanced antibody clearance	18
hIgG2e1-Fc	Human IgG2	K322A	Reduced binding to C1q	Reduced CDC	8
hIgG4e1-Fc	Human IgG4	S228P	-	Reduced Fab-arm exchange	14
mIgG2ae1-Fc	Mouse IgG2a	L235E + E318A/K320A/K322A	Reduced binding to FcγRI and C1q	Reduced ADCC and CDC	9

Applications

Choose a pFUSE-Fc plasmid according to your application:

- **Protein purification -** All pFUSE-Fc can be used for Protein A or Protein G affinity chromatography (see table below).
- **Long term expression *in vivo* -** Choose a pFUSE-Fc with an Fc engineered to display an increased half-life
- **Therapeutic use with cell depletion activity -** Choose a pFUSE-Fc with an Fc engineered to display increased ADCC and CDC
- **Therapeutic use without cell depletion activity -** Choose a pFUSE-Fc with an Fc engineered to display reduced ADCC and CDC

PRODUCT	ISOTYPE	QUANTITY	CAT. CODE (No IL2ss)	CAT. CODE (With IL2ss)
pFUSE-Fc				
Native Fc Regions				
pFUSE-hIgG1-Fc	Human IgG1	20 µg	pfuse-hg1fc1	pfuse-hg1fc2
pFUSE-hIgG2-Fc	Human IgG2	20 µg	pfuse-hfc1	pfuse-hfc2
pFUSE-hIgG3-Fc	Human IgG3	20 µg	pfuse-hg3fc1	pfuse-hg3fc2
pFUSE-hIgG4-Fc	Human IgG4	20 µg	pfuse-hg4fc1	pfuse-hg4fc2
pFUSE-mIgG1-Fc*	Mouse IgG1	20 µg	pfuse-mg1fc1	pfuse-mg1fc2
pFUSE-mIgG2a-Fc	Mouse IgG2a	20 µg	pfuse-mg2afc1	pfuse-mg2afc2
pFUSE-mIgG2b-Fc	Mouse IgG2b	20 µg	pfuse-mg2bfc1	pfuse-mg2bfc2
pFUSE-mIgG3-Fc	Mouse IgG3	20 µg	pfuse-mg3fc1	pfuse-mg3fc2
pFUSE-rIgG-Fc	Rabbit IgG	20 µg	pfuse-rfc1	pfuse-rfc2
pFUSE-rtIgG2b-Fc	Rat IgG2b	20 µg	pfuse-rtg2bfc1	pfuse-rtg2bfc2
Engineered Fc Regions (see decription page 21)				
pFUSE-hIgG1e1-Fc	Human IgG1	20 µg	pfc1-hg1e1	pfc2-hg1e1
pFUSE-hIgG1e2-Fc	Human IgG1	20 µg	pfc1-hg1e2	pfc2-hg1e2
pFUSE-hIgG1e3-Fc**	Human IgG1	20 µg	pfc1-hg1e3	pfc2-hg1e3
pFUSE-hIgG1e4-Fc	Human IgG1	20 µg	pfc1-hg1e4	pfc2-hg1e4
pFUSE-hIgG1e5-Fc	Human IgG1	20 µg	pfc1-hg1e5	pfc2-hg1e5
pFUSE-hIgG1e6-Fc	Human IgG1	20 µg	pfc1-hg1e6	pfc2-hg1e6
pFUSE-hIgG1e7-Fc	Human IgG1	20 µg	pfc1-hg1e7	pfc2-hg1e7
pFUSE-hIgG1e9-Fc	Human IgG1	20 µg	pfc1-hg1e9	pfc2-hg1e9
pFUSE-hIgG1e11-Fc	Human IgG1	20 µg	pfc1-hg1e11	pfc2-hg1e11
pFUSE-hIgG1e12-Fc	Human IgG1	20 µg	pfc1-hg1e12	pfc2-hg1e12
pFUSE-hIgG1e13-Fc	Human IgG1	20 µg	pfc1-hg1e13	pfc2-hg1e13
pFUSE-hIgG2e1-Fc	Human IgG2	20 µg	pfc1-hg2e1	pfc2-hg2e1
pFUSE-hIgG4e1-Fc	Human IgG4	20 µg	pfc1-hg4e1	pfc2-hg4e1
pFUSE-mIgG2ae1-Fc	Mouse IgG2a	20 µg	pfc1-mg2ae1	pfc2-mg2ae1
pINFUSE-Fc				
Native Fc Regions with introns				
pINFUSE-hIgG1-Fc	Human IgG1	20 µg	pfc1-hgin1	pfc2-hgin1
pINFUSE-hIgG2-Fc	Human IgG2	20 µg	pfc1-hgin2	pfc2-hgin2
pINFUSE-hIgG3-Fc	Human IgG3	20 µg	pfc1-hgin3	pfc2-hgin3
pINFUSE-hIgG4-Fc	Human IgG4	20 µg	pfc1-hgin4	pfc2-hgin4
pINFUSE-mIgG2b-Fc	Mouse IgG2b	20 µg	pfc1-mgin2	pfc2-mgin2

PRODUCT	ISOTYPE	QUANTITY	CAT. CODE (No IL2ss)	CAT. CODE (With IL2ss)
pFUSEN-Fc				
N-Terminal Fc Fusions				
pFUSEN-hG1Fc	Human IgG1	20 µg	pfcn-hg1	-
pFUSEN-hG1e2Fc	Human IgG1	20 µg	pfcn-hg1e2	-
pFUSEN-hG2Fc	Human IgG2	20 µg	pfcn-hg2	-
pFUSEN-mG2aFc	Mouse IgG2a	20 µg	pfcn-mg2	-
pFUSE-Lucia-Fc & pFUSEN-Lucia-Fc				
Lucia Luciferase-Tagged Fc Fusions				
pFUSE-Lucia-hG1Fc	Human IgG1	20 µg	pfuse-hg1lc	-
pFUSE-Lucia-mG2aFc	Mouse IgG2a	20 µg	pfuse-mg2alc	-
pFUSEN-Lucia-hG1Fc	Human IgG1	20 µg	pfcn-lchg1	-
pFUSEN-Lucia-hG1e2Fc	Human IgG1	20 µg	pfcn-lchg1e2	-
pFUSEN-Lucia-hG2Fc	Human IgG2	20 µg	pfcn-lchg2	-
pFUSEN-Lucia-mG2aFc	Mouse IgG2a	20 µg	pfcn-lcmg2a	-
pFUSE-SEAP-Fc				
Control Plasmids				
pFUSE-SEAP-hG1Fc	Human IgG1	20 µg	pfuse-hg1sp	-
pFUSE-SEAP-hG2Fc	Human IgG2	20 µg	pfuse-hsp	-
pFUSE-SEAP-hG3Fc	Human IgG3	20 µg	pfuse-hg3sp	-
pFUSE-SEAP-hG4Fc	Human IgG4	20 µg	pfuse-hg4sp	-
pFUSE-SEAP-mG1-Fc	Mouse IgG1	20 µg	pfuse-mg1sp	-
pFUSE-SEAP-mG2aFc	Mouse IgG2a	20 µg	pfuse-mg2asp	-
pFUSE-SEAP-mG2bFc	Mouse IgG2b	20 µg	pfuse-mg2bsp	-
pFUSE-SEAP-mG3Fc	Mouse IgG3	20 µg	pfuse-mg3sp	-
pFUSE-SEAP-rFc	Rabbit IgG	20 µg	pfuse-rsp	-
pFUSE-SEAP-rtFc	Rat IgG2b	20 µg	pfuse-rtsp	-

* The Fc region of mouse IgG1 appears to have a very low affinity for Protein G unlike the entire IgG1 molecule. Therefore, protein G may not be suitable for the purification of mIgG1-Fc-fusion proteins. Protein A may be used after optimization of the purification conditions. High salt concentration and alkaline pH are expected to increase affinity (Nagaoka T. *et al*, 2003 Protein Eng. 16(4):243:245).
** pFUSE-hIgG1e3 plasmids featuring the IgG1e3 engineered Fc are sold by InvivoGen under a limited license from Cambridge Enterprise Limited (International Publication Number WO 99/58572).

RELATED PRODUCTS

PRODUCT	INFO
Zeocin™	See page 14

RECENT CITATIONS

pFUSE-hIgG1-Fc1 - Dubrac A. *et al.*, 2016. Targeting NCK-Mediated Endothelial Cell Front-Rear Polarity Inhibits Neovascularization. Circulation. 133(4):409-21.

Rama N. *et al.*, 2015. Slit2 signaling through Robo1 and Robo2 is required for retinal neovascularization. Nat Med. 21(5):483-91.

pFUSE-hIgG1-Fc2 - Chen X. *et al.*, 2016. Long-acting cocaine hydrolase for addiction therapy. PNAS. 113(2):422-7.

Brügger V. *et al.*, 2015. HDAC1/2-Dependent P0 Expression Maintains Paranodal and Nodal Integrity Independently of Myelin Stability through Interactions with Neurofascins. PLoS Biol. 13(9):e1002258.

Uckun FM. *et al.*, 2015. Recombinant human CD19L-sTRAIL effectively targets B cell precursor acute lymphoblastic leukemia. J Clin Invest. 125(3):1006-18.

pFUSE-hIgG4-Fc - Mitra S. *et al.*, 2015. Interleukin-2 activity can be fine tuned with engineered receptor signaling clamps. Immunity. 42(5):826-38.

pFUSE-mIgG2ae1-Fc - Görtz D. *et al.*, 2015. Anti-interleukin-6 therapy through application of a monogenic protein inhibitor via gene delivery. Sci Rep. 5:14685.

MONOGENIC EXPRESSION

pMONO & pSELECT plasmids are specifically designed for strong and constitutive expression of a gene of interest in a wide variety of mammalian cell lines. They allow the selection of stable transfectants and offer a choice of selectable markers for both E. coli *and mammalian cells.*

pMONO

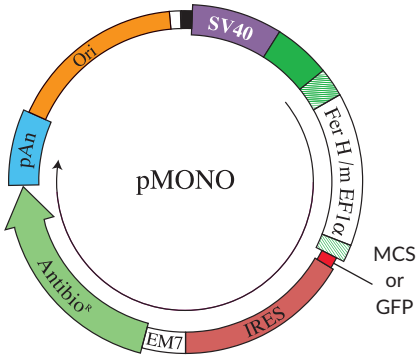
Description

pMONO plasmids contain a unique transcription unit that drives the expression of the gene of interest and the selectable marker through an internal ribosome entry site (IRES). This dual gene expression system ensures that stable clones express the gene of interest.

pMONO plasmids feature the SV40/FerH/mEF-1α promoter, which is composed of the ferritin heavy chain (FerH) core promoter fused at its 5' end to the SV40 enhancer, and at its 3' end to the intron-containing 5'UTR of the mouse elongation factor 1 alpha gene.

pMONO plasmids are selectable with blasticidin, hygromycin, kanamycin/G418 and Zeocin™. All resistance genes are cloned downstream of the IRES from the Foot and Mouth Disease Virus (FMDV), eliminating the need for a second mammalian promoter. The FMDV IRES is in tandem with the constitutive bacterial EM7 promoter conferring resistance both in *E. coli* and mammalian cells.

pMONO plasmids carry a multiple cloning site (MCS) downstream of the SV40/FerH/mEF-1α promoter for convenient cloning of a gene of interest. pMONO plasmids are also available with a GFP allele and can be used as control vectors.



Contents

pMONO plasmids are provided as 20 µg of lyophilized DNA. Product is shipped at room temperature and should be stored at -20°C. Plasmid is stable for up to one year when properly stored.

PRODUCT	QTY	CAT. CODE (MCS)	CAT. CODE (GFP)
pMONO-blasti	20 µg	pmonob-mcs	pmonob-gfp
pMONO-hygro	20 µg	pmonoh-mcs	pmonoh-gfp
pMONO-neo	20 µg	pmonon-mcs	pmonon-gfp
pMONO-zeo	20 µg	pmonoz-mcs	pmonoz-gfp

pSELECT

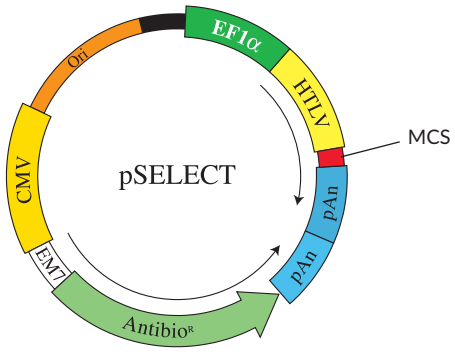
Description

pSELECT plasmids contain two transcription units, the first drives the expression of the gene of interest and the second drives the expression of the resistance gene.

pSELECT plasmids feature the strong EF-1α/HTLV composite promoter, which combines the elongation factor 1 alpha core promoter and the 5'untranslated region of the Human T-cell Leukemia Virus (HTLV).

pSELECT plasmids are selectable with blasticidin, hygromycin, kanamycin/G418, puromycin and Zeocin™. These selectable markers are driven by the CMV promoter in tandem with the bacterial EM7 promoter for selection in both *E. coli* and mammalian cells. pSELECT is also available with the GFPzeo fusion gene that combines the GFP reporter gene and the Zeocin™ resistance gene.

pSELECT plasmids comprise a multiple cloning site (MCS) downstream of the EF-1α/HTLV promoter. The MCS contains several restriction sites that are compatible with many other enzymes to facilitate cloning.



Contents

pSELECT plasmids are provided as 20 µg of lyophilized DNA. Product is shipped at room temperature and should be stored at -20°C. Plasmid is stable for up to one year when properly stored.

PRODUCT	QUANTITY	CAT. CODE
pSELECT-blasti-mcs	20 µg	psetb-mcs
pSELECT-hygro-mcs	20 µg	pseth-mcs
pSELECT-neo-mcs	20 µg	psetn-mcs
pSELECT-puro-mcs	20 µg	psetp-mcs
pSELECT-zeo-mcs	20 µg	psetz-mcs
pSELECT-GFPzeo-mcs	20 µg	psetgz-mcs

pSELECT-Tag

Description

pSELECT-Tag is a family of expression plasmids designed to generate tagged proteins in order to facilitate their detection and/or purification. pSELECT-Tag plasmids feature several tags and allow addition of the tag at either the N or C terminus of the protein of interest.

pSELECT-tag plasmids are available with blasticidin or Zeocin resistance, which are both selectable in *E. coli* and mammalian cells.

A Choice of Four Tags

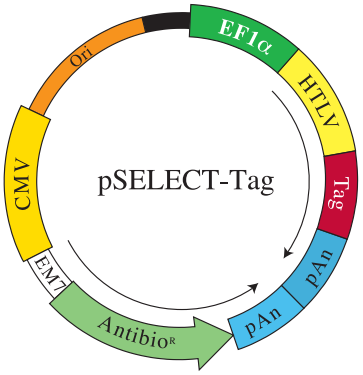
GFP-Tag: the green fluorescent protein (GFP) tag allows visualization of the spatial and temporal localization of the tagged protein by fluorescence microscopy.
HA-Tag: the human influenza hemagglutinin (HA) tag allows detection of the tagged protein by immunocytochemistry, immunoprecipitation or Western blotting, or purification of the tagged protein by affinity chromatography.
His-Tag: the polyhistidine (HIS) allows purification of the tagged protein using an NI-NTA column.
Lucia-Tag: Lucia is a novel secreted luciferase with strong bioluminescent activity.The luciferase activity of Lucia can be detected and quantified directly in the culture medium of transfected cells using InvivoGen's Quanti-Luc™ detection reagent (see p.17). The Lucia-tag allows detection and quantification of the tagged protein by bioluminescence assay.

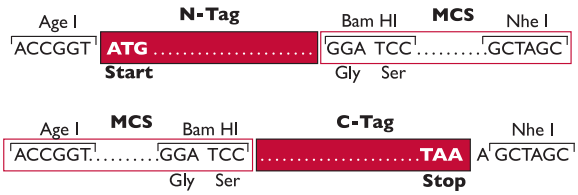
N-Tagged or C-Tagged Protein

N-terminal tag: the tag encompasses the Start codon and is followed by a multiple cloning site (MCS).
C-terminal tag: the tag is cloned downstream of an MCS and followed by a Stop codon.

Contents

pSELECT-Tag plasmids are provided as 20 µg of lyophilized DNA. Product is shipped at room temperature and should be stored at -20°C. Plasmid is stable for up to one year when properly stored.





PRODUCT	QTY	CAT. CODE (N-Tag)	CAT. CODE (C-Tag)
pSELECT-GFP-blasti	20 µg	psetb-ngfp	psetb-cgfp
pSELECT-GFP-zeo	20 µg	psetz-ngfp	psetz-cgfp
pSELECT-HA-blasti	20 µg	psetb-nha	psetb-cha
pSELECT-HA-zeo	20 µg	psetz-nha	psetz-cha
pSELECT-His-blasti	20 µg	psetb-nhis	psetb-chis
pSELECT-His-zeo	20 µg	psetz-nhis	psetz-chis
pSELECT-Lucia-blasti	20 µg	psetb-nlucia	-
pSELECT-Lucia-zeo	20 µg	psetz-nlucia	-

RECENT CITATIONS

pMONO-Hygro-GFP - Lee M. *et al.*, 2015. Silencing of E2F3 suppresses tumor growth of Her2+ breast cancer cells by restricting mitosis. Oncotarget. 6(35):37316-34.

pSELECT-blasti & pSELECT-zeo - Humbert O. *et al.*, 2016. Development of Third-generation Cocal Envelope Producer Cell Lines for Robust Lentiviral Gene Transfer into Hematopoietic Stem Cells and T-cells. Mol Ther. [Ahead of print].

pSELECT-GFPzeo - Anmole G. *et al.*, 2015. A robust and scalable TCR-based reporter cell assay to measure HIV-1 Nef-mediated T cell immune evasion. J Immunol Methods. 426:104-13.

pSELECT-neo-mcs - Mendell JR. *et al.*, 2015. A phase 1/2a follistatin gene therapy trial for becker muscular dystrophy. Mol Ther. 23(1):192-201

RELATED PRODUCTS

PRODUCT	INFO
Selection antibiotics	See pages 12-14

IN VITRO BIGENIC EXPRESSION - pVITRO

pVITRO is a family of plasmids developed mainly for in vitro *studies. They allow ubiquitous and constitutive co-expression of two genes of interest. pVITRO plasmids can be stably transfected in mammalian cells, providing high levels of expression of the genes of interest. Each pVITRO plasmid is available with either two multiple cloning sites or two reporter genes.*

Description

Co-expression of two genes from a single vector

pVITRO are multigenic plasmids that contain two distinct transcription units (TU). They feature promoters of different origins or promoters that are coordinately activated, and strong polyadenylation signals to minimize transcriptional interference.

pVITRO1 plasmids carry two elongation factor 1 alpha (EF-1α) promoters, from rat and mouse origins combined to the CMV and SV40 enhancers, respectively.
pVITRO2 plasmids contain two human ferritin composite promoters, FerH (heavy chain) and FerL (light chain), combined to the SV40 and CMV enhancers, respectively. To eliminate the iron regulation, their 5'UTRs have been replaced by the 5'UTR of the mouse and chimpanzee EF-1α genes.

Single Selection Marker for *E. coli* and Mammalian Cells

pVITRO plasmids are available with different selectable markers that are active both in E. coli and mammalian cells:
- *bsr* gene (blasticidin resistance)
- *hph* gene (hygromycin resistance)
- *neo* gene (kanamycin/G418 resistance)

In bacteria, the resistance gene is expressed from the *E. coli* EM7 promoter. In mammalian cells, it is transcribed from the promoter located at the 3' of the Ori, as a polycistronic mRNA and translated through the IRES of the foot and mouth disease virus.

Available with Two MCS

Each multiple cloning site (MCS) contains several restriction sites that are compatible with many other enzymes, thus facilitating cloning.

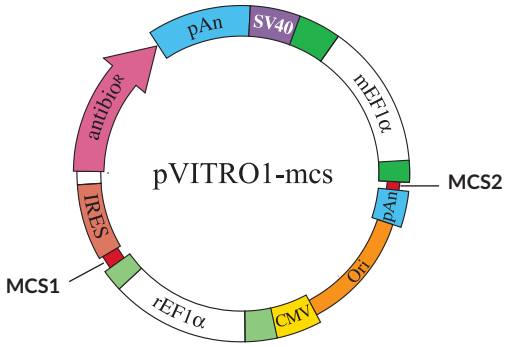
pVITRO1 cloning sites:
- MCS1: 5'- Bsp EI, Bst 1107I, Bam HI, Bsi WI, Avr II -3'
- MCS2: 5'- Age I, Eco RV, Bgl II, Bsr GI, Nhe I -3'

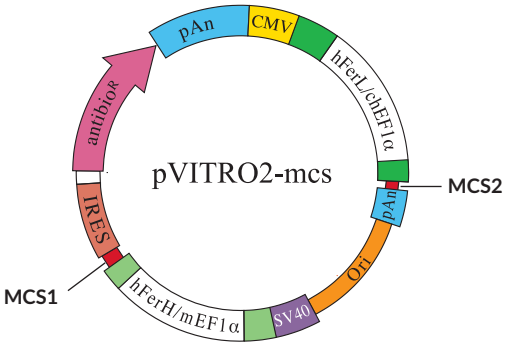
pVITRO2 cloning sites:
- MCS1: 5'- Age I, Eco RV, Bam HI, Mlu I, Cla I, Sal I, Avr II -3'
- MCS2: 5'- Sgr AI, Fsp I, Bgl II, Eco RI, Bst BI, Xho I, Nhe I -3'

Contents

Each pVITRO plasmid is provided as 20 µg of lyophilized DNA. Product is shipped at room temperature and should be stored at -20°C. Plasmid is stable for up to one year when properly stored.

PRODUCT	QUANTITY	CAT. CODE
pVITRO1 Plasmids		
pVITRO1-blasti-mcs	20 µg	pvitro1-bmcs
pVITRO1-hygro-mcs	20 µg	pvitro1-mcs
pVITRO1-neo-mcs	20 µg	pvitro1-nmcs
pVITRO2 Plasmids		
pVITRO2-blasti-mcs	20 µg	pvitro2-bmcs
pVITRO2-hygro-mcs	20 µg	pvitro2-mcs
pVITRO2-neo-mcs	20 µg	pvitro2-nmcs





ALSO AVAILABLE

pVITRO plasmids with two reporter genes

pVITRO1 and pVITRO2 plasmids are also available with the GFP (green fluorescent protein) and SEAP (secreted alkaline phosphatase) reporter genes or the Lucia luciferase (secreted coelenterazine-utilizing luciferase) and SEAP reporter genes. Visit our website for more information.

RECENT CITATIONS

pVITRO1 - Yang Y. *et al.*, **2015.** FANCD2 and REV1 cooperate in the protection of nascent DNA strands in response to replication stress. Nucleic Acids Res. 43(17):8325-39.

pVITRO2 - Brentville VA. *et al.*, **2016.** Citrullinated Vimentin Presented on MHC-II in Tumor Cells Is a Target for CD4+ T-Cell-Mediated Antitumor Immunity. Cancer Res. 76(3):548-60.

IN VIVO BIGENIC EXPRESSION - pVIVO

pVIVO is a family of plasmids specifically designed for in vivo *experiments. They allow strong and sustained co-expression of two genes of interest in many tissues and organs. Each pVIVO is available with two multiple cloning sites or two different reporter genes.*

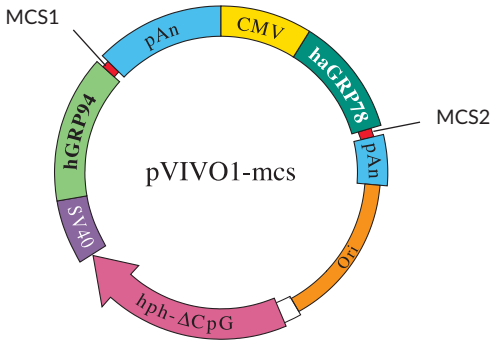
Description

Concomitant Expression of Two Genes of Interest

pVIVO plasmids contain two separate transcription units (TU), each consisting of a strong mammalian promoter and an efficient polyadenylation signal. Both promoters were chosen for their ability to work simultaneously to reduce transcriptional interference.

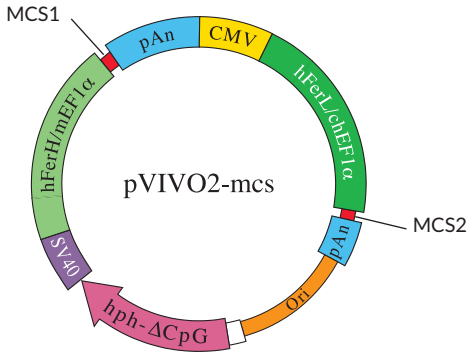
pVIVO1 plasmids contain two glucose-regulated protein (GRP) promoters coupled with either the SV40 or CMV enhancer. The human GRP94 and hamster GRP78 promoters drive weak levels of expression in normal conditions and are induced in stress conditions prevailing inside tumors, such as glucose deprivation and hypoxia.
pVIVO2 plasmids contain two human ferritin composite promoters, FerH (heavy chain) and FerL (light chain), combined with the SV40 and CMV enhancers respectively. To eliminate the iron regulation, their 5'UTRs have been replaced by the 5'UTR of the mouse and chimpanzee EF-1α genes.

pVIVO plasmids carry the hygromycin resistance gene for selection and amplification in *E. coli*. pVIVO plasmids do not contain a selectable marker for mammalian cells.



Sustained Expression of the Transgenes

Both promoters in pVIVO plasmids are of mammalian origin and can yield longer lasting expression of the transgenes in vivo compared to viral promoters. Indeed, viral DNA sequences are recognized by the host immune system leading to their inactivation, usually through methylation. To further increase the duration of expression, immunostimulatory sequences known as CpG motifs, which are present in high numbers in bacterial DNA, have been removed from the plasmid backbone. These specific motifs, found in *E. coli* hygromycin resistance (hph) have been eliminated by chemically synthesizing a new allele of this gene without altering the protein sequence.



Available with Two MCS

Each pVIVO plasmids contain two multiple cloning sites (MCS) for the convenient cloning of cDNAs.
pVIVO1 cloning sites:
- MCS1: 5'- Age I, Bsp HI, Xba I, Hind III, Bst 1107I, Avr II -3'
- MCS2: 5'- Ngo MI, Nco I, Bgl II, Eco RI, Nhe I-3'

pVIVO2 cloning sites:
- MCS1: 5'- Bsp HI, Xba I, Bsr GI, Bst 1107I, Avr II -3'
- MCS2: 5'- Nco I, Bam HI, Eco RI, Eco RV, Nhe I-3'

Contents

Each pVIVO plasmid is provided as 20 µg of lyophilized DNA. Product is shipped at room temperature and should be stored at -20°C. Plasmid is stable for up to one year when properly stored.

PRODUCT	QUANTITY	CAT. CODE
pVIVO1-mcs	20 µg	pvivo1-mcs
pVIVO2-mcs	20 µg	pvivo2-mcs

ALSO AVAILABLE

pVIVO plasmids with two reporter genes

pVIVO1 and pVIVO2 plasmids are also available with the GFP (green fluorescent protein) and SEAP (secreted alkaline phosphatase) reporter genes or the Lucia luciferase (secreted coelenterazine-utilizing luciferase) and SEAP reporter genes. Visit our website for more information.

RECENT CITATIONS

pVIVO1 - Gupta SK. *et al.*, **2016.** Combined administration of the apoptin gene and poly (I:C) induces potent anti-tumor immune response and inhibits growth of mouse mammary tumors. Int Immunopharmacol. 35:163-73.

pVIVO2 - Rogers ML. *et al.*, **2014.** Non-viral gene therapy that targets motor neurons in vivo. Front Mol Neurosci. 7:80.

CpG-FREE PLASMIDS - pCpGfree

Bacterial DNA is rich in unmethylated cytosine-phosphate-guanine (CpG) sites, unlike mammalian DNA, in which most CpG sites are methylated. Unmethylated CpGs in specific sequence contexts activate the vertebrate immune system. Plasmid DNAs with these sequences are recognized as foreign DNA by the vertebrate host, leading to a progressive decline in their expression. InvivoGen has developed a family of CpG-free plasmids designed for long term expression in vivo or to study the effect of promoter CpG methylation in transfection assays.

Description

pCpGfree is a family of plasmids completely devoid of CpG dinucleotides. Typically, the elements required for replication and selection of the plasmid in *E. coli* and gene expression in mammalian cells are rich in CpG. In the pCpGfree plasmids these elements are either naturally CpG-free, or have been modified to remove all CpGs, or entirely synthesized.

Origin of replication: The *E. coli* R6K gamma ori has been modified to remove all CpGs. This origin is activated by the R6K specific initiator protein π, encoded by the pir gene.

Bacterial promoter: EM2K is a CpG-free version of the bacterial EM7 promoter.

Mammalian promoter: The CpG-free promoter combines the mouse CMV enhancer, the human elongation factor 1 alpha core promoter and 5'UTR containing a synthetic intron.

Polyadenylation signal: The polyadenylation signal is a CpG-free form of the late SV40 polyadenylation signal.

MAR: Matrix attached regions (MARs) are sequences that are typically AT-rich and can form barriers between independently regulated domains. pCpGfree plasmids contain two naturally CpG-free MARs from the 5' region of the human IFN-β gene or β-globin gene. The MARs are placed between the bacterial and mammalian transcription units.

Selectable marker: Zeocin™ resistance is conferred by a CpG-free allele of the CpG-rich Zeo gene.

Furthermore, all *Dam* methylation sites (GATC) have been removed to prevent prokaryotic methylation.

A Choice of pCpGfree Plasmids

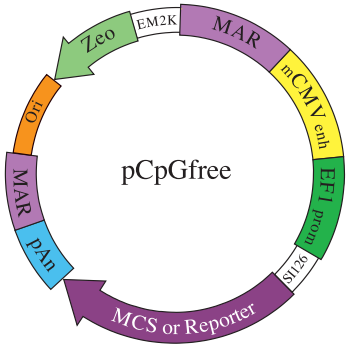
pCpGfree-mcs contains a multiple cloning site (MCS) featuring several commonly used restriction sites for convenient cloning of a CpG-free gene or any open reading frame or cDNA.

pCpGfree-OVA expresses a synthetic OVA gene, a CpG-free allele of the ovalbumin (OVA) gene constructed by chemical synthesis. This plasmid is designed for DNA immunization experiments in animal models. This plasmid possesses dual functions; it can be used as a DNA vaccine carrier for antigen presentation, and as an immunostimulatory adjuvant.

pCpGfree-basic-Lucia lacks the entire promoter region. It contains an MCS upstream of the Lucia luciferase reporter gene. Expression of Lucia luciferase in cells transfected with this plasmid depends on the insertion of a functional promoter or enhancer/promoter cassette upstream from the reporter gene. Thus, the pCpGfree-basic-Lucia plasmid allows the study of the effect of CpG methylation on a promoter, whether it is alone or combined with enhancer elements.

pCpGfree-promoter-Lucia contains the human EF-1α promoter and an MCS in place of the murine CMV enhancer. It is specifically designed to analyze the effect of methylation on CpG residues present in enhancer elements by assessing the expression of the Lucia luciferase gene.

pCpGrich-mcs is a CpG-containing control plasmid. The CpG-free version of the murine CMV enhancer, human EF-1α promoter, ori R6K gamma, bacterial EM2K promoter and Zeocin™ resistance gene have been replaced by their wild-type counterparts. The pCpGrich-mcs plasmid contains 88 CpG dinucleotides.



Due to the presence of the R6K gamma origin of replication, pCpGfree plasmids can only be amplified in an *E. coli* mutant strain expressing a mutant pir gene. They will not replicate in standard *E. coli* strains. Therefore, pCpGfree plasmids are provided with the *E. coli* GT115 strain, a *pir* mutant also deficient in *Dcm* methylation (see page 36).

Contents

pCpGfree and pCpGrich plasmids are provided as 20 µg of lyophilized DNA with a paper disk of lyophilized *E. coli* GT115 strain. Products are shipped at room temperature and should be stored at -20°C.

PRODUCT	QUANTITY	CAT. CODE
pCpGfree-mcs	20 µg	pcpgf-mcs
pCpGfree-OVA	20 µg	pcpgf-ova
pCpGfree-basic-Lucia	20 µg	pcpgf-baslc
pCpGfree-promoter-Lucia	20 µg	pcpgf-promlc
pCpGrich-mcs	20 µg	pcpgr-mcs

RECENT CITATIONS

pCpG-free-mcs - Sharma A. *et al.*, 2016. Principles Governing DNA Methylation during Neuronal Lineage and Subtype Specification. J Neurosci. 36(5):1711-22.

pCpG-free-basic-Lucia - Nguyen A. *et al.*, 2016. Age-Dependent Demethylation of Sod2 Promoter in the Mouse Femoral Artery. Oxid Med Cell Longev. 2016:8627384.

pCpG-free-promoter-Lucia - Weigel C. *et al.*, 2016. Epigenetic regulation of diacylglycerol kinase alpha promotes radiation-induced fibrosis. Nat Commun. 7:10893.

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INDUCIBLE REPORTER PLASMIDS - pNiFty

Description

pNiFty is a family of reporter plasmids expressing a reporter gene under the control of a minimal promoter inducible by either NF-κB or type I interferons.

Two Plasmid Backbones

pNiFty - NF-κB-inducible plasmids

pNiFty-plasmids feature the proximal promoter of the endothelial cell-leukocyte adhesion molecule (ELAM-1; E-selectin) gene contains three NF-κB sites and is truly NF-κB specific, as it lacks an AP1/CREB site found in the full-length promoter[1, 2]. This promoter is fused to five additionnal NF-κB sites.

pNiFty-plasmids contain the bacterial selectable marker ampicillin and can only be used in transient transfection experiments.

pNiFty2 - NF-κB- or IFN-inducible plasmids

pNiFty2 plasmids are available with the composite ELAM-1 promoter described above or the human ISG-56K minimal promoter. ISG-56K is the prototype interferon-stimulated gene (ISG) which expression is highly increased by type I interferons[3].

pNiFty2 plasmids are selectable with Zeocin™ in both *E. coli* and mammalian cells and thus can be used in transient and stable transfection experiments.

1. Schindler U. & Baichwal VR., 1994. Three NF-kappa B binding sites in the human E-selectin gene required for maximal tumor necrosis factor alpha-induced expression. Mol Cell Biol. 14(9):5820-31. **2. Jensen LE. & Whitehead AS., 2003.** ELAM-1/E-selectin promoter contains an inducible AP-1/CREB site and is not NF-kB-specific. Biotechniques 35:54-58. **3. Der SD. et al., 1998.** Identification of genes differentially regulated by interferon alpha, beta, or gamma using oligonucleotide arrays. PNAS. 95(26):15623-8.

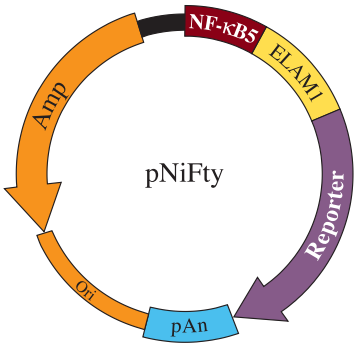
A choice of Reporters

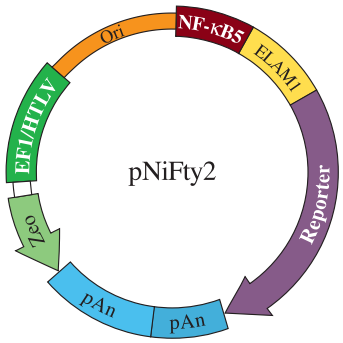
SEAP (secreted embryonic alkaline phosphatase gene) expression can be rapidly and readily measured in supernatants of transfected cells. SEAP levels can be evaluated qualitatively with the naked eye and quantitatively using QUANTI-Blue™ or HEK-Blue™ Detection (see p. 16).

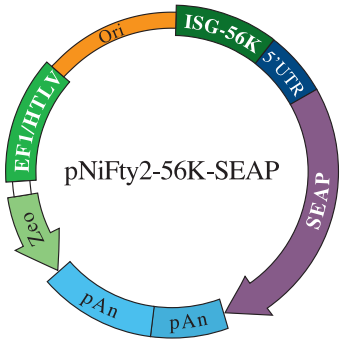
Luc (firefly luciferase) expression can be quantified in cell extracts by using kits commercially available from other companies.

Contents

Each pNiFty plasmid is provided as 20 µg of lyophilized DNA. Product is shipped at room temperature and should be stored at -20°C. Plasmid is stable for up to one year when properly stored.







PRODUCT	QUANTITY	CAT. CODE
pNiFty-SEAP	20 µg	pnifty-seap
pNiFty-Luc	20 µg	pnifty-luc
pNiFty2-SEAP	20 µg	pnifty2-seap
pNiFty2-Luc	20 µg	pnifty2-luc
pNiFty2-56K-SEAP	20 µg	pnf2-56ksp

RELATED PRODUCTS

PRODUCT	INFO
QUANTI-Blue™	See page 16
QUANTI-Luc™	See page 17

RECENT CITATIONS

pNiFty-Luc - Rojas JJ. *et al.*, 2016. Manipulating TLR Signaling Increases the Anti-tumor T Cell Response Induced by Viral Cancer Therapies. Cell Rep. 15(2):264-73.

pNiFty2-Luc - Parvani JG. *et al.*, 2015. Silencing b3 integrin by targeted ECO/siRNA nanoparticles inhibits EMT and metastasis of triple-negative breast cancer. Cancer Res. 75(11):2316-25.

pNiFty-SEAP - Hüttmann J. *et al.*, 2015. Functional Comparison of Molluscum Contagiosum Virus vFLIP MC159 with Murine Cytomegalovirus M36/vICA and M45/vIRA Proteins. J Virol. 90(6):2895-905.

GENES & PROMOTERS

HUMAN & MOUSE GENES - pUNO1

InvivoGen provides a collection of over 1,600 full-length sequenced genes, mainly from human and mouse, involved in immunity and cancer. These genes, provided as open reading frames (ORFs) from the ATG to the Stop codon, are now all available in the mammalian expression plasmid pUNO1. The ORFs are cloned downstream of a strong and ubiquitous mammalian promoter that makes these clones suitable for stable expression and functional studies in various mammalian cell lines.

Description

Full-length Sequenced Open Reading Frames

InvivoGen provides a large collection of fully sequenced human and murine genes. Genes from other species are also available. These genes are provided as open reading frames (ORFs) from the ATG to the Stop codon, excluding introns and untranslated regions. As the ORFs are amplified from cDNA banks, a variant form is sometimes obtained. Most of the variations have been reported in GenBank.

Some of the genes provided encode proteins that are naturally secreted. Their sequences include their native signal sequences, which are generally located at the 5' end of the coding sequence. Other genes, that code for engineered proteins, such as particular fragments of longer proteins (i.e. angiostatic proteins) may include the signal sequence of the human IL-2 gene between the ATG and the second codon to allow their secretion.

Suitable for Expression in Mammalian Cells

Each ORF is cloned in a mammalian expression cassette consisting of a potent and ubiquitous composite promoter and the strong SV40 polyadenylation signal. Each ORF is flanked by a unique restriction site at the 5' end and 3' end to facilitate its subcloning into another vector. Each ORF is supplied in pUNO1, a plasmid selectable in both bacterial and mammalian cells with blasticidin.

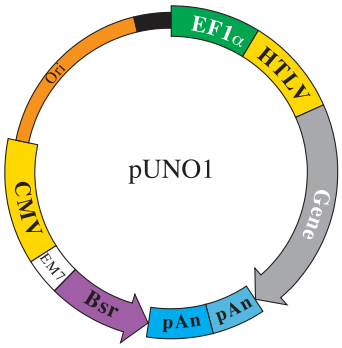
Search the ORF Collection Online

You can easily find your ORF of interest in the ORF Collection at www.invivogen.com/orfs. You can browse the entire list based on HUGO-approved gene symbol (and alias) and function. All sequences are available online or can be emailed upon request.

Engineered Genes also Available

HA-tagged genes contain at their 3' end the influenza hemaglutinine (HA) tag. This short sequence (YPYDVPDYA) encodes a peptide that is the epitope of a very efficient and specific monoclonal antibody. This tag allows for efficient and specific detection of these genes by Western blot using the anti-HA tag antibody. HA-tagged genes are provided in the pUNO1 plasmid. InvivoGen's HA-tagged genes include TLR genes and TLR-related genes.

CpG-free genes: Many non-mammalian genes are widely used as reporter or suicide genes in molecular and cellular studies. However, these genes are recognized as foreign DNA by the vertebrate host leading to a progressive decline of their expression. To circumvent this limitation, InvivoGen has synthesized new alleles of these genes completely devoid of CpG dinucleotides. These synthetic CpG-free genes display higher activity and lower immunogenicity than their wild-type counterparts. CpG-free genes are provided in the pSELECT-Zeo plasmid, which is selectable with Zeocin™ in both *E. coli* and mammalian cells.



Contents

Each pUNO1 plasmid is provided as 20 µg of lyophilized DNA. Product is shipped at room temperature and should be stored at -20°C for up to one year.

PRODUCT	QUANTITY	CAT. CODE
pUNO1-<gene>	20 µg	puno1-<gene>

RECENT CITATIONS

pUNO1-hTLR2 - Adamson SE. *et al.*, 2016. Disabled homolog 2 controls macrophage phenotypic polarization and adipose tissue inflammation. J Clin Invest. 126(4):1311-22.

pUNO1-mVEGF - Stephan SB. *et al.*, 2015. Biopolymer implants enhance the efficacy of adoptive T-cell therapy. Nat Biotechnol. 33(1):97-101.

pUNO1-mATG5 - Jabir MS. *et al.*, 2015. Mitochondrial damage contributes to Pseudomonas aeruginosa activation of the inflammasome and is downregulated by autophagy. Autophagy. ;11(1):166-82

pUNO1-hBDNFa - Hayakawa K. *et al.*, 2015. Intrathecal injection of a therapeutic gene-containing polyplex to treat spinal cord injury. J Control Release. 197:1-9

pUNO1-hNOD1 - Suarez G. *et al.*, 2015. Modification of Helicobacter pylori Peptidoglycan Enhances NOD1 Activation and Promotes Cancer of the Stomach. Cancer Res. 75(8):1749-59.

pUNO1-hcGAS - Ma Z. *et al.*, 2015. Modulation of the cGAS-STING DNA sensing pathway by gammaherpesviruses. PNAS. 112(31):E4306-15.

CELLULAR PROMOTERS - pDRIVE5

InvivoGen offers a large collection of native and composite promoters provided in the pDRIVE5 plasmid. Promoters are valuable tools to study the expression of a given gene both in vitro and in vivo. Now with pDRIVE5, you can choose to express your gene of interest at high or low levels, ubiquitously or specifically, and in a constitutive or inducible manner. Each promoter is fully sequenced.

Description

Native or Composite Promoters

Native promoters, also called minimal promoters, consist of a single fragment from the 5' region of a given gene. Each is comprised of a core promoter and its natural 5'UTR. In some cases, the 5'UTR contains an intron.

Composite promoters combine promoter elements of different origins (e.g. SV40 enhancer/AFP promoter) or were generated by assembling a distal enhancer with a minimal promoter of the same origin (e.g. CEA enhancer/promoter).

Various Expression Patterns

- **Ubiquitous Promoters**, strongly active in a wide range of cells, tissues and cell cycles.
- **Tissue Specific Promoters,** active in specific cell or tissue types.
- **Tumor Specific Promoters,** active specifically in tumor cells.

Easy Subcloning of the Promoters

In every pDRIVE5, several unique restriction sites flank the promoter in order to excise the promoter easily. These restriction sites are compatible with many other enzymes, thus facilitating cloning. Other restriction sites may be introduced by performing PCR using primers containing the restriction sites of interest.

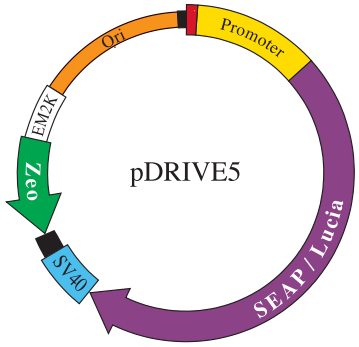
Transient Expression in Mammalian Cells *in vitro*

pDRIVE5 plasmids carry the Zeocin™ resistance gene for selection in *E. coli*. They can be used for transient transfection experiments in mammalian cells in vitro or for expression studies *in vivo*.

A Choice of Reporter Genes

In each pDRIVE5, the promoter drives the expression of a reporter gene, SEAP or Lucia luciferase, to facilitate the evaluation of its activity. Three different reporter genes are available in two different backbones:

- **pDRIVE5-SEAP** plasmids express the secreted embryonic alkaline phosphatase (SEAP) reporter gene. SEAP expression levels can be readily assessed using chromogenic assays, such as QUANTI-Blue™, or luminescence assays.
- **pDRIVE5-Lucia** plasmids feature the Lucia luciferase gene, encoding a secreted luciferase. Its intense sensitivity makes it the reporter of choice for the study of weak promoters, such as tissue-specific promoters. Levels of Lucia luciferase can be determined by measuring the luminescence using coelenterazine-based reagents, such as QUANTI-Luc™.



Contents

pDRIVE5 plasmids are provided as 20 µg of lyophilized DNA. Products are shipped at room temperature. Store at -20°C for up to one year. Each pDRIVE5 plasmid is provided with 10 mg Zeocin™.

PRODUCT	QUANTITY	CAT. CODE
pDRIVE5-SEAP-<promoter>	20 µg	pdrive5s-<prom>
pDRIVE5-Lucia-<promoter>	20 µg	pdrive5lc-<prom>

RELATED PRODUCTS

PRODUCT	INFO
PromTest™	Visit website
Custom-Made pDRIVE	Visit website

RECENT CITATIONS

pDRIVE-hAFP - Zhou J. *et al.*, **2016.** Improvement of the cytotoxic T lymphocyte response against hepatocellular carcinoma by transduction of cancer cells with an adeno-associated virus carrying the interferon-γ gene. Mol Med Rep. 13(4):3197-205.

pDRIVE-hGFAP - Yurek DM. *et al.*, **2015**. Age and lesion-induced increases of GDNF transgene expression in brain following intracerebral injections of DNA nanoparticles. Neuroscience. 284:500-12.

pDRIVE-hMUC1 - Fang CY. *et al.*, **2015.** Inhibition of human bladder cancer growth by a suicide gene delivered by JC polyomavirus virus-like particles in a mouse model. J Urol. 193(6):2100-6.

pDRIVE-hSynapsin - Manchon JF. *et al.*, **2016.** Levetiracetam mitigates doxorubicin-induced DNA and synaptic damage in neurons. Sci Rep. 6:25705.

pDRIVE-mCD68 - Nakatsuji M. *et al.*, **2015.** EP4 Receptor-Associated Protein in Macrophages Ameliorates Colitis and Colitis-Associated Tumorigenesis. PLoS Genet. 11(10):e1005542.

PROMOTER	GENE	TISSUE DISTRIBUTION	SPECIES	SIZE	CAT. CODE
UBIQUITOUS PROMOTERS					
Native Promoters					
β-Act	Beta-Actin	Ubiquitous	Human	2387 bp	pdrive5(s/lc)-hbact
EF-1α	Elongation Factor 1 alpha	Ubiquitous	Chimpanzee Mouse Rat	1365 bp 1315 bp 1314 bp	pdrive5(s/lc)-chef1 pdrive5(s/lc)-mef1 pdrive5(s/lc)-ref1
EGR1	Early Growth Response 1	Ubiquitous, inducible by radiation	Human	1062 bp	pdrive5(s/lc)-hegr1
eIF4A1	Eukaryotic Initiation Factor 4A1	Ubiquitous	Human Mouse	523 bp 482 bp	pdrive5(s/lc)-heif4a1 pdrive5(s/lc)-meif4a1
FerH	Ferritin Heavy Chain	Ubiquitous	Human	370 bp	pdrive5(s/lc)-hferh
FerL	Ferritin Light Chain	Ubiquitous	Human	429 bp	pdrive5(s/lc)-hferl
GAPDH	Glyceraldehyde 3-phosphate Dehydrogenase	Ubiquitous	Human	794 bp	pdrive5(s/lc)-hgapdh
GRP78	Glucose-Regulated Protein 78	Ubiquitous, inducible by stress	Human Mouse Rat	531 bp 545 bp 679 bp	pdrive5(s/lc)-hgrp78 pdrive5(s/lc)-mgrp78 pdrive5(s/lc)-rgrp78
GRP94	Glucose-Regulated Protein 94	Ubiquitous, inducible by stress	Human Mouse Rat	701 bp 696 bp 731 bp	pdrive5(s/lc)-hgrp94 pdrive5(s/lc)-mgrp94 pdrive5(s/lc)-rgrp94
HSP70	Heat Shock Protein 70	Ubiquitous, inducible by heat	Human Mouse	464 bp 1041 bp	pdrive5(s/lc)-hhsp70 pdrive5(s/lc)-mhsp70
β-Kin	Beta-Kinesin	Ubiquitous	Human	539 bp	pdrive5(s/lc)-hbkin
PGK1	Phosphoglycerate Kinase 1	Ubiquitous	Mouse	1428 bp	pdrive5(s/lc)-mpgk
ROSA	Rosa	Ubiquitous	Human Mouse	2815 bp 1926 bp	pdrive5(s/lc)-hrosa pdrive5(s/lc)-mrosa
Ubi B	Ubiquitin B	Ubiquitous	Human	1092 bp	pdrive5(s/lc)-hubiquitinb
Composite Promoters					
β-Act/RU5'	Beta-Actin prom / HTLV 5'UTR	Ubiquitous	Human	1785 bp	pdrive5(s/lc)-hbactru5
EF-1α/RU5'	Elongation Factor 1 prom / HTLV 5'UTR	Ubiquitous	Chimpanzee Rat	672 bp 630 bp	pdrive5(s/lc)-chef1ru5 pdrive5(s/lc)-ref1ru5
FerH/RU5'	Ferritin H prom / HTLV 5'UTR	Ubiquitous	Human	457 bp	pdrive5(s/lc)-hferhru5
SV40/FerH	SV40 enh / Ferritin H prom / EF-1α 5'UTR	Ubiquitous	Human / mouse	1423 bp	pdrive5(s/lc)-sv40ferhef1
FerL/RU5'	Ferritin L prom / HTLV 5'UTR	Ubiquitous	Human	537 bp	pdrive5(s/lc)-hfer1ru5
CMV/FerL	CMV enh / Ferritin L prom / EF-1α 5'UTR	Ubiquitous	Human / chimp.	1666 bp	pdrive5(s/lc)-cmvferlef1
TISSUE-SPECIFIC PROMOTERS					
Native Promoters					
B29	Immunoglobulin Beta	B cells	Human Mouse	1219 bp 1235 bp	pdrive5(s/lc)-hb29 pdrive5(s/lc)-mb29
CD14	Monocyte Receptor for Bacterial LPS	Monocytic cells	Human	612 bp	pdrive5(s/lc)-hcd14
CD43	Leukosialin, Sialophorin	Leukocytes and platelets	Human	775 bp	pdrive5(s/lc)-hcd43
CD45	Leukocyte Common Antigen (LCA)	Hematopoietic cells	Human	856 bp	pdrive5(s/lc)-hcd45
CD68	Human Homolog of Macrosialin	Macrophages	Human Mouse	658 bp 811 bp	pdrive5(s/lc)-hcd68 pdrive5(s/lc)-mcd68
Desmin	Desmin	Muscle	Mouse	983 bp	pdrive5(s/lc)-mdesmin
Elastase	Elastase1	Pancreatic acinar cells	Rat	264 bp	pdrive5(s/lc)-relastase
Endoglin	Endoglin	Endothelial cells	Human	886 bp	pdrive5(s/lc)-hendoglin
FN	Fibronectin	Differentiating cells, healing tissues	Human	1037 bp	pdrive5(s/lc)-hfibronectin
Flt-1	VEGF Receptor 1	Endothelial cells	Human	786 bp	pdrive5(s/lc)-hflt1

PROMOTER	GENE	TISSUE DISTRIBUTION	SPECIES	SIZE	CAT. CODE
Native Promoters					
GFAP	Glial Fibrillary Acidic Protein	Astrocytes	Human Mouse Rat	1675 bp 1679 bp 1588 bp	pdrive5(s/lc)-hgfap pdrive5(s/lc)-mgfap pdrive5(s/lc)-rgfap
GPIIb	Integrin alpha IIb	Megakaryocytes	Human	928 bp	pdrive5(s/lc)-hgp2b
ICAM-2	Intercellular Adhesion Molecule 2	Endothelial cells	Human	397 bp	pdrive5(s/lc)-hicam2
IFN-β	Interferon beta	Hematopoietic cells	Mouse	214 bp	pdrive5(s/lc)-mifnb
Mb	Myoglobin	Muscle	Human Mouse	450 bp 404 bp	pdrive5(s/lc)-hmb pdrive5(s/lc)-mmb
Nphsl	Nephrin	Podocytes	Human	1233 bp	pdrive5(s/lc)-hnphs
OG-2	Osteocalcin 2	Osteoblasts	Mouse	225 bp	pdrive5(s/lc)-mog2
SP-B	Surfactant Protein B	Lung	Human	633 bp	pdrive5(s/lc)-hspb
Synapsin	Synapsin	Neurons	Human	556 bp	pdrive5(s/lc)-hsynapsin
WASP	Wiskott-Aldrich Syndrome Protein	Hematopoietic cells	Human	506 bp	pdrive5(s/lc)-hwasp
Composite Promoters					
SV40/Alb	SV40 enh / Albumin prom	Liver	Bovine Human	443 bp 449 bp	pdrive5(s/lc)-sv40balb pdrive5(s/lc)-sv40halb
SV40/CD43	SV40 enh / Leukosialin prom	Leukocytes and platelets	Human	1014 bp	pdrive5(s/lc)-sv40hcd43
SV40/CD45	SV40 enh / Leukocyte Common Antigen prom	Hematopoietic cells	Human	1096 bp	pdrive5(s/lc)-sv40hcd45
NSE-RU5'	Neuron-Specific Enolase prom / HTLV 5'UTR	Mature neurons	Rat	2043 bp	pdrive5(s/lc)-rnseru5
TUMOR-SPECIFIC PROMOTERS					
Native Promoters					
AFP	Alpha-Fetoprotein	Hepatocellular carcinoma	Human	274 bp	pdrive5(s/lc)-hafp
CCKAR	Cholecystokinin type A Receptor	Pancreatic cancer	Human	724 bp	pdrive5(s/lc)-hcckar
CEA	Carcinoembryonic Antigen	Epithelial cancers	Human	428 bp	pdrive5(s/lc)-hcea
c-erbB2	C-erbB2/neu Oncogen	Breast and pancreas cancer	Human	891 bp	pdrive5(s/lc)-herbb2
COX-2	Cyclo-oxygenase 2	Tumor	Human Mouse	1568 bp 1104 bp	pdrive5(s/lc)-hcox2 pdrive5(s/lc)-mcox2
CXCR4	Receptor for Chemokine SDF1	Tumor	Human	278 bp	pdrive5(s/lc)-hcxcr4
E2F-1	E2F Transcription Factor 1	Tumor	Human	399 bp	pdrive5(s/lc)-he2f1
HE4	Human Epididymis Protein 4	Tumor	Human	652 bp	pdrive5(s/lc)-hhe4
LP	L-Plastin	Tumor	Human	2393 bp	pdrive5(s/lc)-hlp
MUC1	Mucin-like Glycoprotein	Carcinoma cells	Human	757 bp	pdrive5(s/lc)-hmuc1
PSA	Prostate Specific Antigen	Prostate, and prostate cancers	Human	670 bp	pdrive5(s/lc)-hpsa
Survivin	Survivin	Tumor	Human	266 bp	pdrive5(s/lc)-hsurvivin
TRP	Tyrosinase Related Protein	Melanocytes and melanoma	Mouse	1201 bp	pdrive5(s/lc)-mtrp
Tyr	Tyrosinase	Melanocytes and melanoma	use	335 bp	pdrive5(s/lc)-mtyr
Native Promoters					
AFP/AFP	Alpha-Fetoprotein enh-prom	Hepatocellular carcinoma	Human	2144 bp	pdrive5(s/lc)-afphafp
SV40/AFP	SV40 enh /Alpha-Fetoprotein prom	Hepatocellular carcinoma	Human	514 bp	pdrive5(s/lc)-sv40hafp
CEA/CEA	Carcinoembryonic Antigen enh-prom	Epithelial cancers	Human	1861 bp	pdrive5(s/lc)-ceahcea
PSA/PSA	Prostate Specific Antigen enh-prom	Prostate, and prostate cancers	Human	2261 bp	pdrive5(s/lc)-psahpsa
SV40/Tyr	SV40 enh / Tyrosinase prom	Melanocytes and melanoma	Mouse	576 bp	pdrive5(s/lc)-sv40mtyr
Tyr/Tyr	Tyrosinase enh-prom	Melanocytes and melanoma	Mouse	540 bp	pdrive5(s/lc)-tyrmtyr

PLASMID AMPLIFICATION

E. COLI COMPETENT CELLS - ChemiComp *E. coli*

InvivoGen's competent E. coli *strains have been designed to include genotypes that make them efficient and convenient. These isogenic strains contain genetic modifications which are introduced using an elaborate technique of homologous recombination. This technique allows the generation of targeted deletions or insertions in the* E. coli *genome. The resulting strains are made chemically competent by an optimized procedure followed by transformation efficiency verification. They are provided frozen.*

Description

ChemiComp *E. coli* are chemically competent bacteria provided frozen. Their high transformation efficiency makes them ideal for cloning and plasmid propagation. ChemiComp *E. coli* are shipped on dry ice.

ChemiComp GT115 (*Δdcm, uidA::pir-116, sbcCD*)
ChemiComp GT115 cells are high efficiency chemically competent cells specifically designed for cloning and propagation of plasmids containing hairpin structures and the R6K gamma origin of replication, such as pCpGfree plasmids (see p. 28).
Transformation efficiency: 0.1-1 x 10^9 cfu/µg

ChemiComp GT116 (*Δdcm, sbcCD*)
ChemiComp GT116 cells are high efficiency chemically competent cells specifically designed for cloning and propagation of shRNA-expressing plasmids which contain hairpin structures.
Transformation efficiency: 0.1-1 x 10^9 cfu/µg

Contents

ChemiComp *E. coli* cells are provided as 100 µl or 200 µl aliquots. ChemiComp cells are shipped on dry ice. Upon receipt, store ChemiComp cells at -80°C. ChemiComp cells are stable for at least 6 months when properly stored.

PRODUCT	QUANTITY	CAT. CODE
ChemiComp GT115	5 x 0.1 ml (5-10 transf.)	gt115-11
	5 x 0.2 ml (10-20 transf.)	gt115-21
ChemiComp GT116	5 x 0.1 ml (5-10 transf.)	gt116-11
	5 x 0.2 ml (10-20 transf.)	gt116-21

RECENT CITATIONS

GT115 - Hanuszkiewicz A. *et al.* 2015. Identification of the flagellin glycosylation system in Burkholderia cenocepacia and the contribution of glycosylated flagellin to evasion of human innate immune responses. J. Biol Chem. 289(27):19231-44.

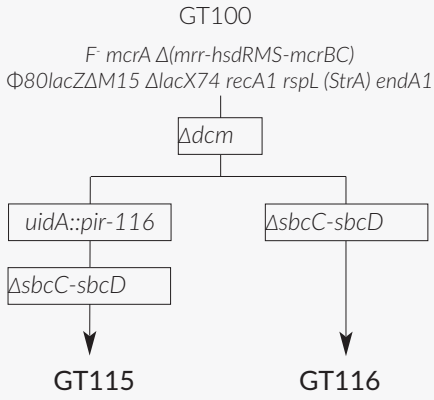
GT116 - Hoppstädter J. *et al.*, 2015. Glucocorticoid-induced leucine zipper: A critical factor in macrophage endotoxin tolerance. J. Immunol. 194: 6057-67.

Different Strains for Different Applications

InvivoGen provides two different *E. coli* competent strains that offer distinct characteristics suitable for a particular application. These strains derive from a common parental strain that features many useful genetic markers making them also versatile strains.

GT115 - *Δdcm, uidA::pir-116, ΔsbcC-sbcD mutant strain*
F⁻ mcrA Δ(mrr-hsdRMS-mcrBC) Φ80lacZΔM15 ΔlacX74 recA1 rspL (StrA) endA1 Δdcm uidA(ΔMluI)::pir-116 ΔsbcC-sbcD

GT116 - *Δdcm, ΔsbcC-sbcD mutant strain*
F⁻ mcrA Δ(mrr-hsdRMS-mcrBC) Φ80lacZΔM15 ΔlacX74 recA1 rspL (StrA) endA1 Δdcm ΔsbcC-sbcD



The *dcm* gene encodes a DNA methylase that methylates the internal cytosine residues in the recognition sequence 5´-C*CAGG-3´ or 5´-C*CTGG-3´. This methylation, which is not found in vertebrates confers a bacterial signature to DNA prepared in *E. coli* making it prone to recognition as foreign DNA by the host immune system. Mutation of the *dcm* gene diminishes the immunostimulatory effects of plasmid DNAs.

The *pir* gene encodes the R6K specific initiator protein π, which is required for the replication of plasmids harboring the R6K gamma origin of replication, such as the pCpG-free plasmid family. The *uidA::pir-116* mutant is the result of the integration of a mutant *pir* gene in the *uidA* locus. This mutant gene increases plasmid copy number several fold.

The *sbcC* and *sbcD* genes encode a dimeric protein called SbcCD that recognizes and cleaves hairpins rendering plasmids with hairpin structures particularly unstable in *E. coli*. Deletion of the *sbcCD* operon significantly improves the number of recombinant clones in plasmids containing hairpin structures.

E. COLI GROWTH & SELECTION MEDIA - Fast-Media®

All you need to make liquid or solid selective E. coli medium are five minutes, a microwave and Fast-Media®. This time-saving product, developed by InvivoGen, comes in individually sealed pouches, each with enough reagents to prepare 200 ml of sterile liquid or agar medium at the appropriate antibiotic concentration. Fast-Media® is extensively tested to guarantee sterility, antibiotic activity and E. coli growth. We subject every ready-to-use Fast-Media® pouch to rigorous quality control to ensure consistent results.

Description

Fast-Media® are pre-mixed, pre-sterilized *E. coli* growth media that can be prepared in just five minutes without autoclaving. Fast-Media® contain everything you need to prepare LB medium, TB medium or agar plates. You'll save hours of medium preparation time because there is:

- No weighing or mixing of media components
- No autoclaving
- No waiting for media to cool before adding antibiotics

These time-saving products, developed by InvivoGen, come in ready-to-use individual pouches. Each pouch contains sufficient reagents for preparing 200 ml of liquid media or 8-10 standard 100 mm^2 agar plates.

For your convenience, Fast-Media® are available with or without (Base) antibiotics. Four selective antibiotics can be chosen from:

- Ampicillin (Amp)
- Blasticidin (Blas)
- Kanamycin (Kan)
- Zeocin™ (Zeo)

Further, to allow the detection of blue/white colonies, Fast-Media® are also available with X-Gal/IPTG.

Quality Control

Each type of Fast-Media® growth medium is extensively tested to ensure *E. coli* growth, antibiotic activity, and sterility. Fast-Media® containing X-Gal/IPTG are tested for efficient blue/white colony screening.

Stability and Storage

After preparation, Fast-Media® keep all their intrinsic properties for 48 hours at 37°C or for 4 weeks at 4°C. Sterility is guaranteed when Fast-Media® are properly prepared and stored.

Contents

Each type of Fast-Media® are provided in a 20- or 30-pouch unit. Each pouch enables the preparation of 200 ml liquid medium or 8-10 agar plates. Store at room temperature. Pouches are stable for 12 months at room temperature.

RECENT CITATIONS

Fast-Media® Amp Agar X-Gal - Novitsky V. *et al.*, **2015.** Long-Range HIV Genotyping Using Viral RNA and Proviral DNA for Analysis of HIV Drug Resistance and HIV Clustering. J Clin Microbiol. 53(8):2581-92.

Fast-Media® Zeo TB and Fast-Media® Zeo Agar X-Gal - Ahmed O. *et al.*, **2015.** Delivery of siRNAs to cancer cells via bacteria. Methods Mol. Biol. 1218:117-29.

Fast-Media® Zeo Agar X-Gal - Pelekanou V. *et al.*, **2016.** Estrogen anti-inflammatory activity on human monocytes is mediated through cross-talk between estrogen receptor ERα36 and GPR30/GPER1. J Leukoc Biol. 99(2):333-47.

Fast-Media® Preparation

1• Empty pouch contents into a clean borosilicate glass bottle. Mix Fast-Media® with 200 ml of distilled water.

2• Heat in microwave oven on medium power (400 watts) for 3 minutes, mix and reheat for 30 seconds.

3• Let cool and pour 8-10 plates.

PRODUCT	QUANTITY	CAT. CODE
Fast-Media® Base LB	30 pouches 500 pouches	fas-b fas-b500
Fast-Media® Base Agar	30 pouches 500 pouches	fas-s fas-s500
Fast-Media® Amp LB	30 pouches 500 pouches	fas-am-b fas-am-b500
Fast-Media® Amp Agar	30 pouches 500 pouches	fas-am-s fas-am-s500
Fast-Media® Amp Agar X-Gal	20 pouches	fas-am-x
Fast-Media® Blas TB	20 pouches	fas-bl-l
Fast-Media® Blas Agar	20 pouches	fas-bl-s
Fast-Media® Kan LB	30 pouches 500 pouches	fas-kn-b fas-kn-b500
Fast-Media® Kan Agar	30 pouches 500 pouches	fas-kn-s fas-kn-s500
Fast-Media® Zeo TB	20 pouches	fas-zn-l
Fast-Media® Zeo Agar	20 pouches	fas-zn-s
Fast-Media® Zeo Agar X-Gal	20 pouches	fas-zn-x

3 REPORTER CELL LINES

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CLR reporter cells 45
Inflammasome reporter cells 46-47

Cytokine Reporter Cells

Interferon reporter cells 48-49
Interleukin reporter cells 50-55
Tumor necrosis factor reporter cells 56-57

Transcription Factor Reporter Cells

IRF reporter cells 58-60
NFAT reporter cells 61
NF-κB reporter cells 62-63
NF-κB and IRF dual reporter cells 64-66

KO Reporter Cells 67-70

REPORTER CELL LINES

InvivoGen provides an expanding collection of recombinant cell lines in which specific genes are over-expressed or have been knocked out. These cell lines are useful for:

- studying gene function;
- investigating cell signaling pathways and signal transduction;
- screening and validating agonists/antagonists of pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs), C-type lectin receptors (CLRs) and cytosolic DNA sensors (CDSs);
- detecting cytokines; and
- validating antibody function.

Recombinant Cells of Various Origins

InvivoGen's recombinant cell lines were derived from human or mouse well-established cell models.

- A549 human lung carcinoma
- B16 mouse melanoma
- HCT116 human colon carcinoma
- HEK293 derived cells
- J774 mouse macrophages
- Jurkat human T lymphocytes
- Ramos human B lymphocytes
- RAW 264.7 mouse macrophages
- THP-1 human monocytes

SEAP and/or Lucia Luciferase Reporter Cell Lines

InvivoGen's reporter cell lines utilize two different reporter systems: secreted embryonic alkaline phosphatase (SEAP) and Lucia luciferase. Both reporter systems offer the great advantage of being secreted outside the cell, allowing their activity to be measured non-destructively and repeatedly over time using an aliquot of the same cell sample. Our cell lines have been stably transfected with reporter constructs containing the SEAP or Lucia luciferase gene downstream of a response element that is specific for the particular cell signaling pathway being studied, such as the NF-κB and IFN pathways. Activation of the signaling pathway leads to the translocation of the transcription factor, whose binding to the response element induces the production of the reporter protein.
To facilitate the read-out of the SEAP and Lucia luciferase activities, InvivoGen has developed the detection reagents HEK-Blue Detection™, QUANTI-Blue™ and QUANTI-Luc™. Both HEK-Blue Detection™ and QUANTI-Blue™ allow the convenient monitoring of SEAP using a spectrophotometer, while QUANTI-Luc™ can be used with a luminometer to readily measure Lucia luciferase.

Stringent Quality Control

All of our cell lines have been extensively validated and are provided with detailed protocols that include instructions for general cell culture and maintenance, as well as example experimental setups. **Each batch is tested for viability, biological activity and absence of mycoplasma** to ensure valid, reproducible results and to minimize the need for end-user optimization and troubleshooting.

Shipping

Cells are shipped on dry ice.

CELL LINE	DESCRIPTION	REPORTER	PARENTAL CELL LINE	CATALOG CODE	INFO
PRR REPORTER CELLS					
CLR Reporter Cells					
HEK-Blue™ hDectin-1b	Human Dectin-1b / NF-κB-reporter cells	SEAP	HEK-Blue™ Null1-v	hkb-hdect1b	p. 45
HEK-Blue™ mDectin-2	Mouse Dectin-2 / NF-κB-reporter cells	SEAP	HEK-Blue™ Null1-v	hkb-mdect2	p. 45
HEK-Blue™ mMincle	Mouse Mincle / NF-κB-reporter cells	SEAP	HEK-Blue™ Null1-v	hkb-mmcl	p. 45
NOD Reporter Cells					
HEK-Blue™ hNOD1	Human NOD1 / NF-κB-reporter cells	SEAP	HEK-Blue™ Null1	hkb-hnod1	p. 44
HEK-Blue™ hNOD2	Human NOD2 / NF-κB-reporter cells	SEAP	HEK-Blue™ Null2	hkb-hnod2	p. 44
HEK-Blue™ mNOD1	Mouse NOD1 / NF-κB-reporter cells	SEAP	HEK-Blue™ Null1	hkb-mnod1	p. 44
HEK-Blue™ mNOD2	Mouse NOD2 / NF-κB-reporter cells	SEAP	HEK-Blue™ Null2	hkb-mnod2	p. 44
TLR Reporter Cells					
HEK-Blue™ hTLR2	Human TLR2 / NF-κB-reporter cells	SEAP	HEK-Blue™ Null1	hkb-htlr2	p. 43
HEK-Blue™ hTLR3	Human TLR3 / NF-κB-reporter cells	SEAP	HEK-Blue™ Null1	hkb-htlr3	p. 43
HEK-Blue™ hTLR4	Human TLR4-MD2-CD14 / NF-κB-reporter cells	SEAP	HEK-Blue™ Null2	hkb-htlr4	p. 43
HEK-Blue™ hTLR5	Human TLR5 / NF-κB-reporter cells	SEAP	HEK-Blue™ Null1	hkb-htlr5	p. 43
HEK-Blue™ hTLR7	Human TLR7 / NF-κB-reporter cells	SEAP	HEK-Blue™ Null1-k	hkb-htlr7	p. 43
HEK-Blue™ hTLR8	Human TLR8 / NF-κB-reporter cells	SEAP	HEK-Blue™ Null1	hkb-htlr8	p. 43
HEK-Blue™ hTLR9	Human TLR9 / NF-κB-reporter cells	SEAP	HEK-Blue™ Null1	hkb-htlr9	p. 43
HEK-Blue™ mTLR2	Mouse TLR2 / NF-κB-reporter cells	SEAP	HEK-Blue™ Null2	hkb-mtlr2	p. 43
HEK-Blue™ mTLR3	Mouse TLR3 / NF-κB-reporter cells	SEAP	HEK-Blue™ Null1-k	hkb-mtlr3	p. 43
HEK-Blue™ mTLR4	Mouse TLR4-MD2-CD14 / NF-κB-reporter cells	SEAP	HEK-Blue™ Null1-v	hkb-mtlr4	p. 43
HEK-Blue™ mTLR5	Mouse TLR5 / NF-κB-reporter cells	SEAP	HEK-Blue™ Null1-v	hkb-mtlr5	p. 43
HEK-Blue™ mTLR7	Mouse TLR7 / NF-κB-reporter cells	SEAP	HEK-Blue™ Null2-k	hkb-mtlr7	p. 43
HEK-Blue™ mTLR8	Mouse TLR8 / NF-κB-reporter cells	SEAP	HEK-Blue™ Null1-v	hkb-mtlr8	p. 43
HEK-Blue™ mTLR9	Mouse TLR9 / NF-κB-reporter cells	SEAP	HEK-Blue™ Null1	hkb-mtlr9	p. 43
HEK-Blue™ mTLR13	Mouse TLR13 / NF-κB-reporter cells	SEAP	HEK-Blue™ Null1-v	hkb-mtlr13	p. 43
Inflammasome Test Cells					
THP1-defASC	ASC knockdown cells	None	THP1-Null	thp-dasc	p. 46
THP1-defCASP1	Caspase-1 knockdown cells	None	THP1-Null	thp-dcasp1	p. 46
THP1-defNLRP3	NLRP3 knockdown cells	None	THP1-Null	thp-dnlp	p. 46
THP1-Null	Control cells	None	-	thp-null	p. 46
CYTOKINE REPORTER CELLS					
Interferon Reporter Cells					
B16-Blue™ IFN-α/β	Type I IFN reporter cells	SEAP	B16-Blue™ ISG	bb-ifnt1	p. 49
HEK-Blue™ IFN-α/β	Human IFN-α/β / ISGF3 reporter cells	SEAP	-	hkb-ifnab	p. 48
HEK-Blue™ IFN-γ	Human IFN-γ / STAT1 reporter cells	SEAP	-	hkb-ifng	p. 49
Interleukin Reporter Cells - NF-κB/AP-1 pathways					
HEK-Blue™ IL-1β	Human IL-1 / NF-κB/AP-1 reporter cells	SEAP	-	hkb-il1b	p. 50
HEK-Blue™ IL-1R	Human-mouse IL-1 / NF-κB-AP-1 reporter cells	SEAP	-	hkb-il1r	p. 51
HEK-Blue™ IL-17	Human-mouse IL-17 / NF-κB-AP-1 reporter cells	SEAP	-	hkb-il17	p. 53
HEK-Blue™ IL-18	Human-mouse IL-18 / NF-κB-AP-1 reporter cells	SEAP	-	hkb-hmil18	p. 52
HEK-Blue™ IL-33	Human IL-33 / NF-κB-AP-1 reporter cells	SEAP	-	hkb-hil33	p. 52
Interleukin Reporter Cells - JAK/STAT pathway					
HEK-Blue™ IL-4/IL-13	Human IL-4 & human-mouse IL-13 / STAT6 reporter cells	SEAP	-	hkb-il413	p. 54

CELL LINE	DESCRIPTION	REPORTER	PARENTAL CELL LINE	CATALOG CODE	INFO
CYTOKINE REPORTER CELLS					
HEK-Blue™ IL-6	Human IL-6 / STAT3 reporter cells	SEAP	-	hkb-hil6	p. 55
HEK-Blue™ IL-12	Human-mouse IL-12 / STAT4 reporter cells	SEAP	-	hkb-il12	p. 55
Tumor Necrosis Factor Reporter Cells					
HEK-Blue™ CD40L	Human CD40 / NF-κB-reporter cells	SEAP	-	hkb-cd40	p. 57
HEK-Blue™ TNF-α	Human TNF-α / NF-κB-AP-1 reporter cells	SEAP	-	hkb-tnfdmyd	p. 56
TRANSCRIPTION FACTOR REPORTER CELLS					
IRF Reporter Cells					
B16-Blue™ ISG	IRF-reporter cells	SEAP	-	bb-ifnabg	p. 58
HEK-Blue™ ISG	IRF-reporter cells	SEAP	-	hkb-isg	p. 59
RAW-Lucia™ ISG	IRF-reporter cells	Lucia luciferase	-	rawl-isg	p. 59
THP1-Blue™ ISG	IRF-reporter cells	SEAP	-	thp-isg	p. 60
NFAT Reporter Cells					
Jurkat-Lucia NFAT	NFAT-reporter cells	Lucia luciferase	-	jktl-nfat	p. 61
NF-κB Reporter Cells					
Ramos-Blue™	NF-κB/AP-1 reporter cells	SEAP	-	rms-sp	p. 62
Ramos-Blue™ KD-MyD	MyD88-KD NF-κB/AP-1 reporter cells	SEAP	Ramos-Blue™	rms-kdmyd	p. 68
RAW-Blue™	NF-κB/AP-1 reporter cells	SEAP	-	raw-sp	p. 63
THP1-Lucia™ NF-κB	NF-κB-reporter cells	Lucia luciferase	-	thpl-nfkb	p. 63
NF-κB & IRF Reporter Cells					
A549-Dual™	NF-κB & IRF-reporter cells	SEAP - Lucia	-	a549d-nfis	p. 64
HCT116-Dual™	NF-κB & IRF-reporter cells	SEAP - Lucia	-	hctd-nfis	p. 65
J774-Dual™	NF-κB & IRF-reporter cells	SEAP - Lucia	-	j774d-nfis	p. 65
Jurkat-Dual™	NF-κB & IRF-reporter cells	Lucia - SEAP	-	jktd-isnf	p. 66
THP1-Dual™	NF-κB & IRF-reporter cells	SEAP - Lucia	-	thpd-nfis	p. 66
KO REPORTER CELLS					
B16-Blue™ ISG-KO-STING	STING knockout IRF-reporter cells	SEAP	B16-Blue™ ISG	bb-kostg	p. 67
HEK-Blue™ ISG-KO-STING	STING knockout IRF-reporter cells	SEAP	HEK-Blue™ ISG	hkb-kostg	p. 68
RAW-Lucia™ ISG-KO-cGAS	cGAS knockout IRF-reporter cells	Lucia luciferase	RAW-Lucia™ ISG	rawl-kocgas	p. 69
RAW-Lucia™ ISG-KO-IFI16	IFI16 knockout IRF-reporter cells	Lucia luciferase	RAW-Lucia™ ISG	rawl-koif16	p. 69
RAW-Lucia™ ISG-KO-IRF3	IRF3 knockout IRF-reporter cells	Lucia luciferase	RAW-Lucia™ ISG	rawl-koirf3	p. 69
RAW-Lucia™ ISG-KO-IRF7	IRF7 knockout IRF-reporter cells	Lucia luciferase	RAW-Lucia™ ISG	rawl-koirf7	p. 69
RAW-Lucia™ ISG-KO-MAVS	MAVS (IPS-1) knockout IRF-reporter cells	Lucia luciferase	RAW-Lucia™ ISG	rawl-komavs	p. 69
RAW-Lucia™ ISG-KO-MDA-5	MDA-5 knockout IRF-reporter cells	Lucia luciferase	RAW-Lucia™ ISG	rawl-komda5	p. 69
RAW-Lucia™ ISG-KO-RIG-I	RIG-I knockout IRF-reporter cells	Lucia luciferase	RAW-Lucia™ ISG	rawl-korigi	p. 69
RAW-Lucia™ ISG-KO-STING	STING knockout IRF-reporter cells	Lucia luciferase	RAW-Lucia™ ISG	rawl-kostg	p. 69
RAW-Lucia™ ISG-KO-TBK1	TBK1 knockout IRF-reporter cells	Lucia luciferase	RAW-Lucia™ ISG	rawl-kotbk	p. 69
RAW-Lucia™ ISG-KO-TREX1	TREX1 knockout IRF-reporter cells	Lucia luciferase	RAW-Lucia™ ISG	rawl-kotrex	p. 69
RAW-Lucia™ ISG-KO-TRIF	TRIF knockout IRF-reporter cells	Lucia luciferase	RAW-Lucia™ ISG	rawl-kotrif	p. 69
THP1-Dual™ KO-cGAS	cGAS knockout NF-κB & IRF-reporter cells	SEAP - Lucia	THP1-Dual™	thpd-kocgas	p. 70
THP1-Dual™ KO-IFI16	IFI16 knockout NF-κB & IRF-reporter cells	SEAP - Lucia	THP1-Dual™	thpd-koifi16	p. 70
THP1-Dual™ KO-MyD	MyD88 knockout NF-κB & IRF-reporter cells	SEAP - Lucia	THP1-Dual™	thpd-komyd	p. 70
THP1-Dual™ KO-STING	STING knockout NF-κB & IRF-reporter cells	SEAP - Lucia	THP1-Dual™	thpd-kostg	p. 70
THP1-Dual™ KO-TREX1	TREX1 knockout NF-κB & IRF-reporter cells	SEAP - Lucia	THP1-Dual™	thpd-kotrex	p. 70

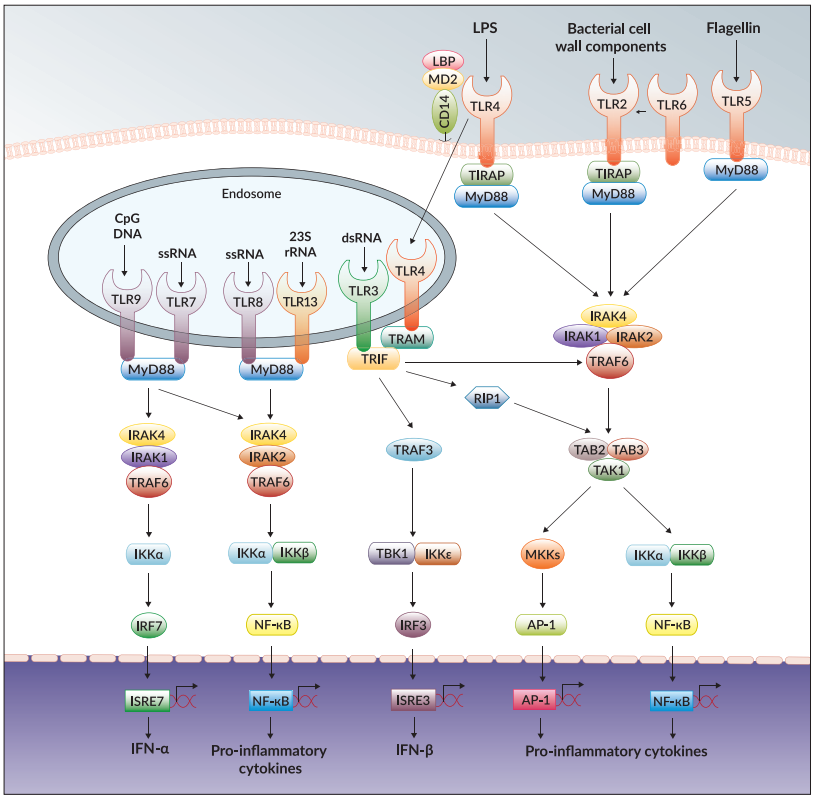
PRR REPORTER CELLS

TLR REPORTER CELLS

TLR reporter cells are a family of HEK293-derived cells engineered to stably express a Toll-like receptor (TLR) gene. TLRs are the first identified and best characterized family of pattern recognition receptors (PRRs). They recognize specific pathogen-associated molecular patterns (PAMPs) present exclusively on microbes, such as viruses, bacteria, parasites and fungi. The recognition of PAMPs by TLRs triggers a signaling cascade leading to the activation of NF-κB and AP-1, and the production of pro-inflammatory cytokines.

In addition to a given TLR gene, TLR reporter cells express an NF-κB-AP-1-inducible SEAP (secreted embryonic alkaline phosphatase) reporter construct. Recognition of a TLR agonist by its cognate receptor induces the production of SEAP. Levels of the reporter protein present in the cell supernatant can be determined spectrophotometrically using the SEAP detection reagents HEK-Blue™ Detection or QUANTI-Blue™, that turn purple/blue in the presence of alkaline phosphatase.

TLR reporter cells can be used for high-throughput screening.



HEK-Blue™ TLR Cells

Description

HEK-Blue™ TLR cells are engineered HEK293 cells that stably co-express a human or murine TLR gene and an NF-κB-AP-1-inducible SEAP reporter gene. To increase the sensitivity to their cognate agonists, HEK-Blue™ TLR2 and HEK-Blue™ TLR4 cells were further transfected with the co-receptors CD14 and MD2/CD14, respectively. HEK-Blue™ TLR cells are resistant to blasticidin and Zeocin™. HEK-Blue™ TLR2 and HEK-Blue™ TLR4 cells are additionally resistant to hygromycin. They are grown in DMEM medium, 2 mM L-glutamine, 10% FBS and supplemented with 100 µg/ml Zeocin™, 30 µg/ml blasticidin and/or 200 µg/ml Hygromycin B Gold (ultra-pure hygromycin), depending on the cell line.

HEK-Blue™ TLR2 cells stably express the human or mouse TLR2 and CD14 genes. They respond to a wide array of microbial molecules, such as lipoteichoic acid (LTA), peptidoglycan (PGN) and lipoproteins from Gram-positive bacteria.

HEK-Blue™ TLR3 cells stably express the human or mouse TLR3 gene. They respond specifically to double-stranded (ds) RNA and to synthetic analogs of dsRNA, such as polyinosine-polycytidylic acid (poly(I:C)).

HEK-Blue™ TLR4 cells stably express the human or mouse TLR4, MD2 and CD14 genes. They respond to lipopolysaccharide (LPS, also known as endotoxin) and lipid A found in Gram-negative bacteria.

HEK-Blue™ TLR5 cells stably express the human or mouse TLR5 gene. They respond specifically to flagellin, the major component of the bacterial flagellar filament, from both Gram-positive and Gram-negative bacteria.

HEK-Blue™ TLR7 cells stably express the human or mouse TLR7 gene. HEK-Blue™ mTLR7 cells respond to viral short GU-rich short single-stranded RNA, as well as to small synthetic molecules, such as imiquimod and R848. HEK-Blue™ hTLR7 cells respond solely to synthetic molecules.

HEK-Blue™ TLR8 cells stably express the human or mouse TLR8 gene. HEK-Blue™ hTLR8 cells respond to viral short GU-rich short single-stranded RNA, as well as small to synthetic molecules, such as CL075 and R848. HEK-Blue™ mTLR8 cells require poly(dT) to respond to TLR8 agonists.

HEK-Blue™ TLR9 cells stably express the human or mouse TLR9 gene. They respond specifically to particular CpG ODNs. The best ligands for HEK-Blue™ hTLR9 and HEK-Blue™ mTLR9 cells are ODN 2006 and ODN 1826, respectively

HEK-Blue™ TLR13 cells stably express the murine TLR13 gene. They respond to Staphylococcus aureus 23S ribosomal RNA.

Control Cell Lines

HEK-Blue™ TLR cells derive from different but closely related parental cell lines, named HEK-Blue™ Null cells.
HEK-Blue™ Null1, HEK-Blue™ Null1-k and HEK-Blue™ Null1-v cells express the SEAP reporter gene under the control of the IFN-β minimal promoter fused to five AP-1 and five NF-κB binding sites.
HEK-Blue™ Null2 and HEK-Blue™ Null2-k cells express the SEAP reporter gene under the control of the IL-12 p40 minimal promoter fused to five AP-1 and five NF-κB binding sites.

HEK-Blue™ Null1-derived cells:

- HEK-Blue™ hTLR2
- HEK-Blue™ hTLR3
- HEK-Blue™ hTLR5
- HEK-Blue™ hTLR8
- HEK-Blue™ hTLR9
- HEK-Blue™ mTLR9

HEK-Blue™ Null1-k-derived cells:

- HEK-Blue™ hTLR7
- HEK-Blue™ mTLR3

HEK-Blue™ Null1-v-derived cells:

- HEK-Blue™ mTLR4
- HEK-Blue™ mTLR5
- HEK-Blue™ mTLR8
- HEK-Blue™ mTLR13

HEK-Blue™ Null2-derived cells:

- HEK-Blue™ hTLR4
- HEK-Blue™ mTLR2

HEK-Blue™ Null2-k-derived cells:

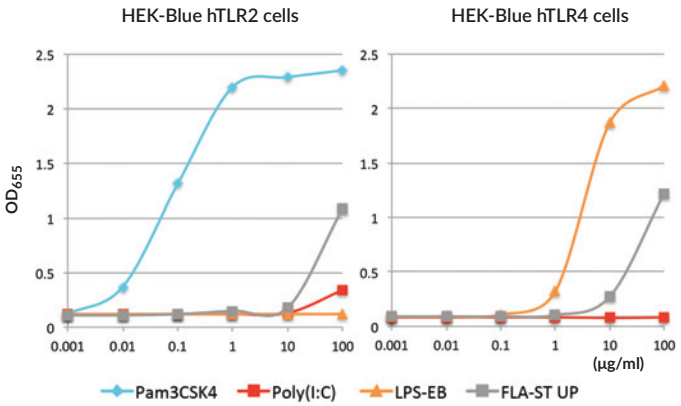
- HEK-Blue™ mTLR7

Quality Control

Transgene expression is confirmed by flow cytometry or RT-PCR. The functionality of each cell line is validated through stimulation assays performed with a selection of TLR agonists using the SEAP detection media HEK-Blue™ Detection or QUANTI™-Blue.
HEK-Blue™ TLR express endogenous levels of TLR1, TLR3, TLR5 and TLR6.

Contents

HEK-Blue™ TLR and HEK-Blue™ Null cells are provided frozen in a cryotube containing 3-7 x 10^6 cells and supplied with the corresponding selection antibiotic(s), 1 ml Normocin™ (50 mg/ml) and 1 pouch of HEK-Blue™ Detection.



Response of HEK-Blue™ hTLR2 and HEK-Blue™ hTLR4 cells to various TLR agonists. Cells were incubated with increasing concentrations of Pam3CSK4 (TLR2), poly(I:C) (TLR3), LPS-EB (TLR4) or FLA-ST (TLR5). After overnight incubation, NF-κB/AP-1 activation was assessed by measuring the levels of SEAP in the supernatant, using HEK-Blue™ Detection.

PRODUCT	QUANTITY	CAT. CODE
HUMAN		
HEK-Blue™ hTLR2 Cells	3-7 x 10^6 cells	hkb-htlr2
HEK-Blue™ hTLR3 Cells	3-7 x 10^6 cells	hkb-htlr3
HEK-Blue™ hTLR4 Cells	3-7 x 10^6 cells	hkb-htlr4
HEK-Blue™ hTLR5 Cells	3-7 x 10^6 cells	hkb-htlr5
HEK-Blue™ hTLR7 Cells	3-7 x 10^6 cells	hkb-htlr7
HEK-Blue™ hTLR8 Cells	3-7 x 10^6 cells	hkb-htlr8
HEK-Blue™ hTLR9 Cells	3-7 x 10^6 cells	hkb-htlr9
MOUSE		
HEK-Blue™ mTLR2 Cells	3-7 x 10^6 cells	hkb-mtlr2
HEK-Blue™ mTLR3 Cells	3-7 x 10^6 cells	hkb-mtlr3
HEK-Blue™ mTLR4 Cells	3-7 x 10^6 cells	hkb-mtlr4
HEK-Blue™ mTLR5 Cells	3-7 x 10^6 cells	hkb-mtlr5
HEK-Blue™ mTLR7 Cells	3-7 x 10^6 cells	hkb-mtlr7
HEK-Blue™ mTLR8 Cells	3-7 x 10^6 cells	hkb-mtlr8
HEK-Blue™ mTLR9 Cells	3-7 x 10^6 cells	hkb-mtlr9
HEK-Blue™ mTLR13 Cells	3-7 x 10^6 cells	hkb-mtlr13
CONTROL		
HEK-Blue™ Null1 Cells	3-7 x 10^6 cells	hkb-null1
HEK-Blue™ Null1-k Cells	3-7 x 10^6 cells	hkb-null1k
HEK-Blue™ Null1-v Cells	3-7 x 10^6 cells	hkb-null1v
HEK-Blue™ Null2 Cells	3-7 x 10^6 cells	hkb-null2
HEK-Blue™ Null2-k Cells	3-7 x 10^6 cells	hkb-null2k

RELATED PRODUCTS

PRODUCT	INFO
HEK-Blue™ hMD2-CD14 cells	Visit website
293/TLR cells	Visit website
TLR agonists	See pages 88-95

RECENT CITATIONS

HEK-Blue™ mTLR2 and mTLR4 - Kurnellas MP. *et al.*, 2015. Amyloid fibrils activate B-1a lymphocytes to ameliorate inflammatory brain disease. PNAS. 112(49):15016-23

HEK-Blue™ hTLR4 - Guillou C. *et al.*, 2016. Soluble alpha-enolase activates monocytes by CD14-dependent TLR4 signalling pathway and exhibits a dual function. Sci Rep. 6:23796.

HEK-Blue™ hTLR7 - O'Brien M. *et al.*, 2016. CD4 Receptor is a Key Determinant of Divergent HIV-1 Sensing by Plasmacytoid Dendritic Cells. PLoS Pathog. 11(7):e1005032

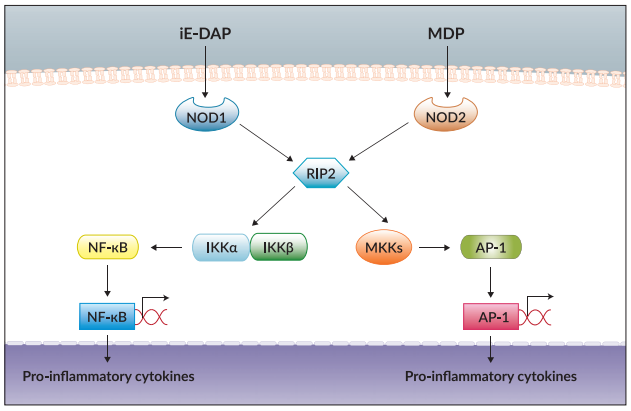
HEK-Blue™ mTLR4 and HEK-Blue™ mTLR5 - Chassaing B. *et al.*, 2015. Dietary emulsifiers impact the mouse gut microbiota promoting colitis and metabolic syndrome. Nature. 519(7541):92-6.

HEK-Blue™ mTLR3 - Zhang P. *et al.*, 2015. Polyelectrolyte Multilayers Assembled Entirely from Immune Signals on Gold Nanoparticle Templates Promote Antigen-Specific T Cell Response. ACS Nano. 9(6):6465-77.

NOD REPORTER CELLS

NOD reporter cells are HEK293-derived cells that stably express the NOD1 or NOD2 gene. NOD1 and NOD2, the founding members of the intracellular NOD-like receptor (NLR) family, sense conserved motifs in bacterial peptidoglycan (PGN) and induce the activation of NF-κB leading to pro-inflammatory responses.

NOD reporter cells also express an NF-κB-AP-1-inducible SEAP (secreted embryonic alkaline phosphatase) reporter gene. Activation of NOD1/NOD2 by PGN-derived PAMPs induces the translocation of NF-κB and AP-1, and the subsequent production of SEAP. Levels of SEAP in the cell supernatant can be determined spectrophotometrically using the SEAP detection reagents HEK-Blue Detection or QUANTI-Blue™.



HEK-Blue™ NOD Cells

Description

HEK-Blue™ NOD cells are engineered HEK293 cells that stably co-express the human and murine NOD1 or NOD2 gene and an NF-κB-AP-1-inducible SEAP reporter gene.
HEK-Blue™ NOD cells are resistant to blasticidin and Zeocin™. They are grown in DMEM medium,10% FBS, 100 µg/ml Zeocin™ and 30 µg/ml blasticidin.

HEK-Blue™ NOD1 cells stably express the NOD1 gene. They respond specifically to molecules containing D-glutamyl-meso-diaminopimelic acid (iE-DAP) moiety, such as C12-iE-DAP and Tri-DAP. They do not respond to NOD2 ligands.

HEK-Blue™ NOD2 cells stably express the NOD2 gene. They respond to molecules containing muramyl dipeptide (MDP). Due to the presence of endogenous NOD1, HEK-Blue™ NOD2 cells show a weak response to NOD1 agonists.

Control Cell Lines

HEK-Blue™ NOD cells derive from different but closely related parental cell lines, named HEK-Blue™ Null cells.
HEK-Blue™ Null1 cells express the SEAP reporter gene under the control of the IFN-β minimal promoter fused to five NF-κB binding sites.
HEK-Blue™ Null2 express the SEAP reporter gene under the control of the IL-12 p40 minimal promoter fused to five NF-κB binding sites.

HEK-Blue™ Null1-derived cells:
- HEK-Blue™ hNOD1
- HEK-Blue™ mNOD1

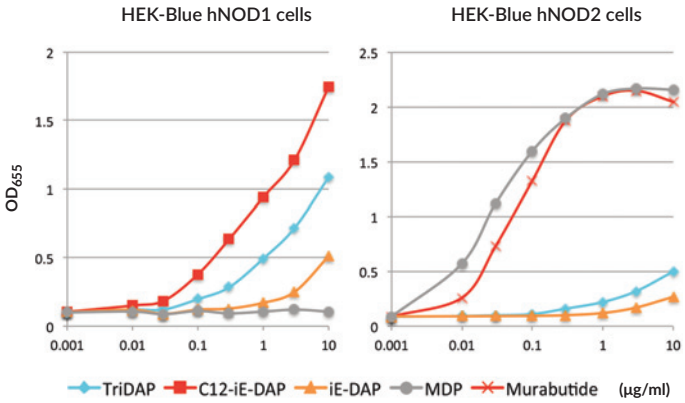
HEK-Blue™ Null2-derived cells:
- HEK-Blue™ hNOD2
- HEK-Blue™ mNOD2

Quality Control

Transgene expression is confirmed by RT-PCR. The functionality of each cell line is validated through stimulation assays performed with a selection of NOD agonists using the SEAP detection reagents HEK-Blue™ Detection or QUANTI™-Blue.

Contents

HEK-Blue™ NOD and HEK-Blue™ Null cells are provided in cryotubes containing 3-7 x 10^6 cells and they come supplied with 10 mg Zeocin™, 1 mg blasticidin (not provided with HEK-Blue™ Null cells), 50 mg Normocin™ and 1 pouch of HEK-Blue™ Detection.



Response of HEK-Blue™ hNOD1 and HEK-Blue™ hNOD2 cells to various PGN-derived PAMPs. Cells were incubated with increasing concentrations of Tri-DAP (NOD1), c12-iE-DAP (NOD1), iE-DAP (NOD1), MDP (NOD2) or murabutide (NOD2). After overnight incubation, NF-κB/AP-1 activation was assessed by measuring the levels of SEAP in the supernatant using HEK-Blue™ Detection.

PRODUCT	QUANTITY	CAT. CODE
HUMAN		
HEK-Blue™ hNOD1 cells	3-7 x 10^6 cells	hkb-hnod1
HEK-Blue™ hNOD2 cells	3-7 x 10^6 cells	hkb-hnod2
MOUSE		
HEK-Blue™ mNOD1 cells	3-7 x 10^6 cells	hkb-mnod1
HEK-Blue™ mNOD2 cells	3-7 x 10^6 cells	hkb-mnod2
CONTROL		
HEK-Blue™ Null1 cells	3-7 x 10^6 cells	hkb-null1
HEK-Blue™ Null2 cells	3-7 x 10^6 cells	hkb-null2

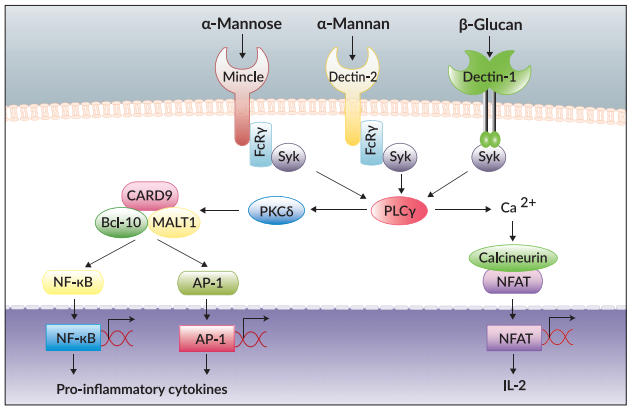
RELATED PRODUCTS

PRODUCT	INFO
293/NOD cells	Visit website
NOD agonists	See pages 96-97

CLR REPORTER CELLS

CLR reporter cells are HEK293-derived cells that stably express Dectin-1b, Dectin-2 or Mincle. These proteins are members of the C-type lectin receptors (CLRs), a large family of receptors that bind to carbohydrates in a calcium-dependent manner. They play an important in the immune response against fungi.

CLR reporter cells also express an NF-κB-AP-1-inducible SEAP (secreted embryonic alkaline phosphatase) reporter gene. Upon binding of a CLR agonist, the receptor triggers signaling cascades leading to the activation of NF-κB and AP-1, and the subsequent production of SEAP. Reporter activity can be determined spectrophotometrically using the SEAP detection reagents HEK-Blue Detection or QUANTI-Blue™.



HEK-Blue™ CLR Cells

Description

HEK-Blue™ CLR cells are engineered HEK293 cells that stably co-express the human Dectin-1b, mouse Dectin-2 or mouse Mincle gene and multiple genes of the signaling pathway leading to NF-κB-AP-1 activation. They also express an NF-κB-AP-1-inducible SEAP gene.
HEK-Blue™ CLR cells are resistant to several selective antibiotics, some of which are combined in a solution called HEK-Blue™ CLR Selection. They are grown in DMEM medium, 10% FBS, 1X HEK-Blue™ CLR Selection, 1 µg/ml puromycin and/or 30 µg/ml blasticidin depending on the cell line.

HEK-Blue™ hDectin-1b cells stably express the human Dectin-1b gene. They respond specifically to β-glucans, in particular to particulate β-glucans, such as curdlan and zymosan. They do not respond to the soluble β-glucans, laminarin and WGP soluble.

HEK-Blue™ mDectin-2 cells stably express the mouse Dectin-2 gene. They respond specifically to agonists of Dectin-2, a receptor that binds to mannose carbohydrates, such as those present in furfurman, a cell wall preparation of *Malassezia furfur*.

HEK-Blue™ mMincle cells stably express the murine Mincle gene. They respond specifically to Mincle ligands, which encompass a variety of carbohydrate structures, such as trehalose-6,6-dibehenate (TDB), a synthetic analog of Mycobacterium tuberculosis cord factor.

Control Cell Lines

HEK-Blue™ CLR cells derive from **HEK-Blue™ Null1-v**, a cell line that expresses the SEAP reporter gene under the control of the IFN-β minimal promoter fused to five NF-κB binding sites.

Quality Control

Transgene expression is confirmed by flow cytometry or RT-PCR. The functionality of each cell line is validated through stimulation assays performed with a selection of CLR agonists using the SEAP detection media HEK-Blue™ Detection or QUANTI™-Blue.

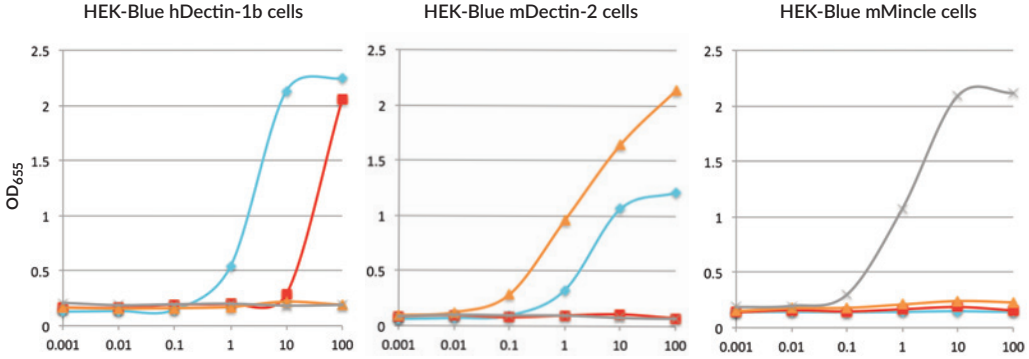
Contents

HEK-Blue™ CLR and HEK-Blue™ Null1-v cells are provided in cryotubes containing 3-7 x 10^6 cells and they come supplied with the corresponding selective antibiotic(s), 50 mg Normocin™ and 1 pouch of HEK-Blue™ Detection.

PRODUCT	QUANTITY	CAT. CODE
HEK-Blue™ hDectin-1b cells	3-7 x 10^6 cells	hkb-hdect1b
HEK-Blue™ mDectin-2 cells	3-7 x 10^6 cells	hkb-mdect2
HEK-Blue™ mMincle cells	3-7 x 10^6 cells	hkb-mmcl
HEK-Blue™ Null1v cells	3-7 x 10^6 cells	hkb-null1v
HEK-Blue™ CLR Selection (250X)	10 x 1 ml	hb-csm

RELATED PRODUCTS

PRODUCT	INFO
CLR agonists	See pages 99-101

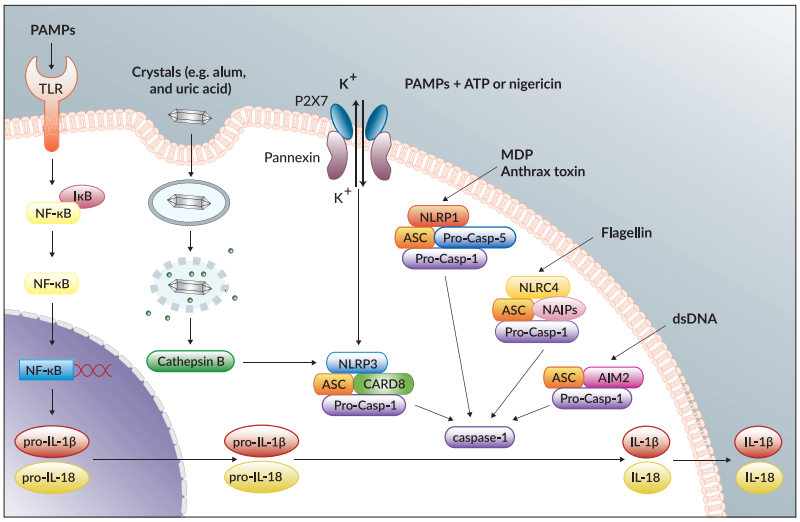


Response of CLR reporter cells to various fungi or mycobacteria-derived PAMPs. Cells were incubated with increasing concentrations of Zymosan (*S. cerevisiae* cell wall preparation), Zymosan depleted (hot alkali treated zymosan), Furfurman (*M. furfur* cell wall preparation) or TDB (synthetic cord factor). After overnight incubation, NF-κB/AP-1 activation was assessed by measuring the levels of SEAP in the supernatant using HEK-Blue™ Detection.

INFLAMMASOME TEST CELLS

The inflammasome is a multiprotein complex that activates caspase 1, leading to the processing and secretion of the pro-inflammatory cytokines interleukin-1β (IL-1β) and IL-18. A number of inflammasomes have been described, including the NLRP1, NLRP3 and AIM2 inflammasomes. The NLRP3 inflammasome is the most extensively studied and is also the most versatile due to its broad recognition of a wide range of pathogen-associated molecular patterns (PAMPs) and non-microbial danger-associated molecular patterns (DAMPs). It is however still unclear how these highly diverse signals can be detected by a single inflammasome.

InvivoGen has developed a cellular assay, based on two cell lines, to test the activation of inflammasomes. The first cell line is a THP-1-derived cell line, which upon activation of the inflammasome secretes IL-1β in the supernatant. The second cell line is HEK-Blue™ IL-1β, a HEK293-derived reporter cell line that detects IL-1β (see page 50).



THP1 Cells

Description

THP-1 human monocytic cells represent the most commonly used model cell line for the study of inflammasome activation, as they express high levels of NLRP3, ASC and pro-caspase-1. InvivoGen provides a series of THP-1 cell lines as tools to determine whether a signal activates the inflammasome and in particular the NLRP3 inflammasome.

THP1-defASC cells were generated from THP-1 cells through knockdown of the ASC gene. They express reduced levels of ASC (~3 fold reduction) but native levels of NLRP3 and pro-caspase-1. THP1-defASC cells are unable to respond to inducers of ASC-dependent inflammasomes, such as inducers of the NLRP3, NLRC4 and AIM2 inflammasomes.
THP1-defASC cells can be used with THP1-Null cells to determine whether a given compound is an inflammasome inducer or inhibitor.

THP1-defCASP1 cells were generated from THP-1 cells through knockdown of the caspase-1 gene. They are highly deficient for caspase-1 activity (~7 fold reduction). They produce significantly less IL-1β in response to stimuli that activate the inflammasomes, such as ATP and MSU, (NLRP3 inflammasome) or transfected poly(dA:dT) (AIM2 inflammasome), when compared to the control cell line THP1-Null.
THP1-defCASP1 cells, together with THP1-Null cells, allow to determine if a compound is an inflammasome inducer.

THP1-defNLRP3 cells were generated from THP-1 cells through knockdown of the NLRP3 gene. They express reduced levels of NLRP3 but native levels of ASC and pro-caspase-1. THP1-defNLRP3 cells are unable to respond to inducers of the NLRP3 inflammasome, such as ATP and MSU. However, they may respond to stimuli that activate other ASC-dependent inflammasomes such as the AIM2, NLRP1 and NLRC4 inflammasomes.

THP1-Null cells are fully efficient for NLRP3 and ASC activities. They produce IL-1β upon stimulation with inflammasome inducers. They can be used as a positive control cell line for inflammasome studies.

InvivoGen's THP1 cells are resistant to hygromycin. They are grown in RPMI medium with 10% FBS, 100 µg/ml Normocin™ and 200 µg/ml Hygromycin B Gold (ultrapure Hygromycin).

Quality Control

ASC, Caspase-1 or NLRP3 deficiency in THP1-defASC, THP1-defCASP1 or THP1-defNLRP3 cells, respectively, was confirmed by qRT-PCR and a functional assay using NLRP3 inflammasome inducers.

Contents

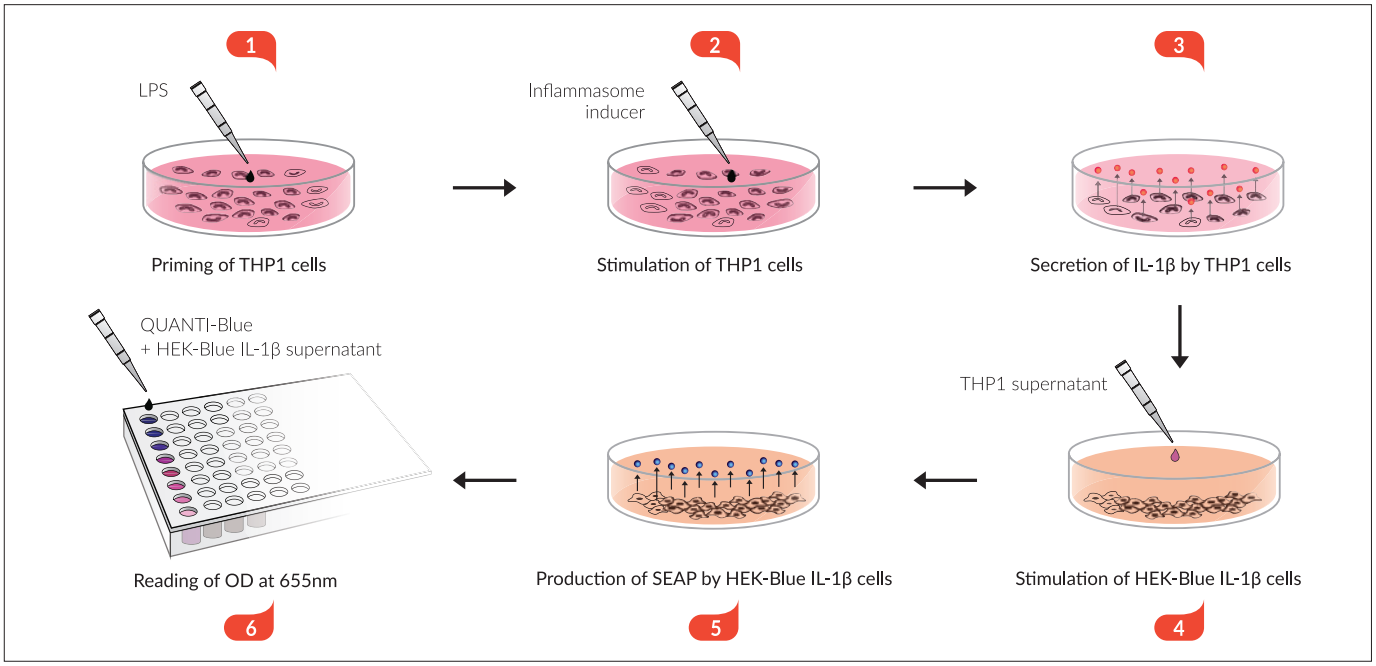
THP1 cells are provided in cryotubes containing 3-7 x 10^6 cells and they come supplied with 10 mg Hygromycin B Gold and 50 mg Normocin™.

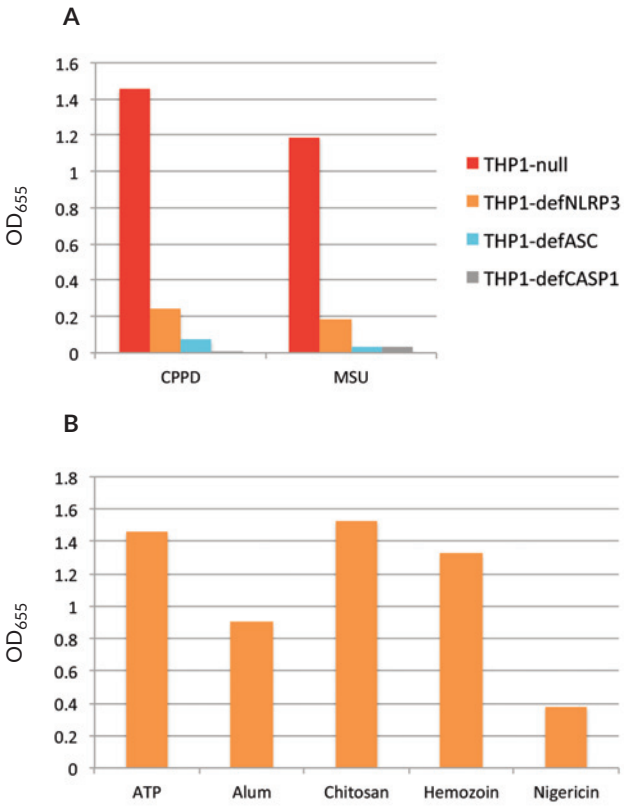
PRODUCT	QUANTITY	CAT. CODE
THP1-defASC	3-7 x 10^6 cells	thp-dasc
THP1-defCASP1	3-7 x 10^6 cells	thp-dcasp1
THP1-defNLRP3	3-7 x 10^6 cells	thp-dnlp
THP1-Null	3-7 x 10^6 cells	thp-null

RELATED PRODUCTS

PRODUCT	INFO
HEK-Blue™ IL-1β cells	See page 50
Inflammasome inducers	See pages 97-98
Inflammasome inhibitors	See pages 117-118

THP1 / HEK-Blue™ IL-1β cell-based assay





Response of THP1 cells to inflammasome inducers. LPS-primed THP1 cells were stimulated with various NLRP3 inflammasome inducers. After 24h incubation, THP1 cell supernatants were added to HEK-Blue™ IL-1β cells. IL-1β-induced NF-κB activation was assessed by measuring the levels of SEAP using QUANTI-Blue™. **A)** THP1-null, THP1-defNLRP3, THP1-defASC and THP1-defCASP1 cells were incubated with 12.5 µg/ml CPPD or 100 µg/ml MSU. **B)** THP1-null were stimulated with 5 mM ATP, 125 µg/ml alum, 100 µg/ml chitosan, 125 µg/ml hemozoin or 5 µM nigericin.

RECENT CITATIONS

TTHP1-defNLRP3 - Queen J. *et al.*, 2015. Mechanisms of inflammasome activation by Vibrio cholerae secreted toxins vary with strain biotype. Infect Immun. 83(6):2496-506.

Steverson D Jr. *et al.*, 2016. Tribbles Homolog 3 Promotes Foam Cell Formation Associated with Decreased Proinflammatory Cytokine Production in Macrophages: Evidence for Reciprocal Regulation of Cholesterol Uptake and Inflammation. Metab Syndr Relat Disord. 14(1):7-15.

THP1-defNLRP3 and THP1-null - Dietsch GN. *et al.*, 2016. Coordinated Activation of Toll-Like Receptor8 (TLR8) and NLRP3 by the TLR8 Agonist, VTX-2337, Ignites Tumoricidal Natural Killer Cell Activity. PLoS One. 11(2):e0148764.

Schaale K. *et al.*, 2016. Strain- and host species-specific inflammasome activation, IL-1β release, and cell death in macrophages infected with uropathogenic Escherichia coli. Mucosal Immunol. 9(1):124-36.

THP1-defASC and THP1-defNLRP3 - Lee MS. *et al.*, 2015. Shiga Toxins Activate the NLRP3 Inflammasome Pathway To Promote Both Production of the Proinflammatory Cytokine Interleukin-1β and Apoptotic Cell Death. Infect Immun. 84(1):172-86.

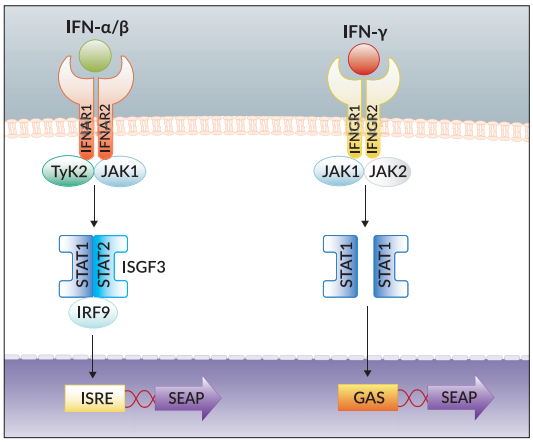
Sun B. *et al.*, 2015. NADPH Oxidase-Dependent NLRP3 Inflammasome Activation and its Important Role in Lung Fibrosis by Multiwalled Carbon Nanotubes. Small. 11(17):2087-97.

THP1-defASC, THP1-defCASP1 and THP1-defNLRP3 - Naji A. *et al.*, 2015. Endocytosis of indium-tin-oxide nanoparticles by macrophages provokes pyroptosis requiring NLRP3-ASC-Caspase1 axis that can be prevented by mesenchymal stem cells. Sci Rep. 2016 May 19;6:26162.

CYTOKINE REPORTER CELLS

INTERFERON REPORTER CELLS

Interferons (IFNs) play a vital role in host resistance to viral infections. They are divided into three groups designated type I, type II and type III IFNs. Type I IFNs consist of two major classes: IFN-α and IFN-β. There is only one IFN-β but multiple IFN-α species. IFN-γ is the only type II IFN. The recently classified type III IFN group consists of three IFN-λ members. IFNs signal mainly through the JAK-STAT pathway. IFN-α and IFN-β bind to a common receptor (IFNAR), while IFN-γ binds to the IFNGR receptor. Upon binding to its receptor, each IFN activates a distinct combination of members of the JAK and STAT families. Type I IFN-induced JAK-STAT signaling leads to the formation of the ISGF3 complex. ISGF3 binds to IFN-stimulated response elements (ISRE) in the promoters of IFN-stimulated genes (ISG) to regulate their expression. Type II IFN signaling induces the formation of STAT1-STAT1 homodimers, which bind GAS (interferon-gamma-activated site) elements that are present in the promoter of certain ISGs, thereby initiating the transcription of these genes.



- B16-Blue™ IFN-α/β
- HEK-Blue™ IFN-α/β
- HEK-Blue™ IFN-γ

HEK-Blue™ IFN-α/β Cells - Human type I IFN reporter cells

Description

HEK-Blue™ IFN-α/β cells allow the detection of bioactive human type I IFNs by monitoring the activation of the ISGF3 pathway. These cells were generated by stable transfection of HEK293-derived cells with the human STAT2 and IRF9 genes to obtain a fully active type I IFN signaling pathway. The other genes of the pathway are naturally expressed in sufficient amounts. The cells were further transfected with a SEAP reporter gene under the control of the IFN-α/β-inducible ISG54 promoter.
Stimulation of HEK-Blue™ IFN-α/β cells with human IFN-α or IFN-β activates the JAK/STAT/ISGF3 pathway and subsequently induces the production of SEAP.

Features

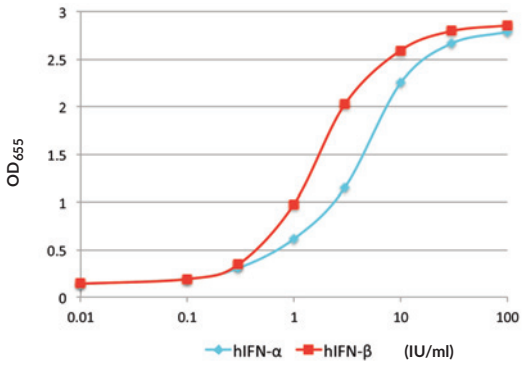
Ligand	EC50	Fold response*
Human IFN-α	8 ± 4 IU/ml	20
Human IFN-β	5 ± 4 IU/ml	20

HEK-Blue™ IFN-α/β cells are resistant to blasticidin and Zeocin™. They are grown in standard DMEM medium, 10% FBS supplemented with 100 µg/ml Normocin, 30 µg/ml blasticidin and 100 µg/ml Zeocin™.

Contents

HEK-Blue™ IFN-α/β cells are provided frozen in a cryotube containing 3-7 x 10^6 cells and they come supplied with 1 mg blasticidin, 10 mg Zeocin™, 50 mg Normocin™ and 1 pouch of QUANTI-Blue™.

PRODUCT	QUANTITY	CAT. CODE
HEK-Blue IFN-α/β	3-7 x 10^6 cells	hkb-ifnab



Response of HEK-Blue™ IFN-α/β cells to human type I IFNs. HEK-Blue™ IFN-α/β cells were incubated with increasing concentrations of recombinant human IFN-α or IFN-β. After 24h incubation the levels of ISGF3-induced SEAP were determined using QUANTI-Blue™.

RECENT CITATIONS

Bego MG. *et al.*, 2015. Vpu Exploits the Cross-Talk between BST2 and the ILT7 Receptor to Suppress Anti-HIV-1 Responses by Plasmacytoid Dendritic Cells. PLoS Pathog. 11(7):e1005024.

Herzner AM. *et al.*, 2015. Sequence-specific activation of the DNA sensor cGAS by Y-form DNA structures as found in primary HIV-1 cDNA. Nat Immunol. 16(10):1025-33.

Wang H. *et al.*, 2015. A frequent hypofunctional IRAK2 variant is associated with reduced spontaneous hepatitis C virus clearance. Hepatology. 62(5):1375-87.

B16-Blue™ IFN-α/β Cells - Mouse type I IFN reporter cells

Description

B16-Blue™ IFN-α/β cells allow the detection of bioactive murine type I IFNs by monitoring the activation of the JAK/STAT/ISGF3 pathway. They were derived from the murine B16 melanoma cell line of C57B1/6 origin after stable transfection with a SEAP reporter gene under the control of the IFN-α/β-inducible ISG54 promoter.
Stimulation of B16-Blue™ IFN-α/β cells with murine IFN-α or IFN-β, but not human IFN-α or IFN-β, activates the JAK/STAT/ISGF3 pathway and triggers the subsequent production of SEAP.

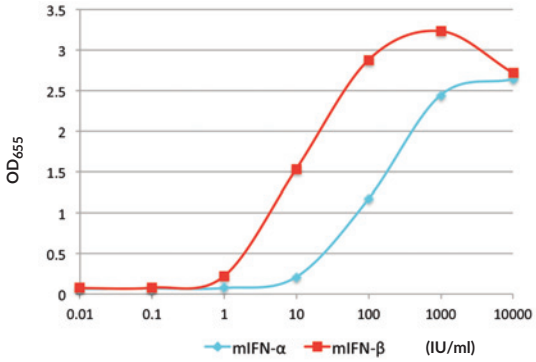
Features

Ligand	EC50	Fold response*
Mouse IFN-α	100 ± 10 IU/ml	30
Mouse IFN-β	10 ± 3 IU/ml	40

B16-Blue™ IFN-α/β cells are resistant to Zeocin™. They are grown in RPMI medium, 10% FBS supplemented with 100 µg/ml Normocin™ and 100 µg/ml Zeocin™.

Contents

B16-Blue™ IFN-α/β cells are provided frozen in a cryotube containing 3-7 x 10^6 cells and they come supplied with 10 mg Zeocin™, 50 mg Normocin™ and 1 pouch of QUANTI-Blue™.



Response of B16-Blue™ IFN-α/β cells to mouse type I IFNs. B16-Blue™ IFN-α/β cells were incubated with increasing concentrations of recombinant mouse IFN-α or IFN-β. After 24h incubation the levels of ISGF3-induced SEAP were determined using QUANTI-Blue™.

PRODUCT	QUANTITY	CAT. CODE
B16-Blue IFN-α/β	3-7 x 10^6 cells	bb-ifnt1

HEK-Blue™ IFN-γ Cells - Human IFN-γ reporter cells

Description

HEK-Blue™ IFN-γ cells allow the detection of bioactive human IFN-γ by monitoring the activation of the JAK/STAT-1 pathway. They were generated by stable transfection of HEK293-derived cells with the human STAT1 gene to obtain a fully active STAT1 pathway. The other genes of the pathway are naturally expressed in sufficient amounts. The cells were further transfected with a SEAP reporter gene under the control of an ISG54 promoter fused to four GAS elements. HEK-Blue™ IFN-γ cells produce SEAP in response to human IFN-γ stimulation and human IFN-β at very high concentrations (≥ 10^4 IU/ml).

Features

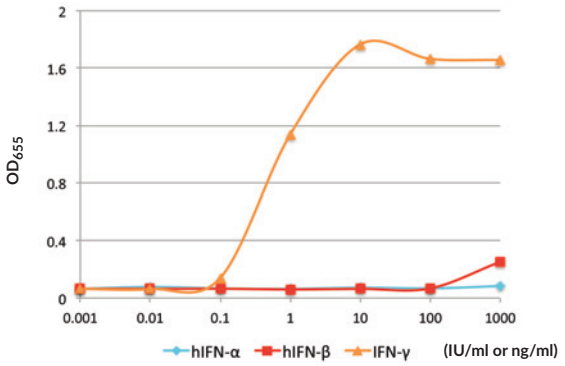
Ligand	EC50	Fold response*
Human IFN-γ	0.7 ± 0.3 ng/ml	30

HEK-Blue™ IFN-γ cells are resistant to blasticidin and Zeocin™. They are grown in standard DMEM medium, 10% FBS supplemented with 100 µg/ml Normocin, 30 µg/ml blasticidin and 100 µg/ml Zeocin™.

Contents

HEK-Blue™ IFN-γ cells are provided frozen in a cryotube containing 3-7 x 10^6 cells and they come supplied with 1 mg blasticidin, 10 mg Zeocin™, 50 mg Normocin™ and 1 pouch of QUANTI-Blue™.

*The fold response was calculated by dividing the OD at 655 nm for the treated cells by the OD at 655 nm for the untreated cells.



Response of HEK-Blue™ IFN-γ cells to human IFNs. HEK-Blue™ IFN-γ cells were incubated with increasing concentrations of recombinant human IFN-α, IFN-β (IU/ml) or IFN-γ (ng/ml). After 24h incubation the levels of GAS-induced SEAP were determined using QUANTI-Blue™.

PRODUCT	QUANTITY	CAT. CODE
HEK-Blue IFN-γ	3-7 x 10^6 cells	hkb-ifng

RELATED PRODUCTS

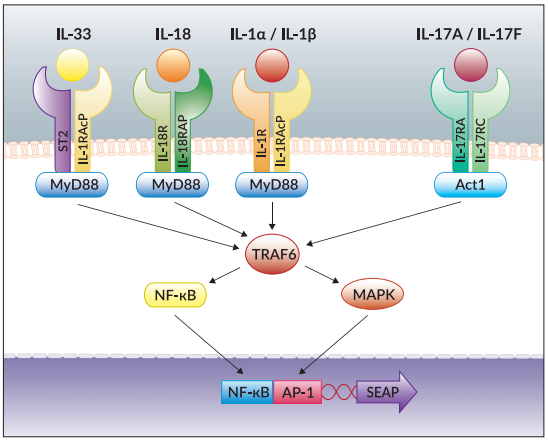
PRODUCT	INFO
Recombinant human IFN-γ	See page 136
B16-Blue IFN-γ	Visit website

INTERLEUKIN REPORTER CELLS

Interleukins Signaling by the NF-κB and AP-1 Pathways

Interleukins (IL) that signal through the NF-κB and AP-1 pathways include the members of the IL-1 and IL-17 families. IL-1 cytokines (e.g. IL-1α, IL-1β, IL-18 and IL-33) and IL-17 cytokines (IL-17A and IL-17F) play important roles in inflammation. They bind to their correspondent receptors and induce signaling cascades leading to the expression of inflammatory and catabolic genes. IL-1 cytokines recruit a common adaptor, MyD88, which is also involved in TLR signaling, while IL-17 cytokines use the adaptor Act1 as a common mediator. Recruitment of MyD88 or Act1 results in the activation of important signaling proteins, such as TRAF6, as well as transcription factors, including NF-κB and c-Jun (a subunit of AP-1).

InvivoGen provides a collection of HEK293-derived reporter cells that provide a convenient means of detecting human and/or mouse bioactive IL-1α, IL-1β, IL-17, IL-18 and IL-33 by measuring the activity of NF-κB-AP-1-induced SEAP (secreted embryonic alkaline phosphatase).



3

- HEK-Blue™ IL-1β
- HEK-Blue™ IL-1R
- HEK-Blue™ IL-17
- HEK-Blue™ IL-18
- HEK-Blue™ IL-33

HEK-Blue™ IL-1β Cells - Human IL-1α/β reporter cells

Description

HEK-Blue™ IL-1β cells allow the detection of bioactive human interleukin-1 cytokines (IL-1α and IL-1β) by monitoring the activation of the NF-κB/AP-1 pathway. They were generated from HEK293-derived cells, which naturally express the IL-1R, by stable transfection with a NF-κB-AP-1-inducible reporter contruct. This construct consists of the SEAP reporter gene under the control of the IFN-β minimal promoter fused to five NF-κB and five AP-1 binding sites.

Binding of IL-1β (or IL-1α) to its receptor on the surface of HEK-Blue™ IL-1β cells triggers a signaling cascade leading to the activation of NF-κB and AP-1, and the subsequent production of SEAP.

HEK-Blue™ IL-1β respond to low concentrations of human IL-1α and IL-1β. They do not respond to human TNF-α. They can be used to detect IL-1β in inflammasome studies (see page 46).

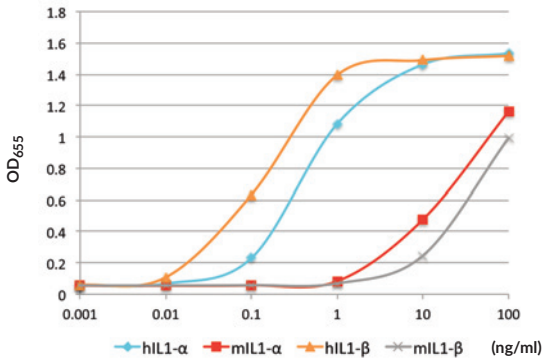
Features

Ligand	EC50	Fold response*
Human IL-1α	0.4 ± 0.1 ng/ml	30
Human IL-1β	0.15 ± 0.02 ng/ml	30

HEK-Blue™ IL-1β cells are resistant to Zeocin™ and hygromycin B. They are grown in standard DMEM medium, 10% FBS supplemented with 100 µg/ml Normocin, 100 µg/ml Zeocin™ and 200 µg/ml hygromycin B Gold (ultrapure hygromycin).

Contents

HEK-Blue™ IL-1β cells are provided frozen in a cryotube containing 3-7 x 10^6 cells and they come supplied with 10 mg Zeocin™, 10 mg hygromycin B Gold, 50 mg Normocin™ and 1 pouch of QUANTI-Blue™.



Response of HEK-Blue™ IL-1β cells to interleukin-1 cytokines. HEK-Blue™ IL-1β cells were incubated with increasing concentrations of recombinant human IL-1α or IL-1β, or mouse IL-1α or IL-1β. After 24h incubation the levels of NF-κB-AP-1-induced SEAP were determined using QUANTI-Blue™.

PRODUCT	QUANTITY	CAT. CODE
HEK-Blue IL-1β	3-7 x 10^6 cells	hkb-il1b

*The fold response was calculated by dividing the OD at 655 nm for the treated cells by the OD at 655 nm for the untreated cells.

HEK-Blue™ IL-1R Cells - Human & mouse IL-1α/β reporter cells

Description

HEK-Blue™ IL-1R cells were designed to detect bioactive human and mouse interleukin-1 cytokines (IL-1α and IL-1β) by monitoring the activation of the NF-κB and AP-1 pathways. Additionally, these cells detect bioactive IL-1β from cynomolgus monkeys, dogs, and rats. They were derived from the HEK-Blue™ IL-1β cell line by stable transfection with the mouse IL-1 receptor.

HEK-Blue™ IL-1R cells express a SEAP reporter gene under the control of the IFN-β minimal promoter fused to five NF-κB and five AP-1 binding sites. Activation of the human and mouse IL-1 receptors leads to the translocation of NF-κB and AP-1 and the production of SEAP.

HEK-Blue™ IL-1R are very sensitive to both human and mouse IL-1 cytokines. They can detect high levels (≥10 ng/ml) of mouse TNF-α. They do not respond to human TNF-α.

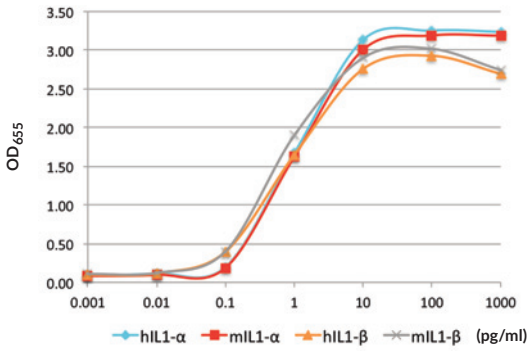
Features

Ligand	EC50	Fold response*
Human IL-α	1 ± 0.2 pg/ml	30
Mouse IL-α	1 ± 0.2 pg/ml	30
Human IL-β	0.8 ± 0.2 pg/ml	30
Mouse IL-β	0.6 ± 0.2 pg/ml	30

HEK-Blue™ IL-1R cells are resistant to Zeocin™, hygromycin B and puromycin. They are grown in standard DMEM medium, 10% FBS supplemented with 100 µg/ml Normocin, 100 µg/ml Zeocin™, 200 µg/ml hygromycin B Gold (ultrapure hygromycin) and 1 µg/ml puromycin.

Contents

HEK-Blue™ IL-1R cells are provided frozen in a cryotube containing 3-7 x 10^6 cells and they come supplied with 10 mg Zeocin™, 10 mg hygromycin B Gold, 1 mg puromycin, 50 mg Normocin™ and 1 pouch of QUANTI-Blue™.



Response of HEK-Blue™ IL-1R cells to interleukin-1 cytokines. HEK-Blue™ IL-1R cells were incubated with increasing concentrations of recombinant human IL-1α or IL-1β, or mouse IL-1α or IL-1β. After 24h incubation the levels of NF-κB-AP-1-induced SEAP were determined using QUANTI-Blue™.

PRODUCT	QUANTITY	CAT. CODE
HEK-Blue IL-1R	3-7 x 10^6 cells	hkb-il1r

3

RELATED PRODUCTS

PRODUCT	INFO
Recombinant human IL-1β	See page 136

RECENT CITATIONS

HEK-Blue™ IL-1β cells

Ramirez-Carrozzi V. *et al.*, 2015. Functional analysis of protective IL1RL1 variants associated with asthma risk. J Allergy Clin Immunol. 135(4):1080-3.e3.

Edye ME. *et al.*, 2015. Acid-dependent Interleukin-1 (IL-1) Cleavage Limits Available Pro-IL-1β for Caspase-1 Cleavage. J Biol Chem. 290(42):25374-81.

Kimura T. *et al.*, 2015. TRIM-mediated precision autophagy targets cytoplasmic regulators of innate immunity. J Cell Biol. 210(6):973-89.

HEK-Blue™ IL-1R cells

Karmakar M. *et al.*, 2016. Neutrophil P2X7 receptors mediate NLRP3 inflammasome-dependent IL-1β secretion in response to ATP. Nat Commun. 7:10555.

Antonopoulos C. *et al.*, 2015. Caspase-8 as an Effector and Regulator of NLRP3 Inflammasome Signaling. J Biol Chem. 290(33):20167-84.

HEK-Blue™ IL-18 cells

Lee KC. *et al.*, 2015. Extracorporeal liver assist device to exchange albumin and remove endotoxin in acute liver failure: Results of a pivotal pre-clinical study. J Hepatol. 63(3):634-42.

HEK-Blue™ IL-33 cells

Nadeau-Vallée M. *et al.*, 2015. Novel Noncompetitive IL-1 Receptor-Biased Ligand Prevents Infection- and Inflammation-Induced Preterm Birth. J Immunol. 195(7):3402-15.

HEK-Blue™ IL-18 Cells - Human IL-18 reporter cells

Description

HEK-Blue™ IL-18 cells are designed to detect bioactive human IL-18 and to a lesser extent mouse IL-18 by monitoring the activation of the NF-κB and AP-1 pathways. They were generated by stable transfection of HEK293 derived cells with the genes encoding IL-18R and IL-18RAP. In addition, the TNF-α and the IL-1β responses have been blocked. Therefore, HEK-Blue™ IL-18 cells respond specifically to IL-18. These cells express the SEAP reporter gene under the control of the IFN-β minimal promoter fused to five NF-κB and five AP-1 binding sites.
Binding of IL-18 to IL-18R/IL-18RAP on the surface of HEK-Blue™ IL-18 cells triggers a signaling cascade leading to the activation NF-κB and AP-1 with the subsequent production of SEAP.

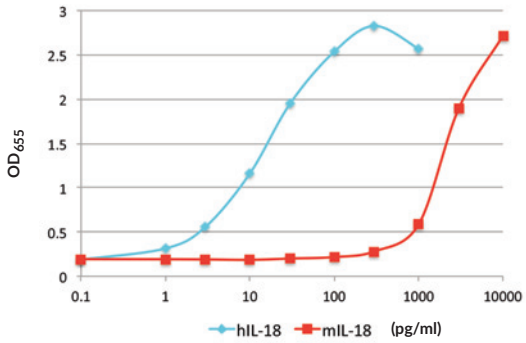
Features

Ligand	EC50	Fold response*
Human IL-18	18 ± 6 pg/ml	20
Mouse IL-18	2 ± 1 ng/ml	20

HEK-Blue™ IL-18 cells are resistant to blasticidin, hygromycin and Zeocin™. They are grown in standard DMEM medium, 10% FBS supplemented with HEK-Blue™ Selection, a solution that combines several selective antibiotics.

Contents

HEK-Blue™ IL-18 cells are provided frozen in a cryotube containing 3-7 x 10^6 cells and they come supplied with 2 ml HEK-Blue™ Selection (250X), 50 mg Normocin™ and 1 pouch of QUANTI-Blue™.



Response of HEK-Blue™ IL-18 cells to IL-18. HEK-Blue™ IL-18 cells were incubated with increasing concentrations of recombinant human IL-18. After 24h incubation the levels of NF-κB-AP-1-induced SEAP were determined using QUANTI-Blue™.

PRODUCT	QUANTITY	CAT. CODE
HEK-Blue IL-18	3-7 x 10^6 cells	hkb-hmil18

RELATED PRODUCTS

PRODUCT	INFO
Recombinant human IL-18	See page 136

HEK-Blue™ IL-33 Cells - Human IL-33 reporter cells

Description

HEK-Blue™ IL-33 cells are designed to detect human bioactive IL-33 by monitoring the activation of the NF-κB and AP-1 pathways. They were generated by stable transfection of HEK293-derived cells with the human IL1RL1 gene. In addition, the TNF-α and the IL-1β responses have been blocked. Therefore, HEK-Blue™ IL-33 cells respond specifically to IL-33. These cells express the SEAP reporter gene under the control of the IFN-β minimal promoter fused to five NF-κB and five AP-1 binding sites.
Binding of human IL-33 to the heterodimeric IL-33 receptor on the surface of these cells triggers a signaling cascade leading to the activation NF-κB/AP-1 and the subsequent production of SEAP.

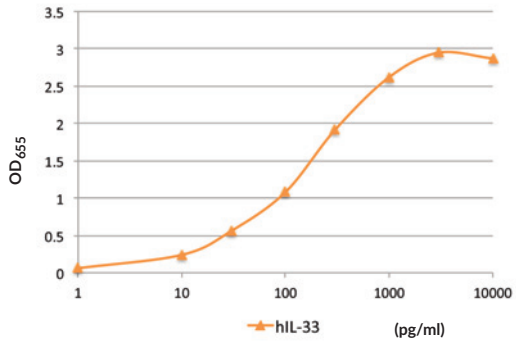
Features

Ligand	EC50	Fold response*
Human IL-33	0.2 ± 0.1 ng/ml	50

HEK-Blue™ IL-33 cells are resistant to blasticidin, hygromycin and Zeocin™. They are grown in standard DMEM medium, 10% FBS supplemented with HEK-Blue™ Selection, a solution that combines several selective antibiotics.

Contents

HEK-Blue™ IL-33 cells are provided frozen in a cryotube containing 3-7 x 10^6 cells and they come supplied with 2 ml HEK-Blue™ Selection (250X), 50 mg Normocin™ and 1 pouch of QUANTI-Blue™.



Response of HEK-Blue™ IL-33 cells to IL-33. HEK-Blue™ IL-33 cells were incubated with increasing concentrations of recombinant human IL-33. After 24h incubation the levels of NF-κB-AP-1-induced SEAP were assessed using QUANTI-Blue™.

PRODUCT	QUANTITY	CAT. CODE
HEK-Blue IL-33	3-7 x 10^6 cells	hkb-hil33

RELATED PRODUCTS

PRODUCT	INFO
Recombinant human IL-33	See page 137

HEK-Blue™ IL-17 Cells - Human & mouse IL-17 reporter cells

Description

HEK-Blue™ IL-17 cells are designed to detect bioactive human and mouse IL-17 by monitoring the activation of the NF-κB and AP-1 pathways. They were generated by stably introducing the human genes for the IL-17RA/IL-17RC heterodimer and the Act1 adaptor molecule into HEK293-derived cells. Furthermore, in these cells the TNF-α and MyD88-dependent IL-1β responses are blocked, hence, HEK-Blue™ IL-17 cells respond specifically to IL-17. They also express a SEAP reporter gene under the control of the IFN-β minimal promoter fused to five NF-κB and five AP-1 binding sites.
Binding of IL-17 to IL-17RA/IL-17RC on the surface of HEK-Blue™ IL-17 cells triggers a signaling cascade leading to the activation NF-κB and AP-1 with the subsequent production of SEAP.

Features

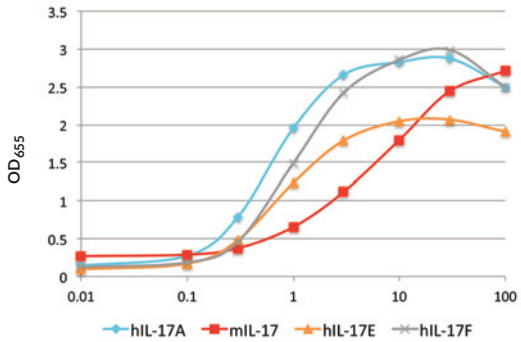
Ligand	EC50	Fold response*
Human IL-17A	0.7 ± 0.4 ng/ml	25
Human IL-17E (IL-25)	0.7 ± 0.2 ng/ml	20
Human IL-17F	0.9 ± 0.3 ng/ml	30
Mouse IL-17	7 ± 4 ng/ml	10

HEK-Blue™ IL-17 cells are resistant to blasticidin, hygromycin and Zeocin™. They are grown in standard DMEM medium, 10% FBS supplemented with HEK-Blue™ Selection, a solution that combines several selective antibiotics.

Contents

HEK-Blue™ IL-17 cells are provided frozen in a cryotube containing 3-7 x 10^6 cells and they come supplied with 2 ml HEK-Blue™ Selection (250X), 50 mg Normocin™ and 1 pouch of QUANTI-Blue™.

*The fold response was calculated by dividing the OD at 655 nm for the treated cells by the OD at 655 nm for the untreated cells.



Response of HEK-Blue™ IL-17 cells to IL-17 cytokines. HEK-Blue™ IL-17 cells were incubated with increasing concentrations of recombinant human IL-17A, IL17E (IL-25), IL-17F or mouse IL-17. After 24h incubation the levels of NF-κB-AP-1-induced SEAP were determined using QUANTI-Blue™.

PRODUCT	QUANTITY	CAT. CODE
HEK-Blue IL-17	3-7 x 10^6 cells	hkb-il17

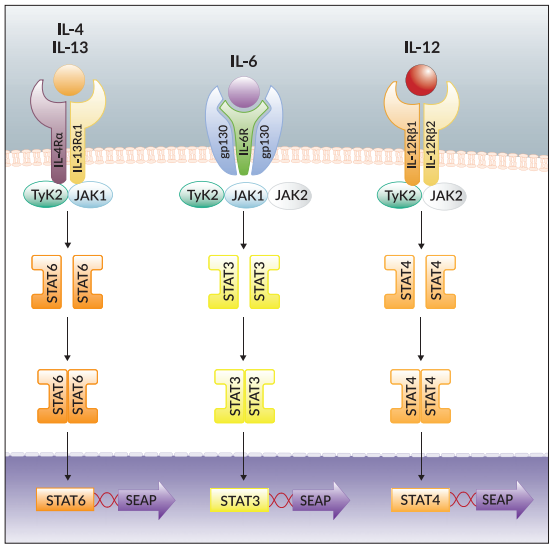
RELATED PRODUCTS

PRODUCT	INFO
Recombinant human IL-17A	See page 136

Interleukins Signaling by the JAK/STAT Pathway

Interleukins (IL) that signal through the JAK/STAT pathway include IL-4, IL-6, IL-12 and IL-13. IL-4 and IL-13 are closely related cytokines, that play a central role in the development of allergic inflammation and asthma. They use a common receptor complex composed of two subunits, IL-4Rα and IL-13Rα1, which signals through Janus kinase 1 (JAK1) and tyrosine kinase 2 (Tyk2), leading to the activation of signal transducer and activator of transcription 6 (STAT6). IL-6 is one of the most important mediators of fever and of the acute phase response. It signals through a cytokine complex consisting of the ligand-binding IL-6Rα chain and the signal-transducing component gp130, that recruits JAK1, JAK2, and Tyk2 inducing the activation of STAT3. IL-12 promotes the development of Th1 responses and is a powerful inducer of interferon gamma (IFN-γ). It binds to the IL-12 receptor (IL-12R), which is a heterodimeric receptor composed IL-12R-β1 and IL-12R-β2. Binding of IL-12 to its receptor activates JAK2, TyK2 and STAT4 resulting in the production of IFN-γ.

InvivoGen provides a collection of HEK293-derived reporter cells that are useful for the detection of human/mouse bioactive IL-4/IL-13, IL-6 and IL-12 by measuring the activity of STAT-induced SEAP (secreted embryonic alkaline phosphatase).



- HEK-Blue™ IL-4/IL-13
- HEK-Blue™ IL-6
- HEK-Blue™ IL-12

HEK-Blue™ IL-4/IL-13 Cells - Human IL-4, human & mouse IL-13 reporter cells

Description

HEK-Blue™ IL-4/IL-13 cells allow the detection of bioactive IL-4 and IL-13 by monitoring the activation of the STAT-6 pathway. These cells were generated by stable transfection of HEK293 cells with the human STAT6 gene to obtain a fully active STAT6 pathway. The other genes of the pathway are naturally expressed in sufficient amounts. The cells were further transfected with a SEAP reporter gene under the control of the IFN-β minimal promoter fused to four STAT6 binding sites. HEK-Blue™ IL-4/IL-13 cells produce SEAP in response to IL-4 or IL-13 stimulation.

Features

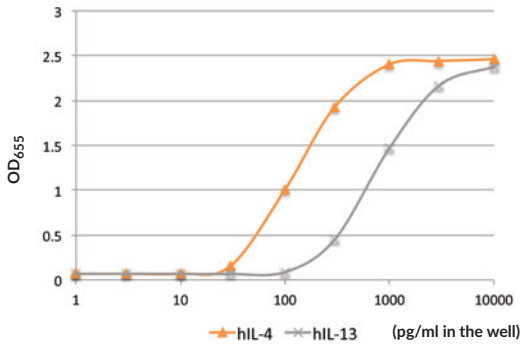
Ligand	EC50	Fold response*
Human IL-4	0.15 ± 0.1 ng/ml	35
Human IL-13	1 ± 0.5 ng/ml	35
Mouse IL-13	0.8 ± 0.1 ng/ml	30

HEK-Blue™ IL-4/13 cells are resistant to blasticidin and Zeocin™. They are grown in standard DMEM medium, 10% FBS supplemented with 100 µg/ml Normocin, 10 µg/ml blasticidin and 100 µg/ml Zeocin™.

Contents

HEK-Blue™ IL-4/13 cells are provided frozen in a cryotube containing 3-7 x 10^6 cells and they come supplied with 1 mg blasticidin, 10 mg Zeocin™, 50 mg Normocin™ and 1 pouch of QUANTI-Blue™.

*The fold response was calculated by dividing the OD at 655 nm for the treated cells by the OD at 655 nm for the untreated cells.



Response of HEK-Blue™ IL-4/IL-13 cells to IL-4 and IL-13. HEK-Blue™ IL-4/IL-13 cells were incubated with increasing concentrations of recombinant human IL-4, human IL-13 or mouse IL-13. After 24h incubation the levels of STAT6-induced SEAP were determined using QUANTI-Blue™.

PRODUCT	QUANTITY	CAT. CODE
HEK-Blue IL-4/IL-13	3-7 x 10^6 cells	hkb-il413

RELATED PRODUCTS

PRODUCT	INFO
Recombinant human IL-4	See page 136
Recombinant human IL-13	See page 136

HEK-Blue™ IL-6 Cells - Human IL-6 reporter cells

Description

HEK-Blue™ IL-6 cells allow the detection of bioactive IL-6 by monitoring the activation of the STAT-3 pathway. These cells were generated by stable transfection of HEK293 cells with the human IL-6R gene. They were further transfected with a SEAP reporter gene under the control of the IFN-β minimal promoter fused to four STAT3 binding sites. Upon IL-6 stimulation, HEK-Blue™ IL-6 cells trigger the activation of STAT3 and the subsequent secretion of SEAP.

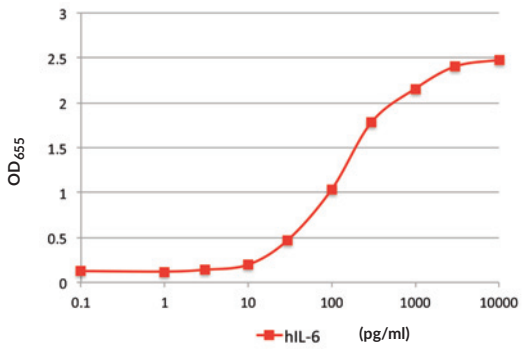
Features

Ligand	EC50	Fold response*
Human IL-6	0.2 ± 0.1 ng/ml	20

HEK-Blue™ IL-6 cells are resistant to hygromycin and Zeocin™. They are grown in standard DMEM medium, 10% FBS supplemented with 100 µg/ml Normocin, 200 µg/ml hygromycin B Gold (ultrapure hygromycin) and 100 µg/ml Zeocin™.

Contents

HEK-Blue™ IL-6 cells are provided frozen in a cryotube containing 3-7 x 10^6 cells and they come supplied with 10 mg hygromycin B Gold, 10 mg Zeocin™, 50 mg Normocin™ and 1 pouch of QUANTI-Blue™.



Response of HEK-Blue™ IL-6 cells to IL-6. HEK-Blue™ IL-6 cells were incubated with increasing concentrations of recombinant human IL-6. After 24h incubation the levels of STAT3-induced SEAP were determined using QUANTI-Blue™.

PRODUCT	QUANTITY	CAT. CODE
HEK-Blue IL-6	3-7 x 10^6 cells	hkb-hil6

RELATED PRODUCTS

PRODUCT	INFO
Recombinant human IL-6	See page 136

HEK-Blue™ IL-12 Cells - Human & mouse IL-12 reporter cells

Description

HEK-Blue™ IL-12 cells are designed to detect bioactive human and mouse IL-12 by monitoring the activation of the STAT-4 pathway. These cells were generated by stably introducing the human genes for the IL-12 receptor and the genes of the IL-12 signaling pathway into HEK293-derived cells. Furthermore, these cells express a STAT4-inducible SEAP reporter gene.
Binding of IL-12 to the IL-12R on the surface of HEK-Blue™ IL-12 cells triggers a signaling cascade leading to the activation STAT-4 and the subsequent production of SEAP.

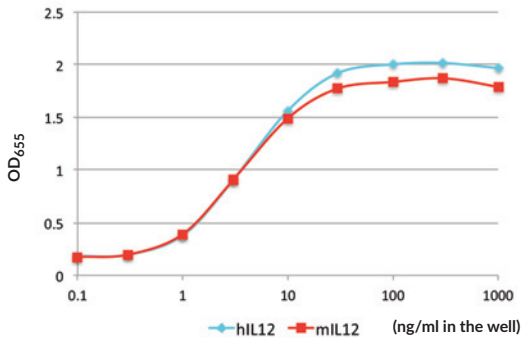
Features

Ligand	EC50	Fold response*
Human IL-12	4 ± 1 ng/ml	10
Mouse IL-12	2 ± 2 ng/ml	10

HEK-Blue™ IL-12 cells are resistant to blasticidin, hygromycin and Zeocin™. They are grown in standard DMEM medium, 10% FBS supplemented with 100 µg/ml Normocin, 30 µg/ml blasticidin, 200 µg/ml hygromycin B Gold (ultrapure hygromycin) and 100 µg/ml Zeocin™.

Contents

HEK-Blue™ IL-12 cells are provided frozen in a cryotube containing 3-7 x 10^6 cells and they come supplied with 1 mg blasticidin, 10 mg hygromycin B Gold, 10 mg Zeocin™, 50 mg Normocin™ and 1 pouch of QUANTI-Blue™.



Response of HEK-Blue™ IL-12 cells to IL-12. HEK-Blue™ IL-12 cells were incubated with increasing concentrations of recombinant human or mouse IL-12. After 24h incubation the levels of STAT4-induced SEAP were assessed using QUANTI-Blue™.

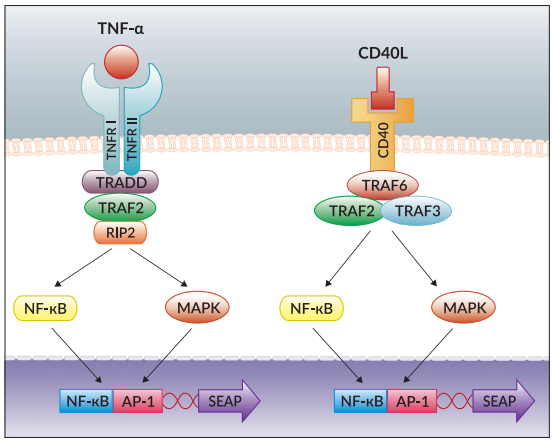
PRODUCT	QUANTITY	CAT. CODE
HEK-Blue IL-12	3-7 x 10^6 cells	hkb-il12

RELATED PRODUCTS

PRODUCT	INFO
Recombinant human IL-12	See page 136

TUMOR NECROSIS FACTOR REPORTER CELLS

Tumor-necrosis factor (TNF)-α and cluster of differentiation 40 ligand (CD40L) are members of the TNF superfamily. After binding to their receptors, both cytokines mediate cell proliferation and inflammation through activation of the NF-κB and MAPK pathways. TNF-α plays an important role in the immune response to microbial invasions and in the necrosis of specific tumors. TNF-α binds two receptors, TNFR1 and TNFR2, inducing signaling that involves TRADD, TRAF2 and RIP, and leads to the activation of the NF-κB and the MAPK pathways. CD40L is a transmembrane molecule expressed mainly by activated T-lymphocytes. It interacts with CD40 on antigen-presenting cells to regulate both humoral and cellular immune responses. The CD40 cytoplasmic domain binds directly to several TNF receptor-associated factors (TRAFs) initating a signaling that leads to the activation of several transcription factors, including NF-κB and c-Jun (a subunit of AP-1).



- HEK-Blue™ TNF-α
- HEK-Blue™ CD40L

HEK-Blue™ TNF-α Cells - Human & mouse TNF-α reporter cells

Description

HEK-Blue™ TNF-α cells enable the detection of bioactive human and murine TNF-α by monitoring the activation of the NF-κB and AP-1 pathways. They were generated by stable transfection of HEK293-derived cells with the TNFRI gene and an inducible SEAP reporter construct. Expression of the reporter gene is driven by the IFN-β minimal promoter fused to five NF-κB and five AP-1 binding sites. The cells were further rendered unresponsive to IL-1β by knockingout the MyD88 gene.
Stimulation of HEK-Blue™ TNF-α cells with TNF-α triggers the activation of the NF-κB-AP-1-inducible promoter and the subsequent production of SEAP.

Features

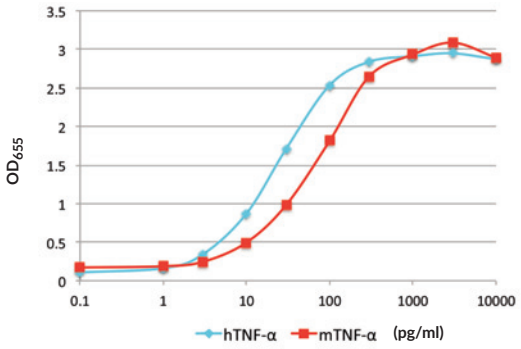
Ligand	EC50	Fold response*
Human TNF-α	25 ± 15 pg/ml	35
Mouse TNF-α	60 ± 20 pg/ml	20

Sensitivity: 25 pg/ml (in the sample)

HEK-Blue™ IFN-α/β cells are resistant to puromycin and Zeocin™. They are grown in standard DMEM medium, 10% FBS supplemented with 100 µg/ml Normocin, 1 µg/ml puromycin and 100 µg/ml Zeocin™.

Contents

HEK-Blue™ TNF-α cells are provided frozen in a cryotube containing 3-7 x 10^6 cells and they come supplied with 1 mg puromycin, 10 mg Zeocin™, 50 mg Normocin™ and 1 pouch of QUANTI-Blue™.



Response of HEK-Blue™ TNF-α cells to TNF-α. HEK-Blue™ TNF-α cells were incubated with increasing concentrations of recombinant human or mouse TNF-α. After 24h incubation the levels of NF-κB-AP-1-induced SEAP were determined using QUANTI-Blue™.

PRODUCT	QUANTITY	CAT. CODE
HEK-Blue TNF-α	3-7 x 10^6 cells	hkb-tnfdmyd

RELATED PRODUCTS

PRODUCT	INFO
Recombinant human TNF-α	See page 137

HEK-Blue™ CD40L Cells - Human CD40L reporter cells

Description

HEK-Blue™ CD40L cells can serve to measure the bioactivity of human CD40L based on measuring secreted embryonic alkaline phosphatase (SEAP) upon NF-κB/AP-1 activation following CD40 stimulation. These cells were generated by stable transfection of HEK293-derived cells with the human CD40 gene and an NF-κB-AP-1 inducible SEAP construct. Binding of CD40L to its receptor CD40 triggers a signaling cascade leading to the activation of NF-κB and AP-1, and the subsequent production of SEAP.
HEK293 cells express endogenously the receptors for the cytokines IL-1β and TNF-α, which share a common signaling pathway with CD40L. Consequently, HEK-Blue™ CD40L cells also respond to IL-1β and TNF-α.

Features

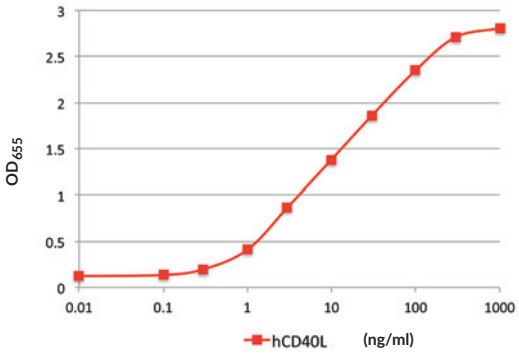
Ligand	EC50	Fold response*
Human CD40L	10 ± 6 ng/ml	20

HEK-Blue™ CD40L cells are resistant to blasticidin and Zeocin™. They are grown in standard DMEM medium, 10% FBS supplemented with 100 µg/ml Normocin, 10 µg/ml blasticidin and 100 µg/ml Zeocin™.

Contents

HEK-Blue™ CD40L cells are provided frozen in a cryotube containing 3-7 x 10^6 cells and they come supplied with 1 mg blasticidin, 10 mg Zeocin™, 50 mg Normocin™ and 1 pouch of QUANTI-Blue™.

*The fold response was calculated by dividing the OD at 655 nm for the treated cells by the OD at 655 nm for the untreated cells.



Response of HEK-Blue™ CD40L cells to CD40L. HEK-Blue™ CD40L cells were incubated with increasing concentrations of recombinant human CD40L. After 24h incubation the levels of NF-κB-AP-1-induced SEAP were determined using QUANTI-Blue™.

PRODUCT	QUANTITY	CAT. CODE
HEK-Blue CD40L	3-7 x 10^6 cells	hkb-cd40

RELATED PRODUCTS

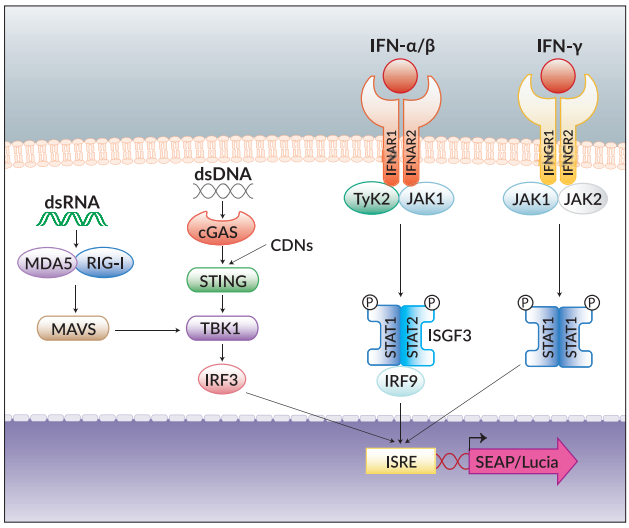
PRODUCT	INFO
Recombinant human CD40L	See page 136

TRANSCRIPTION FACTOR REPORTER CELLS

IRF REPORTER CELLS

Interferon regulatory factors (IRFs) constitute a family of transcription factors that play an important role in host defense. Interferons (IFNs) play a vital role in host resistance to viral infections. They are produced following the recognition of viral nucleic acids in the cytosol by cytosolic DNA sensors (CDSs), such as RIG-I and cGAS, which signal through the TBK1/IRF3 pathway. IFNs are grouped into three classes: type I (IFN-α and IFN-β), type II (IFN-γ) and type III IFNs. The type I IFNs bind to a common receptor (IFNAR) and recruit the Janus kinases (JAK1 and TyK2), which phosphorylate STAT1 and STAT2, which then dimerize and interact with IFN regulatory factor 9 (IRF9), forming a complex named ISGF3. ISGF3 binds to IFN-stimulated response elements (ISRE) in the promoters of IFN-stimulated genes (ISG) to regulate their expression. IFN-γ also signals through the JAK/STAT pathway and regulates the transcription of ISGs. The regulation of the IFN pathway is crucial for maintaining a balance between the protective anti-viral effects of IFNs and the destructive effects of IFN overproduction, which has been associated with autoimmune disease.

To help you study IFN production and IRF activation, InvivoGen provides a collection of reporter cells that stably express an IRF-inducible secreted embryonic alkaline phosphatase (SEAP) or Lucia luciferase gene.



- B16-Blue™ ISG
- HEK-Blue™ ISG
- RAW-Lucia™ ISG
- THP1-Blue™ ISG

B16-Blue™ ISG Cells - Mouse melanoma cells

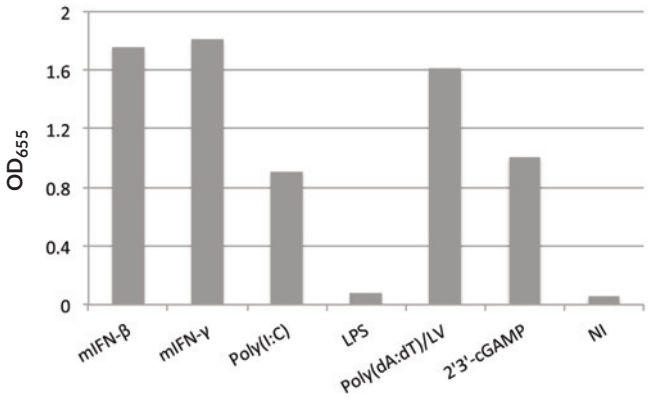
Description

B16-Blue™ ISG cells were derived from the murine B16 F1 melanoma cell line. These cells can be used to study the activation of the TBK1/IRF3 pathway by cytosolic DNA, double-stranded RNA or cyclic dinucleotides (CDNs). They express the SEAP reporter gene under the control of the I-ISG54 promoter which is comprised of the IFN-inducible ISG54 promoter enhanced by a multimeric ISRE.
Stimulation of B16-Blue™ ISG cells with IFNs, CDNs such as 2'3'-cGAMP or type I IFN inducers such as transfected poly(dA:dT) leads to the production of SEAP. Levels of SEAP in the supernatant can be easily determined using QUANTI- Blue™.
B16-Blue™ ISG cells are resistant to Zeocin™. They are grown in RPMI medium, 2 mM L-glutamine, 10% FBS with 100 µg/ml Zeocin™.

Contents

B16-Blue™ ISG cells are provided in vials containing 3-7 x 10^6 cells, and they come supplied with 10 mg Zeocin™, 50 mg Normocin™ and 1 pouch of QUANTI-Blue™.

PRODUCT	QUANTITY	CAT. CODE
B16-Blue™ ISG Cells	3-7 x 10^6 cells	bb-ifnabg



Response of B16-Blue™ ISG cells to IFNs and various PAMPs. Cells were incubated with 100 IU/ml mIFN-β, 1 ng/ml mIFN-γ, 10 µg/ml poly(I:C), 10 µg/ml LPS, 1 µg/ml poly(dA:dT)/LyoVec™ or 10 µg/ml 2'3'-cGAMP. After 24h incubation, IRF activation was assessed by measuring the levels of SEAP in the supernatant using QUANTI-Blue™.

HEK-Blue™ ISG Cells - HEK293 derived cells

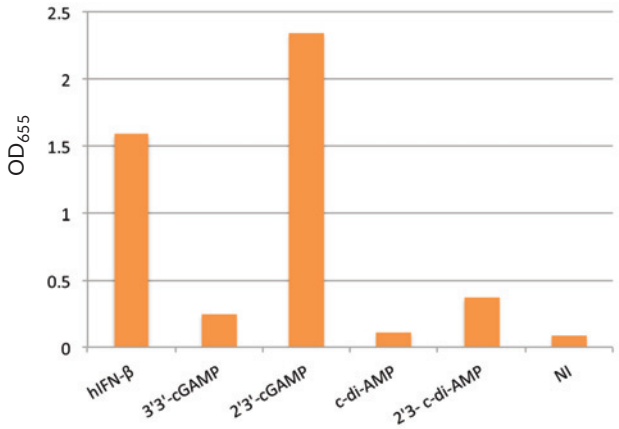
Description

HEK-Blue™ ISG cells were derived from HEK293, a human embryonic kidney cell line. They stably express the SEAP reporter gene under the control of an IRF-inducible promoter comprised of five IFN-stimulated response elements (ISRE) fused to an ISG54 minimal promoter.
Stimulation of HEK-Blue™ ISG cells by type I IFNs or CDNs triggers the activation of IRF9 or IRF3, respectively, leading to the subsequent production of SEAP in the cell supernatant. Levels of SEAP can be easily monitored using QUANTI-Blue™.
HEK-Blue™ ISG cells are resistant to Zeocin™. They are grown in DMEM medium, 2mM L-glutamine, 10% FBS with 100 µg/ml Zeocin™.

Contents

HEK-Blue™ ISG cells are provided in vials containing 3-7 x 10^6 cells, and they come supplied with 10 mg Zeocin™, 50 mg Normocin™ and 1 pouch of QUANTI-Blue™.

PRODUCT	QUANTITY	CAT. CODE
HEK-Blue™ ISG Cells	3-7 x 10^6 cells	hkb-isg



Response of HEK-Blue™ ISG cells to type I IFNs and CDNs. Cells were incubated with 100 IU/ml hIFN-β or 10 µg/ml each of 3'3'-cGAMP, 2'3'-cGAMP, c-di-AMP, or 2'3'-c-di-AMP. After 24h incubation, the levels of IRF-induced SEAP were measured using QUANTI-Blue™.

RAW-Lucia™ ISG Cells - Mouse macrophages

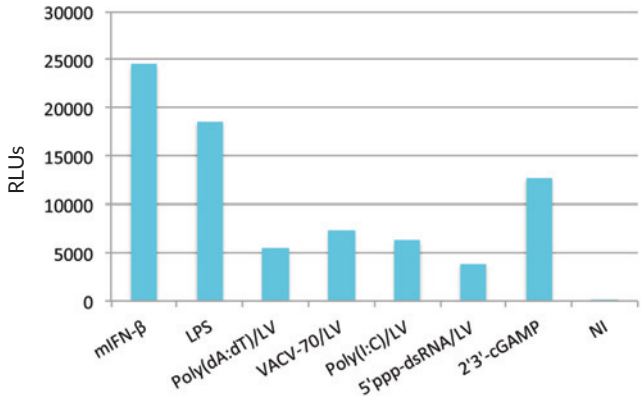
Description

RAW-Lucia™ ISG cells were generated from the murine RAW 264.7 macrophage cell line by stable integration of an IRF-inducible Lucia luciferase reporter construct. Expression of Lucia luciferase is driven by an ISG54 minimal promoter fused to five ISREs.
RAW-Lucia™ ISG cells are responsive to type I IFNs and PRR ligands that trigger the IFN signaling pathway, including CDS ligands (transfected double stranded DNA), RIG-I ligands (transfected double stranded RNA) and STING ligands (cyclic dinucleotides). The levels of IRF-induced Lucia luciferase in the cell culture supernatant can be easily monitored using QUANTI-Luc™, a luciferase detection reagent.
RAW-Lucia™ ISG cells are resistant to Zeocin™. They are grown in DMEM medium, 2mM L-glutamine, 10% FBS with 200 µg/ml Zeocin™.

Contents

RAW-Lucia™ ISG cells are provided in vials containing 5-7 x 10^6 cells, and they come supplied with 10 mg Zeocin™, 50 mg Normocin™ and 1 pouch of QUANTI-Luc™.

PRODUCT	QUANTITY	CAT. CODE
RAW-Lucia™ ISG Cells	3-7 x 10^6 cells	rawl-isg



Response of RAW-Lucia™ ISG cells to type I IFNs and PRR agonists. Cells were incubated with 10^4 IU/ml IFN-β, 100 ng/ml LPS, 1 µg/ml poly(dA:dT)/LyoVec™, 1 µg/ml VACV70/LyoVec™, 1 µg/ml poly(I:C)/LyoVec™, 1 µg/ml 5'ppp-dsRNA/LyoVec™ or 3 µg/ml 2'3'-cGAMP. After 24h incubation, IRF activation was assessed by measuring the levels of Lucia luciferase in the supernatant using QUANTI-Luc™.

RELATED PRODUCTS

PRODUCT	INFO
B16-Blue™ ISG KO-STING cells	See page 67
HEK-Blue™ ISG KO-STING cells	See page 68
RAW-Lucia™ ISG KO cells	See page 69
RAW-Blue™ ISG cells	Visit website

RECENT CITATIONS

B16-Blue™ ISG - Li L. *et al.*, 2014. Hydrolysis of 2'3'-cGAMP by ENPP1 and design of nonhydrolyzable analogs. Nat Chem Biol. 10(12):1043-8.

RAW-Lucia™ ISG - Dey B. *et al.*, 2015. A bacterial cyclic dinucleotide activates the cytosolic surveillance pathway and mediates innate resistance to tuberculosis. Nat Med. 21(4):401-6.

THP1-Blue™ ISG Cells - Human monocytes

Description

THP1-Blue™ ISG cells were specifically designed for monitoring the interferon signaling pathway in a physiologically relevant cell line. They were derived from the human THP-1 monocyte cell line by stable integration of an IRF-inducible SEAP reporter construct. As a result, THP1-Blue™ ISG cells allow the monitoring of IRF activation by determining the activity of SEAP.

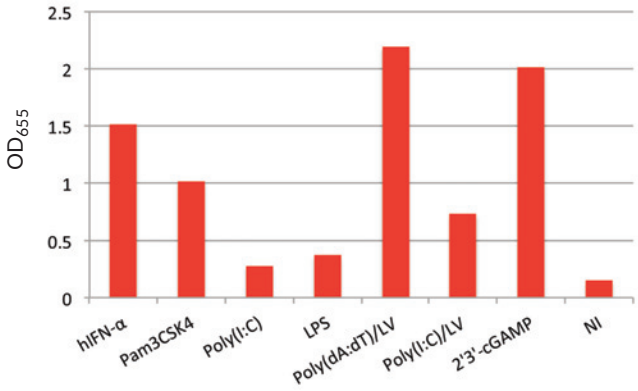
THP1-Blue™ ISG cells are highly responsive to PRR agonists that trigger the IFN signaling pathway, such as LPS and transfected double-stranded nucleic acid. When stimulated, they produce SEAP in the supernatant, that can be readily monitored using QUANTI-Blue™.

THP1-Blue™ ISG cells are resistant to Zeocin™. They are grown in RPMI medium, 2mM L-glutamine, 10% FBS with 100 µg/ml Zeocin™.

Contents

THP1-Blue™ ISG cells are provided in vials containing 3-7 x 10^6 cells, and they come supplied with 10 mg Zeocin™, 50 mg Normocin™ and 1 pouch of QUANTI-Blue™.

PRODUCT	QUANTITY	CAT. CODE
THP1-Blue™ ISG Cells	3-7 x 10^6 cells	thp-isg



Response of THP1-Blue™ ISG cells to type I IFNs and PRR ligands. Cells were incubated with 100 IU/ml hIFN-α, 1 µg/ml Pam3CSK4, 10 µg/ml poly(I:C), 10 µg/ml LPS, 1 µg/ml poly(dA:dT)/LyoVec™, 1 µg/ml poly(I:C)/LyoVec™ or 10 µg/ml 2'3'-cGAMP. After 24h incubation, IRF activation was assessed by measuring the levels of SEAP in the supernatant using QUANTI-Blue™.

RELATED PRODUCTS

PRODUCT	INFO
THP1-Lucia™ ISG cells	Visit website

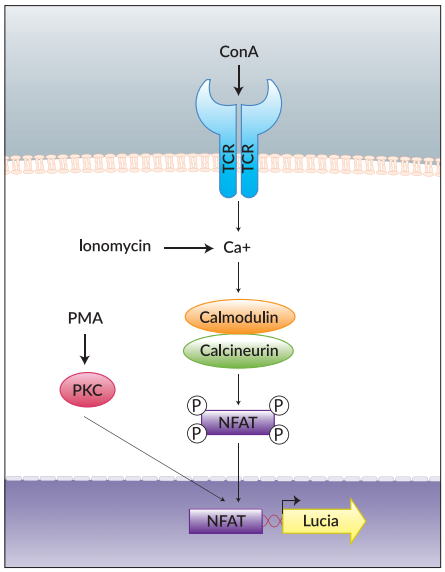
RECENT CITATIONS

THP1-Blue™ ISG - Tosolini M. et al., 2015. Human monocyte recognition of adenosine-based cyclic dinucleotides unveils the A2a Gαs protein-coupled receptor tonic inhibition of mitochondrially induced cell death. Mol Cell Biol. 35(2):479-95.

NFAT REPORTER CELLS

NFAT (nuclear factor of activated T cells) proteins are a family of transcription factors that are important in the development and function of the immune system. In T cells, NFAT proteins regulate thymocyte development, T cell activation, differentiation and self-tolerance. Their activation is induced by the engagement of the T cell receptor (TCR) that is coupled to the calcium-signaling pathway. Calcium binds calmodulin and activates calcineurin, which in turn dephosphorylates NFAT proteins inducing their nuclear translocation and activation, and the transcription of specific responsive genes.

InvivoGen has developed an NFAT reporter cell line derived from the immortalized T-cell line Jurkat that expresses an NFAT-inducible Lucia luciferase reporter construct. NFAT activation can be readily monitored using the Lucia luciferase detection reagent QUANTI-Luc.



Jurkat-Lucia™ NFAT

Jurkat-Lucia™ NFAT Cells- Human T lymphocytes

Description

Jurkat-Lucia™ NFAT cells were derived from the human T lymphocyte-based Jurkat cell line by stable integration of an NFAT-inducible Lucia reporter construct. The Lucia gene, which encodes a secreted coelenterazine-utilizing luciferase, is driven by an ISG54 minimal promoter fused to six copies of the NFAT consensus transcriptional response element.

Jurkat-Lucia™ NFAT cells induce the activation of NFAT in response to phorbol 12-myristate 13-acetate (PMA) and ionomycin, and T-lymphocyte mitogens, such as concanavalin A (ConA) and phytohemagglutinin.

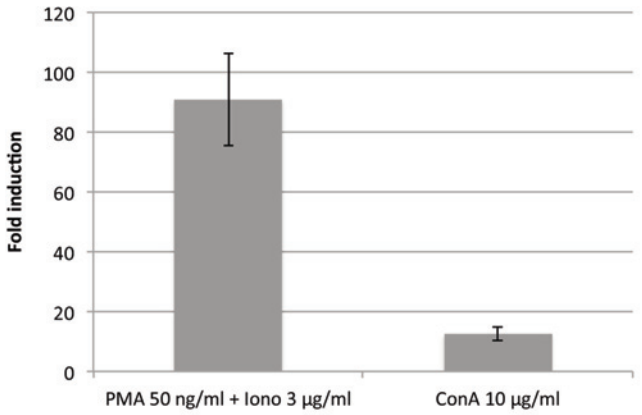
Jurkat-Dual™ cells can be used for the study of NFAT activation following stimuli treatments by monitoring Lucia luciferase activity. Levels of Lucia luciferase are readily measurable in the cell culture supernatant when using QUANTI-Luc™, a Lucia luciferase detection reagent.

Jurkat-Lucia™ NFAT cells are resistant to Zeocin™. They are grown in RPMI medium, 2mM L-glutamine, 10% FBS with 100 µg/ml Zeocin™.

Contents

Jurkat-Lucia™ NFAT cells are provided in vials containing 3-7 x 10^6 cells, and they come supplied with 10 mg Zeocin™, 50 mg Normocin™ and 1 pouch of QUANTI-Luc™.

PRODUCT	QUANTITY	CAT. CODE
Jurkat-Lucia™ NFAT Cells	3-7 x 10^6 cells	jktl-nfat



Response of Jurkat-Lucia™ NFAT cells to PMA/ionomycin and concanavalin A. Cells were incubated with 50 ng/ml PMA and 3 µg/ml ionomycin, or 10 µg/ml conA. After 24h incubation, NFAT activation was assessed by measuring the levels of Lucia luciferase in the supernatant using QUANTI-Luc™.

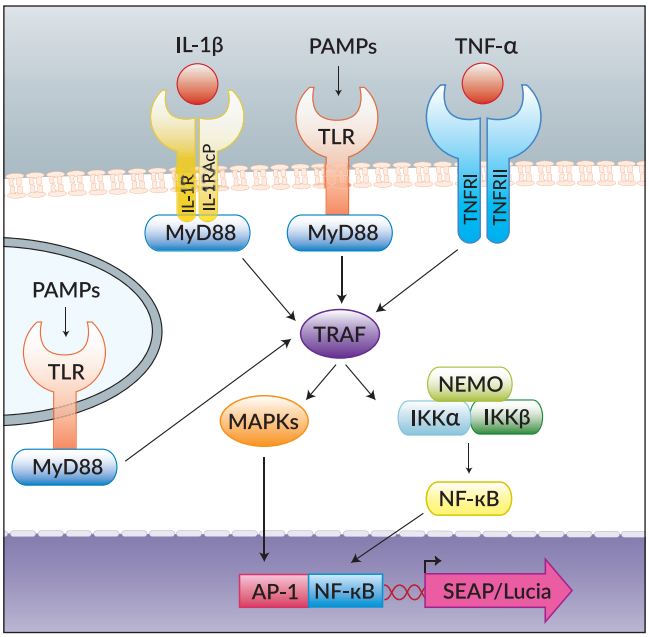
RELATED PRODUCTS

PRODUCT	INFO
Concanavalin A	Visit website
Ionomycin	Visit website
PMA	Visit website

NF-κB REPORTER CELLS

The nuclear factor-κB (NF-κB) family of transcription factors has an essential role in inflammation and innate immunity. The NF-κB pathway can be activated by pro-inflammatory cytokines, such as tumor necrosis factor α (TNF-α), interleukin-1β (IL-1β), and by pathogen-associated molecular patterns (PAMPs). PAMPs are recognized by pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs). Stimulation through these receptors leads to the activation of TNF receptor (TNFR) associated factor (TRAF) proteins causing nuclear translocation of activator protein 1 (AP-1) via mitogen-activated protein kinases (MAPKs). Activation of TRAF proteins also leads to the assembly of the IκB kinase (IKK) complex, which consists of two kinase subunits, IKKα (IKK1) and IKKβ (IKK2), and a regulatory subunit IKKγ (NEMO). The IKK complex promotes the association of the two NF-κB subunits, p50 and p65 (RelA), and the translocation of NF-κB to the nucleus where, either alone or in combination with other transcription factors such as AP-1, it induces the expression of pro-inflammatory and cell survival genes.

To help you study NF-κB activation, InvivoGen provides a collection of reporter cells that stably express an NF-κB-inducible secreted embryonic alkaline phosphatase (SEAP) or Lucia luciferase.



- Ramos-Blue™
- RAW-Blue™
- THP1-Lucia™ NF-κB

Ramos-Blue™ Cells - Human B lymphocytes

Description

Ramos-Blue™ cells were derived from a human Burkitt's lymphoma which is negative for Epstein Barr virus. They have the characteristics of B lymphocytes and are routinely used as a model of B lymphocytes and for apoptosis studies.

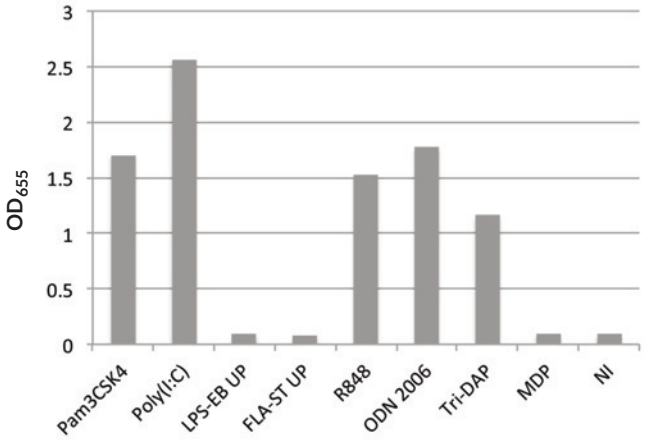
Ramos-Blue™ cells stably express an NF-κB/AP-1-inducible SEAP reporter gene. They are responsive to various PRR agonists that activate the NF-κB pathway, such as CpG ODNs, Pam3CSK4 and poly(I:C). When stimulated, they produce SEAP in the supernatant, that can be readily monitored using QUANTI-Blue™.

Ramos-Blue™ cells are resistant to Zeocin™. They are grown in IMDM medium, 2 mM L-glutamine, 10% FBS with 100 µg/ml Zeocin™.

Contents

Ramos-Blue™ cells are provided in vials containing 3-7 x 10^6 cells, and they come supplied with 10 mg Zeocin™, 50 mg Normocin™ and 1 pouch of QUANTI-Blue™.

PRODUCT	QUANTITY	CAT. CODE
Ramos-Blue™ Cells	3-7 x 10^6 cells	rms-sp



Response of Ramos-Blue™ cells to PRR agonists. Cells were incubated with 10 µg/ml each of Pam3CSK4 (TLR2), poly(I:C) (TLR3), LPS-EB (TLR4), FLA-ST UP (TLR5), R848 (TLR7/8), ODN2006 (TLR9), Tri-DAP (NOD1) or MDP (NOD2). After 24h incubation, NF-κB/AP-1 activation was assessed by measuring the levels of SEAP in the supernatant using QUANTI-Blue™.

RELATED PRODUCTS

PRODUCT	INFO
Ramos-Blue™ KD-MyD cells	See page 68

RECENT CITATIONS

Goldstein RL. et al., 2015. Pharmacoproteomics identifies combinatorial therapy targets for diffuse large B cell lymphoma. J Clin Invest.125(12):4559-71.

RAW-Blue™ Cells - Mouse macrophages

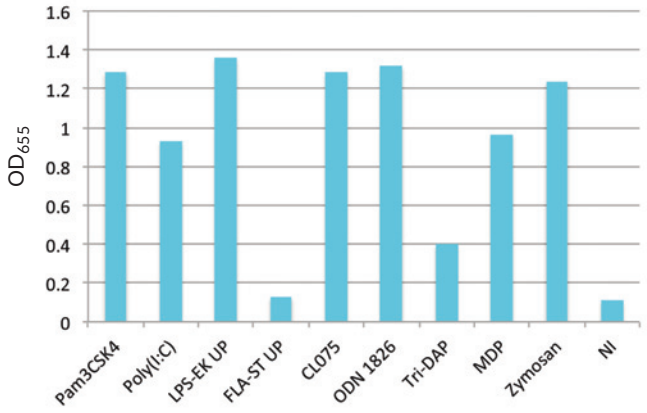
Description

RAW-Blue™ cells were derived from RAW 264.7, a mouse monocyte macrophage cell line, by chromosomal integration of a SEAP reporter construct inducible by NF-κB and AP-1. RAW 264.7 cells are capable of phagocytosis and express many pattern recognition receptors, including TLRs and cytosolic DNA sensors.
Stimulation of RAW-Blue™ cells by PRR agonists (e.g. LPS and zymosan) or cytokines (e.g. TNF-α and IL-1β) triggers the activation of NF-κB and AP-1 and leads to the release of SEAP in the cell supernatant. Levels of SEAP can be easily monitored using QUANTI-Blue™.
RAW-Blue™ cells are resistant to Zeocin™. They are grown in DMEM medium, 2mM L-glutamine, 10% FBS with 200 µg/ml Zeocin™.

Contents

RAW-Blue™ cells are provided in vials containing 3-7 x 10^6 cells, and they come supplied with 10 mg Zeocin™, 50 mg Normocin™ and 1 pouch of QUANTI-Blue™.

PRODUCT	QUANTITY	CAT. CODE
RAW-Blue™ Cells	3-7 x 10^6 cells	raw-sp



Response of RAW-Blue™ cells to PRR agonists. Cells were incubated with 10 ng/ml Pam3CSK4 (TLR2), 10 µg/ml poly(I:C) (TLR3), 1 ng/ml LPS-EK (TLR4), 1 µg/ml FLA–ST UP (TLR5), 300 ng/ml CL075 (TLR7/8), 100 ng/ml ODN1826 (TLR9), 10 µg/ml Tri-DAP (NOD1), 1 µg/ml MDP (NOD2) or 1 µg/ml zymosan. After 24h incubation, NF-κB/AP-1-induced SEAP levels were measured using QUANTI-Blue™.

THP1-Lucia™ NF-κB Cells - Human monocytes

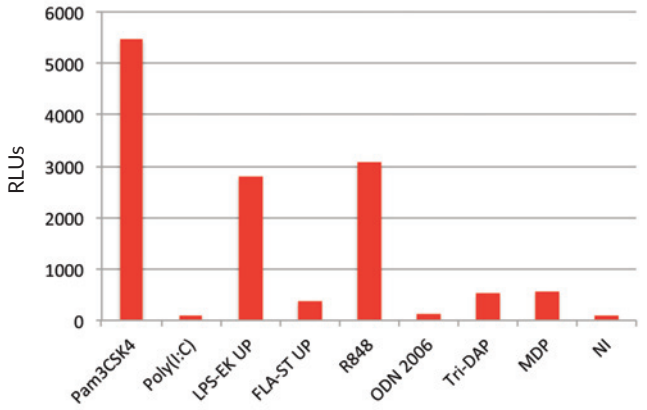
Description

THP1-Lucia™ NF-κB cells were derived from the human THP-1 monocyte cell line by stable integration of an NF-κB-inducible Lucia luciferase reporter construct. The Lucia luciferase gene is driven by an IFN-β minimal promoter fused to five copies of the NF-κB consensus transcriptional response element and three copies of the c-Rel binding site.
THP1-Lucia™ NF-κB cells induce the activation of NF-κB in response to various stimuli, including PRR ligands, such as Pam3CSK4 and LPS. The levels of NF-κB-induced Lucia luciferase in the supernatant are readily assessed with QUANTI-Luc™, a Lucia™ detection reagent.
THP1-Lucia™ NF-κB cells are resistant to Zeocin™. They are grown in RPMI medium, 2mM L-glutamine, 10% FBS with 100 µg/ml Zeocin™.

Contents

THP1-Lucia™ NF-κB cells are provided in vials containing 3-7 x 10^6 cells, and they come supplied with 10 mg Zeocin™, 50 mg Normocin™ and 1 pouch of QUANTI-Luc™.

PRODUCT	QUANTITY	CAT. CODE
THP1-Lucia™ NF-κB Cells	3-7 x 10^6 cells	thpl-nfkb



Response of THP1-Lucia™ NF-κB cells to PRR agonists. Cells were incubated with 10 ng/ml Pam3CSK4 (TLR2), 10 µg/ml poly(I:C) (TLR3), 100 ng/ml LPS-EK UP (TLR4), 10 µg/ml FLA–ST UP (TLR5), 10 µg/ml R848 (TLR7/8), 10 µg/ml ODN2006 (TLR9), 10 µg/ml Tri-DAP (NOD1) or 10 µg/ml MDP (NOD2). After 24h incubation, NF-kB/AP-1 activation was assessed by measuring the levels of Lucia luciferase in the supernatant using QUANTI-Luc™.

RELATED PRODUCTS

PRODUCT	INFO
THP1-Blue™ NF-κB cells	Visit website

RECENT CITATIONS

RAW-Blue™ - van der Plas M. *et al.*, 2016. APseudomonas aeruginosa elastase cleaves a C-terminal peptide from human thrombin that inhibits host inflammatory responses. Nat Commun. 7:11567.

Gause KT. *et al.*, 2015. Physicochemical and immunological assessment of engineered pure protein particles with different redox states. ACS Nano. 9(3):2433-44.

THP1-Lucia™ NF-kB - Tikhanovich I. et al., 2015. Dynamic Arginine Methylation of Tumor Necrosis Factor (TNF) Receptor-associated Factor 6 Regulates Toll-like Receptor Signaling. J Biol Chem.290(36):22236-49.

NF-κB & IRF DUAL REPORTER CELLS

Dual reporter cells have been developed to allow the simultaneous study of the NF-κB and IRF/IFN pathways. They express two secreted reporter genes encoding secreted embryonic alkaline phosphatase (SEAP) and Lucia luciferase to provide convenient read-outs to follow the activation of both pathways. NF-κB and IRF dual reporter cells express the SEAP reporter gene under the control of the IFN-β minimal promoter fused to five NF-κB binding sites, and the Lucia luciferase gene under the control of an ISG54 minimal promoter in conjunction with five IFN-stimulated response elements. Thus, these cells allow the simultaneous study of the NF-κB pathway, by assessing the activity of SEAP, and the interferon regulatory factor (IRF) pathway, by monitoring the activity of Lucia luciferase. SEAP and Lucia luicferase are readily measurable in the cell culture supernatant when using QUANTI-Blue™ and QUANTI-Luc™, respectively.

- A549-Dual™
- HCT116-Dual™
- J774-Dual™
- Jurkat-Dual™
- THP1-Dual™

A549-Dual™ Cells - Human lung carcinoma (NF-κB-SEAP / IRF-Lucia)

Description

A549-Dual™ cells are adherent epithelial cells that have been derived from the human A549 lung carcinoma cell line by stable integration of two inducible reporter constructs. The A549 cell line is a well-characterized cellular model for asthma, allergies and respiratory infections.

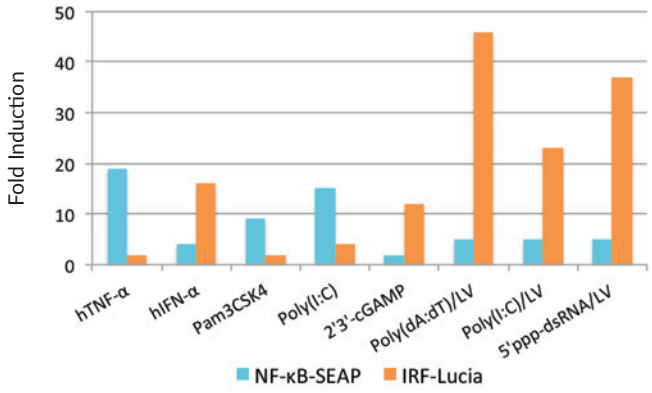
A549-Dual™ cells express an NF-κB-inducible SEAP gene and an IRF-inducible Lucia luciferase gene. SEAP is produced upon stimulation by poly(I:C) or TNF-α, whereas Lucia luciferase is produced in response to cytosolic nucleic acids, such as transfected poly(dA:dT) or poly(I:C).

A549-Dual™ cells are resistant to blasticidin and Zeocin™. They are grown in DMEM medium, 2 mM L-glutamine, 20% FBS with 10 µg/ml blasticidin and 100 µg/ml Zeocin™.

Contents

A549-Dual™ cells are provided in vials containing 3-7 x 10^6 cells, and they come supplied with 1 mg blasticidin, 10 mg Zeocin™, 50 mg Normocin™, 1 pouch of of QUANTI-Blue™ and 1 pouch of QUANTI-Luc™.

PRODUCT	QUANTITY	CAT. CODE
A549-Dual™ Cells	3-7 x 10^6 cells	a549d-nfis



NF-κB and IRF responses of A549-Dual™ cells to cytokines and PRR agonists. Cells were incubated with 1 ng/ml TNF-α, 10^3 IU/ml IFN-α, 300 ng/ml Pam3CSK4 (TLR2), 3 µg/ml poly(I:C) (TLR3), 10 µg/ml 2'3'-cGAMP (STING), 100 ng/ml poly(dA:dT)/LyoVec™ (CDS), 100 ng/ml poly(I:C)/LyoVec™ (RLR) or 1 µg/ml 5'ppp-dsRNA/LyoVec™ (RIG-I). After 24h incubation, NF-kB and IRF activation was assessed by measuring the levels of SEAP and Lucia luciferase in the supernatant using QUANTI-Blue™ or QUANTI-Luc™, respectively.

RELATED PRODUCTS

PRODUCT	INFO
Recombinant human cytokines	See pages 136-137
PRR ligands	See pages 76-83

RECENT CITATIONS

Ezzat SM. et al., 2016. The Cardenolide Glycoside Acovenoside A Affords Protective Activity in Doxorubicin-Induced Cardiotoxicity in Mice. J Pharmacol Exp Ther. 358(2):262-70.

HCT116-Dual™ Cells - Human colorectal carcinoma (NF-κB-SEAP / IRF-Lucia)

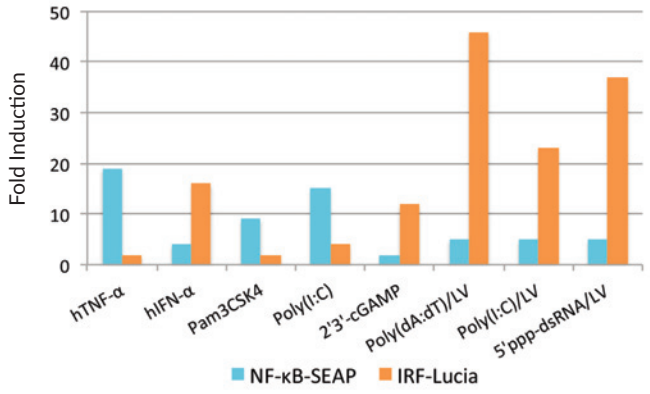
Description

HCT116-Dual™ cells are adherent epithelial cells that have been derived from the human HCT116 colorectal carcinoma cell line by stable integration of two inducible reporter constructs.
HCT116-Dual™ cells express an NF-κB-inducible SEAP gene and an IRF-inducible Lucia luciferase gene. They produce SEAP in response to poly(I:C) and IL-1β, and Lucia luciferase upon stimulation with poly(I:C) (transfected and non-transfected), poly(dA:dT) (transfected) and type IFNs.
HCT116-Dual™ cells are resistant to blasticidin and Zeocin™. They are grown in DMEM medium, 2 mM L-glutamine, 20% FBS with 10 µg/ml blasticidin and 100 µg/ml Zeocin™.

Contents

HCT116-Dual™ cells are provided in vials containing 3-7 x 10^6 cells, and they come supplied with 1 mg blasticidin, 10 mg Zeocin™, 50 mg Normocin™, 1 pouch of of QUANTI-Blue™ and 1 pouch of QUANTI-Luc™.

PRODUCT	QUANTITY	CAT. CODE
HCT116-Dual™ Cells	3-7 x 10^6 cells	hctd-nfis



NF-κB and IRF responses of HCT116-Dual™ cells to cytokines and PRR agonists. Cells were incubated with 1 ng/ml IL-1β, 10^3 IU/ml IFN-α, 10 µg/ml Pam3CSK4 (TLR2), 100 ng/ml poly(I:C) (TLR3), 10 µg/ml 2'3'-cGAMP (STING), 100 ng/ml poly(dA:dT)/LyoVec™ (CDS), 100 ng/ml poly(I:C)/LyoVec™ (RLR) or 1 µg/ml 5'ppp-dsRNA/LyoVec™ (RIG-I). After 24h incubation, NF-κB and IRF activation was assessed by measuring the levels of SEAP and Lucia luciferase in the supernatant using QUANTI-Blue™ or QUANTI-Luc™, respectively.

J774-Dual™ Cells - Mouse macrophage (NF-κB-SEAP / IRF-Lucia)

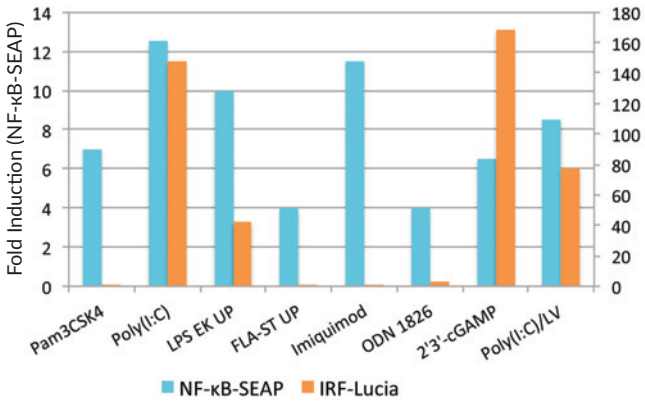
Description

J774-Dual™ cells have been derived from the mouse J774.1 macrophage-like cell line by stable integration of two inducible reporter constructs. J774.1 cell line is widely used in host-pathogen interaction study.
J774-Dual™ cells express an NF-κB-inducible SEAP gene and an IRF-inducible Lucia luciferase gene. They are responsive to a large variety of PRR ligands. Some of these ligands, such as Pam3CSK4 and imiquimod, only activate the NF-κB pathway leading to the production of SEAP, while others, such as poly(I:C) and 2'3'-cGAMP, activate the NF-κB and IFN pathways inducing the production of both reporters.
J774-Dual™ cells are resistant to blasticidin and Zeocin™. They are grown in DMEM medium, 2 mM L-glutamine, 20% FBS with 5 µg/ml blasticidin and 100 µg/ml Zeocin™.

Contents

J774-Dual™ cells are provided in vials containing 3-7 x 10^6 cells, and they come supplied with 1 mg blasticidin, 10 mg Zeocin™, 50 mg Normocin™, 1 pouch of of QUANTI-Blue™ and 1 pouch of QUANTI-Luc™.

PRODUCT	QUANTITY	CAT. CODE
J774-Dual™ Cells	3-7 x 10^6 cells	j774d-nfis



NF-κB and IRF responses of J774-Dual™ cells to PRR agonists. Cells were incubated with 10 ng/ml Pam3CSK4 (TLR2), 100 ng/ml poly(I:C) (TLR3), 100 ng/ml LPS-EK UP (TLR4), 1 µg/ml FLA-ST UP (TLR6), 1 µg/ml Imiquimod, 1 µg/ml ODN 1826, 3 µg/ml 2'3'-cGAMP (STING) and 100 ng/ml poly(I:C)/LyoVec™ (RIG-I/MDA-5). After 24h incubation, NF-κB and IRF activation was assessed by measuring the levels of SEAP and Lucia luciferase in the supernatant using QUANTI-Blue™ or QUANTI-Luc™, respectively.

Jurkat-Dual™ Cells - Human T lymphocytes (NF-kB-Lucia / IRF-SEAP)

Description

Jurkat-Dual™ cells were derived from the human T lymphocyte-based Jurkat cell line by stable integration of two inducible reporter constructs. Jurkat cells are commonly used for the study of T cell signaling.

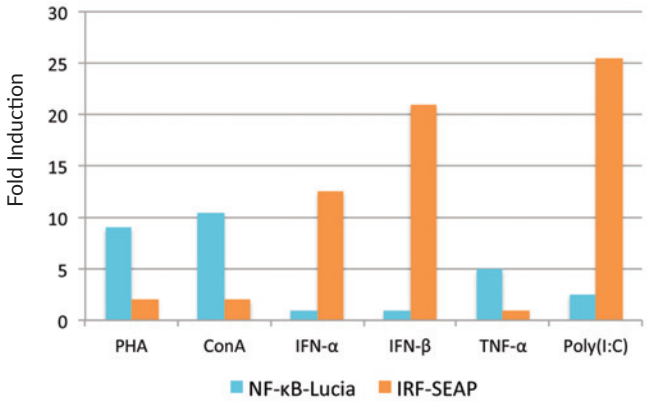
Jurkat-Dual™ cells express an NF-κB-inducible Lucia luciferase gene and an IRF-inducible SEAP gene. They produce Lucia luciferase in response to the mitogens, phytohemagglutinin (PHA) and concanavalin A (ConA), and SEAP upon stimulation with type I IFNs or poly(I:C).

Jurkat-Dual™ cells are resistant to blasticidin and Zeocin™. They are grown in RPMI medium, 2 mM L-glutamine, 20% FBS with 10 µg/ml blasticidin and 100 µg/ml Zeocin™.

Contents

Jurkat-Dual™ cells are provided in vials containing 3-7 x 10^6 cells, and they come supplied with 1 mg blasticidin, 10 mg Zeocin™, 50 mg Normocin™, 1 pouch of of QUANTI-Blue™ and 1 pouch of QUANTI-Luc™.

PRODUCT	QUANTITY	CAT. CODE
RAW-Blue™ Cells	3-7 x 10^6 cells	raw-sp



NF-κB and IRF responses of Jurkat-Dual™ cells to various stimuli. Cells were incubated with 20 µg/ml of the nonspecific mitogens phytohaemagglutinin (PHA) or concanavalin A (ConA), 300 IU/ml IFN-α, 300 IU/ml IFN-α, 100 ng/ml TNF-α or 10 µg/ml poly(I:C). After 24h incubation, NF-κB and IRF activation was assessed by measuring the levels of SEAP and Lucia luciferase in the supernatant using QUANTI-Luc™ or QUANTI-Blue™, respectively.

THP1-Dual™ Cells - Human monocyte (NF-κB-SEAP / IRF-Lucia)

Description

THP1-Dual™ cells were derived from the human THP-1 monocyte cell line by stable integration of two inducible reporter constructs. As a result, THP1-Dual™ cells allow the simultaneous study of the NF-κB pathway, by monitoring the activity of SEAP, and the IRF pathway, by assessing the activity of Lucia luciferase.

Both reporter proteins are readily measurable in the cell culture supernatant when using QUANTI-Blue™, a SEAP detection reagent, and QUANTI-Luc™, a luciferase detection reagent.

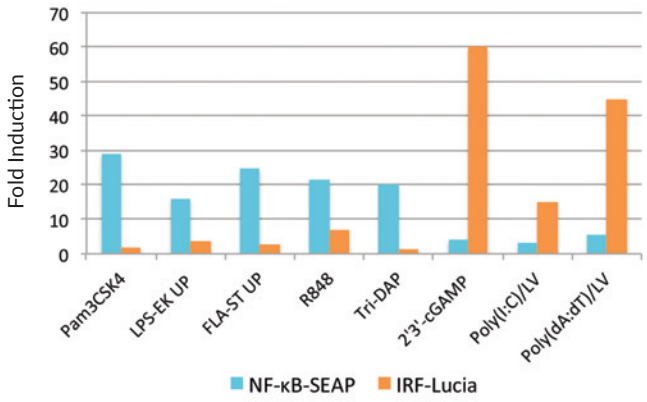
THP1-Dual™ cells induce the activation of NF-κB in response to certain TLR agonists, such as Pam3CSK4 and flagellin. They trigger the IRF pathway upon stimulation with type I IFNs and RLR, CDS or STING agonists, such as transfected poly(I:C) or poly(dA:dT) and 2'3'-cGAMP respectively.

THP1-Dual™ cells are resistant to to blasticidin and Zeocin™. They are grown in RPMI medium, 2mM L-glutamine, 10% FBS with 10 µg/ml blasticidin and 100 µg/ml Zeocin™.

Contents

THP1-Dual™ cells are provided in vials containing 3-7 x 10^6 cells, and they come supplied with 1 mg blasticidin, 10 mg Zeocin™, 50 mg Normocin™, 1 pouch of of QUANTI-Blue™ and 1 pouch of QUANTI-Luc™.

PRODUCT	QUANTITY	CAT. CODE
THP1-Dual™ Cells	3-7 x 10^6 cells	thpd-nfis



NF-κB and IRF responses of THP1-Dual™ cells to PRR agonists. Cells were incubated with 1 ng/ml Pam3CSK4 (TLR2), 100 ng/ml LPS-EK UP (TLR4), 100 ng/ml FLA-ST UP (TLR5), 10 µg/ml R848 (TLR7/8), 10 µg/ml Tri-DAP (NOD1), 3 µg/ml 2'3'-cGAMP (STING), 1 µg/ml poly(I:C)/LyoVec™ (RLR) or 100 ng/ml poly(dA:dT)/LyoVec™ (CDS). After 24h incubation, NF-κB and IRF activation was assessed by measuring the levels of SEAP and Lucia luciferase in the supernatant using QUANTI-Luc™ or QUANTI-Blue™, respectively.

RELATED PRODUCTS

PRODUCT	INFO
Recombinant human cytokines	See pages 136-137
PRR ligands	See pages 76-83

RECENT CITATIONS

Dey B. *et al.*, 2015. A bacterial cyclic dinucleotide activates the cytosolic surveillance pathway and mediates innate resistance to tuberculosis. Nat Med. 21(4):401-6.

Yang H. *et al.*, 2015. Amino Acid-Dependent Attenuation of Toll-like Receptor Signaling by Peptide-Gold Nanoparticle Hybrids. ACS Nano. 9(7):6774-84.

KO CELL LINES

InvivoGen provides a series of knockout (KO) cell lines generated by gene editing in popular cell lines, such as RAW 264.7 mouse macrophage and THP-1 human monocyte. The genes targeted for KO are involved in the interferon (IFN) response to nucleic acids (e.g. double-stranded DNA or RNA) and to cyclic dinucleotides (CDNs). These genes include those encoding for the cytosolic DNA sensor cGAS, the cytosolic RNA sensor RIG-I, the cytosolic CDN sensor (and cGAS adaptor) STING, and the IFN signaling mediator TBK1. Coupled with their parental cell lines, InvivoGen's KO cell lines are valuable tools for studying the function of these genes and for screening for molecules that activate pathways involving the proteins encoded by these genes.

The KO status of each cell line is thoroughly tested as follows:

- *Biallelic mutation/deletion confirmed by PCR and DNA sequencing;*
- *Reporter activity validated by functional assays;*
- *KO stability guaranteed up to 20 passages.*

- **B16-Blue™ ISG-KO-STING Cells**
- **HEK-Blue™ ISG-KO-STING Cells**
- **RAW-Lucia™ ISG-KO Cells:** KO-cGAS, KO-IRF3, KO-MDA5, KO-RIG-I, KO-STING, KO-TRIF
- **THP1-Dual™ KO Cells:** KO-cGAS, KO-IFI16, KO-MyD, KO-STING, KO-TREX1

B16-Blue™ ISG-KO-STING Cells - STING-KO mouse melanoma cells

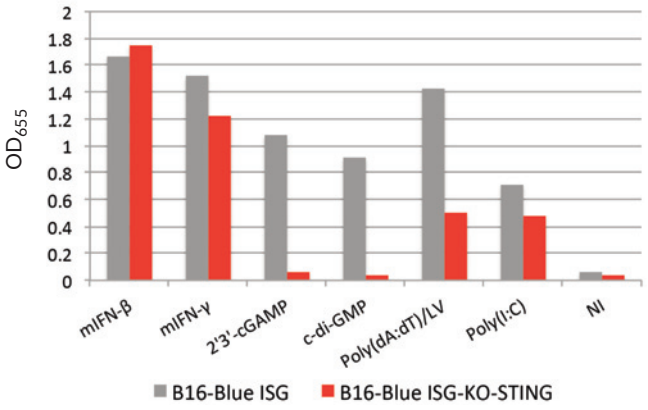
Description

B16-Blue™ ISG-KO-STING cells were generated from the B16-Blue™ ISG cell line through stable knockout of the STING gene. STING (stimulator of interferon genes, also known as MPYS, MITA or ERIS) works as a direct sensor of cyclic dinucleotides CDNs, such as cGAMP and c-di-GMP, and an adaptor in type I IFN signaling. Unlike their parental cells, these STING-KO cells do not respond to cytosolic DNA or CDNs; however, like their parental cells, they respond to type I and type II IFNs.
B16-Blue™ ISG-KO-STING cells are resistant to Zeocin™. They are grown in RPMI medium, 2 mM L-glutamine, 10% FBS with 100 µg/ml Zeocin™.

Contents

B16-Blue™ ISG-KO-STING cells are provided in vials containing 3-7 x 10^6 cells, and they come supplied with 10 mg Zeocin™, 50 mg Normocin™ and 1 pouch of of QUANTI-Blue™.

PRODUCT	QUANTITY	CAT. CODE
B16-Blue™ ISG-KO-STING Cells	3-7 x 10^6 cells	bb-kostg



Responses of B16-Blue™ ISG and B16-Blue™ ISG-KO-STING cells to interferons and PRR agonists. Cells were incubated with 100 IU/ml mIFN-β, 1 ng/ml mIFN-γ, 30 µg/ml 2'3'-cGAMP, 30 µg/ml c-di-GMP, 1 µg/ml poly(dA:dT)/LyoVec™ or 10 µg/ml poly(I:C). After 24h incubation, activation was assessed by measuring the levels of SEAP in the supernatant using QUANTI-Blue™.

RELATED PRODUCTS

PRODUCT	INFO
B16-Blue™ ISG cells	See page 58

HEK-Blue™ ISG-KO-STING Cells - STING-KO HEK293 derived cells

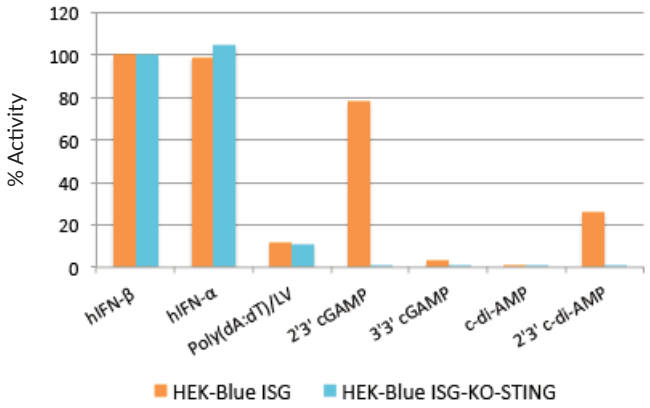
Description

HEK-Blue™ ISG-KO-STING cells were generated from the HEK-Blue™ ISG cell line through the stable knockout of the STING gene. STING (stimulator of interferon genes, also known as MPYS, MITA or ERIS) works as a direct sensor of cyclic dinucleotides CDNs, such as cGAMP and c-di-GMP, and an adaptor in type I IFN signaling. Unlike their parental cell line, HEK-Blue™ ISG-KO-STING cells do not respond to cytosolic DNA and CDNs but still respond to type I and type II IFNs.
HEK-Blue™ ISG-KO-STING cells are resistant to Zeocin™. They are grown in DMEM medium, 2mM L-glutamine, 10% FBS with 100 µg/ml Zeocin™.

Contents

HEK-Blue™ ISG-KO-STING cells are provided in vials containing 3-7 x 10^6 cells, and they come supplied with 10 mg Zeocin™, 50 mg Normocin™ and 1 pouch of QUANTI-Blue™.

PRODUCT	QUANTITY	CAT. CODE
HEK-Blue™ ISG-KO-STING Cells	3-7 x 10^6 cells	hkb-kostg



Responses of HEK-Blue™ ISG and HEK-Blue™ ISG-KO-STING cells to CDS/STING agonists. Cells were stimulated with 1 µg/ml poly(dA:dT)/LyoVec™, 1 µg/ml 2'3'-cGAMP, 10 µg/ml 3'3'-cGAMP, 10 µg/ml c-di-AMP or 10 µg/ml 2'3'-c-di-AMP. Human IFN-α ($1x10^3$ U/ml) and IFN-β ($1x10^3$ U/ml) serve as positive controls. After 24h incubation, IRF activation was determined using QUANTI-Blue™ and by reading the optical density (OD) at 655 nm. The IRF induction of each ligand is expressed relative to that of hIFN-β at $1x10^3$ U/ml (taken as 100%).

RELATED PRODUCTS

PRODUCT	INFO
HEK-Blue™ ISG cells	See page 59

Ramos-Blue™ KD-MyD Cells - MyD88-KD human B lymphocytes

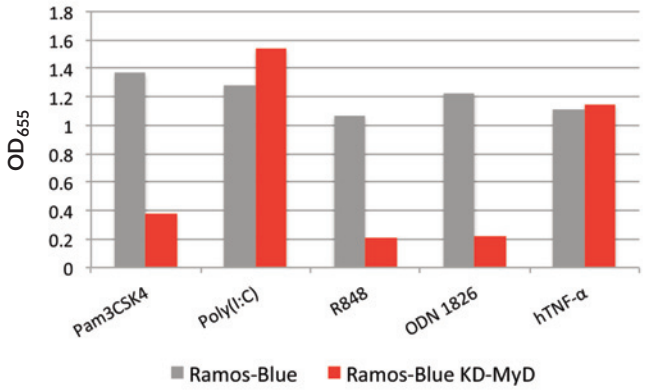
Description

Ramos-Blue™ KD-MyD cells were generated from the human B lymphocyte Ramos-Blue™ cell line through the stable knockdown of the MyD88 gene. MyD88 (myeloid differentiation primary response 88) is a key downstream adapter for most Toll-like receptors (TLRs) and interleukin-1 receptors (IL1Rs). Ramos-Blue™ KD-MyD cells have lost their ability to respond to TLR2, TLR7/8 and TLR9 ligands, but still respond to MyD88-independent stimuli such as TLR3 ligands and TNF-α.
Ramos-Blue™ KD-MyD cells are resistant to Hygromycin B and Zeocin™. They are grown in IMDM medium, 2 mM L-glutamine, 10% FBS with 200 µg/ml Hygromycin B Gold (ultrapure hygromycin) and 100 µg/ml Zeocin™.

Contents

Ramos-Blue™ KD-MyD cells are provided in vials containing 3-7 x 10^6 cells, and they come supplied with 10 mg Hygromycin B Gold, 10 mg Zeocin™, 50 mg Normocin™ and 1 pouch of QUANTI-Blue™.

PRODUCT	QUANTITY	CAT. CODE
Ramos-Blue™ KD-MyD Cells	3-7 x 10^6 cells	rms-kdmyd



Response of Ramos-Blue™ and Ramos-Blue™ KD-MyD cells to PRR agonists. Cells were incubated with 10 µg/ml Pam3CSK4 (TLR2), 10 ng/ml poly(I:C) (TLR3), 1 µg/ml R848 (TLR7/8), 10 µg/ml ODN2006 (TLR9) or 10 ng/ml TNF-α. After 24h incubation, NF-κB/AP-1 activation was assessed by measuring the levels of SEAP in the supernatant using QUANTI-Blue™.

RELATED PRODUCTS

PRODUCT	INFO
Ramos-Blue™ cells	See page 62

RAW-Lucia™ ISG-KO Cells - KO mouse macrophages

Description

RAW-Lucia™ ISG-KO cells is a family of knockout cells generated from the RAW-Lucia™ ISG cell line (see page 59). The genes targeted for knockout are involved in the response to nucleic acids, such as double stranded DNA or RNA, and cyclic dinucleotides (CDNs).
RAW-Lucia™ ISG-KO cells express a secreted reporter gene, Lucia luciferase, under the control of the I-ISG54 promoter which is comprised of the IFN-inducible ISG54 promoter enhanced by a multimeric ISRE. The levels of Lucia luciferase, which can be readily monitored using QUANTI-Luc™, are directly proportional to the amount of type I IFNs produced.
RAW-Lucia™ ISG-KO cells are resistant to Zeocin™. They are grown in DMEM medium, 2mM L-glutamine, 10% FBS with 200 µg/ml Zeocin™.

RAW-Lucia™ ISG-KO-cGAS cells were generated by stable knockout of the cGAS gene. Their response to cytosolic DNA, such as transfected poly(dA:dT), is significantly reduced compared to the response of their parental cell line (see figure).

RAW-Lucia™ ISG-KO-IFI16 cells were generated by stable knockout of the IFI16 gene. No significant difference was observed between the response of these cells and their parental cell line to stimulation with nucleic acids, such as transfected poly(dA:dT) or poly(I:C), or 2'3'-cGAMP.

RAW-Lucia™ ISG-KO-IRF3 cells were generated by stable knockout of the IRF3 gene. They have lost their ability to respond to cytosolic nucleic acids, such as transfected poly(dA:dT) or poly(I:C), or 2'3'-cGAMP, while retaining the ability to respond to type I IFNs.

RAW-Lucia™ ISG-KO-IRF7 cells were generated by stable knockout of the IRF7 gene. No significant difference was observed between the response of these cells and their parental cell line to stimulation with nucleic acids, such as transfected poly(dA:dT) or poly(I:C), or 2'3'-cGAMP.

RAW-Lucia™ ISG-KO-MAVS cells were generated by stable knockout of the MAVS gene. Their response to RLR ligands, such as transfected poly(I:C) or 5'ppp-dsRNA, is significantly reduced compared to the response of their parental cell line.

RAW-Lucia™ ISG-KO-MDA5 cells were generated by stable knockout of the MDA-5 gene. No significant difference was observed between the response of these cells and their parental cell line to stimulation with the RLR ligands, transfected poly(I:C) or 5'ppp-dsRNA.

RAW-Lucia™ ISG-KO-RIG-I cells were generated by stable knockout of the RIG-I gene. Their response to RLR ligands, such as transfected poly(I:C) or 5'ppp-dsRNA, is significantly reduced compared to the response of their parental cell line (see figure).

RAW-Lucia™ ISG-KO-STING cells were generated by stable knockout of the STING gene. They are unable to respond to CDNs, such as c-di-GMP and 2'3'-cGAMP, but respond to type I IFNs similarly to their parental cell line (see figure).

RAW-Lucia™ ISG-KO-TBK1 cells were generated by stable knockout of the TBK-1 gene. As expected, they do not to respond to cytosolic nucleic acids, such as transfected poly(dA:dT) or poly(I:C), or 2'3'-cGAMP. Their response to type I IFNs is unaffected by the TBK1-KO.

RAW-Lucia™ ISG-KO-TREX1 cells were generated by stable knockout of the TREX1 gene. No significant differencein response was observed between these cells and their parental cell line to stimulation with nucleic acids, such as transfected poly(dA:dT) or poly(I:C), or 2'3'-cGAMP.

RAW-Lucia™ ISG-KO-TRIF cells were generated by stable knockout of the TRIF gene. Their response to the TLR3 ligand poly(I:C) and the TLR4 ligand LPS is significantly reduced when compared to their parental cell line, while their response to type I IFNs is unaffected by the TRIF-KO.

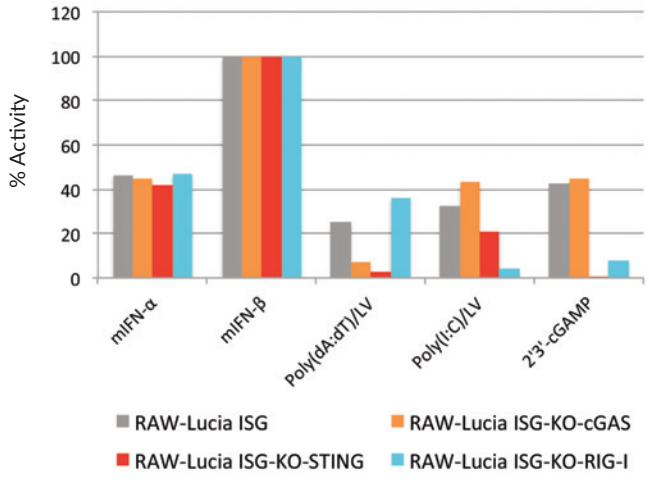
Contents

RAW-Lucia™ ISG-KO cells are provided in vials containing 3-7 x 10^6 cells, and they come supplied with 10 mg Zeocin™, 50 mg Normocin™ and 1 pouch of of QUANTI-Luc™.

PRODUCT	QUANTITY	CAT. CODE
RAW-Lucia™ ISG-KO-cGAS Cells	3-7 x 10^6 cells	rawl-kocgas
RAW-Lucia™ ISG-KO-IFI16 Cells	3-7 x 10^6 cells	rawl-koif16
RAW-Lucia™ ISG-KO-IRF3 Cells	3-7 x 10^6 cells	rawl-koirf3
RAW-Lucia™ ISG-KO-IRF7 Cells	3-7 x 10^6 cells	rawl-koirf7
RAW-Lucia™ ISG-KO-MAVS Cells	3-7 x 10^6 cells	rawl-komavs
RAW-Lucia™ ISG-KO-MDA5 Cells	3-7 x 10^6 cells	rawl-komda5
RAW-Lucia™ ISG-KO-RIG-I Cells	3-7 x 10^6 cells	rawl-korigi
RAW-Lucia™ ISG-KO-STING Cells	3-7 x 10^6 cells	rawl-kostg
RAW-Lucia™ ISG-KO-TBK1 Cells	3-7 x 10^6 cells	rawl-kotbk
RAW-Lucia™ ISG-KO-TREX1 Cells	3-7 x 10^6 cells	rawl-kotrex
RAW-Lucia™ ISG-KO-TRIF Cells	3-7 x 10^6 cells	rawl-kotrif

RELATED PRODUCTS

PRODUCT	INFO
RAW-Lucia™ ISG cells	See page 59
CDS ligands	See page 82
RLR ligands	See page 81
STING ligands	See pages 82-83



Responses of RAW-Lucia™ ISG, RAW-Lucia™ ISG-KO-cGAS, RAW-Lucia™ ISG-KO-STING and RAW-Lucia™ ISG-KO-RIG-I cells to nucleic acids. Cells were stimulated with 1 µg/ml poly(dA:dT)/LyoVec™ (CDS), 1 µg/ml poly(I:C)/LyoVec™ (RLR), or 3 µg/ml 2'3'-cGAMP (STING). Mouse IFN-α (1x10^4 U/ml) and IFN-β (1x10^4 U/ml) serve as positive controls. After 24h incubation, IRF activation was determined using QUANTI-Blue™ and by reading the optical density (OD) at 655 nm. The IRF induction of each ligand is expressed relative to that of mIFN-β at 1x10^3 U/ml (taken as 100%).

THP1-Dual™ KO Cells - KO human monocytes

Description

THP1-Dual™ KO cells are a collection of knockout cells generated from the THP1-Dual™ ISG cell line (see page 66). They derive from human THP-1 monocytes, a cell line often used to study DNA sensing pathways, as they express all the cytosolic DNA sensors identified so far (with the exception of DAI). The genes targeted for knockout are involved in the response to pathogen-associated molecular patterns (PAMPs), and in particular to nucleic acids, such as double-stranded DNA or RNA, and cyclic dinucleotides (CDNs).

THP1-Dual™ KO cells express stably express two inducible secreted reporter genes: Lucia luciferase and SEAP (secreted embryonic alkaline phosphatase). The Lucia luciferase reporter gene is under the control of an ISG54 (interferon-stimulated gene) minimal promoter in conjunction with five IFN-stimulated response elements. The SEAP gene is driven by an IFN-β minimal promoter fused to five copies of the NF-κB response element. As a result, they allow the simultaneous study of the IFN regulatory factor (IRF) pathway, by assessing the activity of Lucia luciferase and the NF-κB pathway, by monitoring the activity of SEAP. Both reporter proteins are readily measurable in the cell culture supernatant when using QUANTI-Luc™, a Lucia luciferase detection reagent and QUANTI-Blue™, a SEAP detection reagent.

THP1-Dual™ KO cells are resistant to blasticidin and Zeocin™. They are grown in RPMI medium, 2mM L-glutamine, 10% FBS with 10 µg/ml blasticidin and 100 µg/ml Zeocin™.

THP1-Dual™ KO-cGAS cells were generated by stable knockout of the cGAS gene. Their response to cytosolic DNA, such as transfected VACV-70, is significantly reduced compared to the response of their parental cell line.

THP1-Dual™ KO-IFI16 cells were generated by stable knockout of the IFI16 gene. No significant difference was observed between the response of these cells and their parental cell line to stimulation with nucleic acids, such as transfected poly(dA:dT) or poly(I:C), or 2'3'-cGAMP.

THP1-Dual™ KO-MyD cells were generated by stable knockout of the MYD88 gene. They have lost their ability to respond to PRR ligands and cytokines that signal through MyD88, such as TLR2 and TLR4 ligands and IL-1 cytokines (see figure 1).

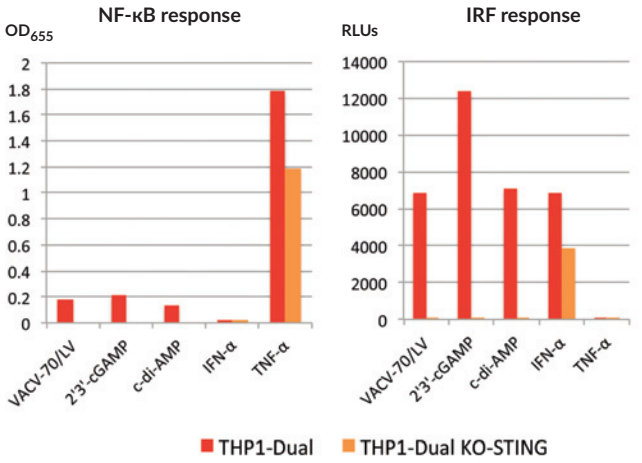
THP1-Dual™ KO-STING cells were generated by stable knockout of the STING gene. They are unable to respond to cytosolic DNA, such as transfected VACV-70, and CDNs, such as c-di-GMP and 2'3'-cGAMP, but respond to type I IFNs similarly to their parental cell line (see figure 2).

THP1-Dual™ KO-TREX1 cells were generated by stable knockout of the TREX1 gene. No significant difference in response was observed between these cells and their parental cell line to stimulation with nucleic acids, such as transfected poly(dA:dT) or poly(I:C), or 2'3'-cGAMP.

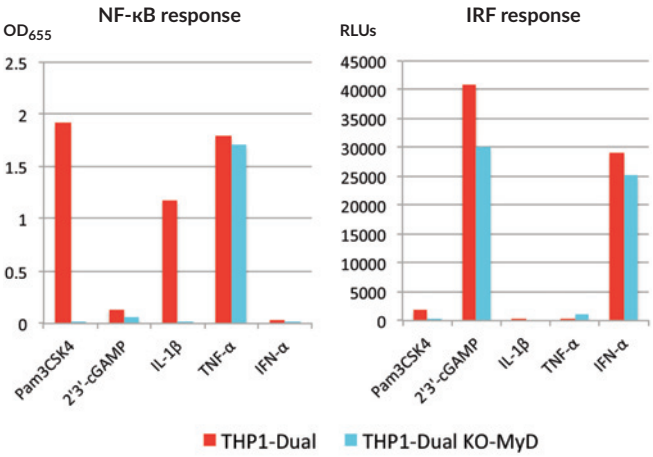
Contents

THP1-Dual™ KO cells are provided in vials containing 3-7 x 10^6 cells, and they come supplied with 1 mg blasticidin, 10 mg Zeocin™, 50 mg Normocin™, 1 pouch of QUANTI-Blue™ and 1 pouch of of QUANTI-Luc™.

PRODUCT	QUANTITY	CAT. CODE
THP1-Dual™ KO-cGAS Cells	3-7 x 10^6 cells	thpd-kocgas
THP1-Dual™ KO-IFI16 Cells	3-7 x 10^6 cells	thpd-koif16
THP1-Dual™ KO-MyD Cells	3-7 x 10^6 cells	thpd-komyd
THP1-Dual™ KO-STING Cells	3-7 x 10^6 cells	thpd-kostg
THP1-Dual™ KO-TREX1 Cells	3-7 x 10^6 cells	thpd-kotrex



1- NF-κB and IRF responses of THP1-Dual™ and THP1-Dual™ KO-STING cells to various PAMPs and cytokines. Cells were stimulated with 1 µg/ml VACV70/LyoVec™ (CDS), 3 µg/ml 2'3'-cGAMP (STING), 10 µg/ml c-di-AMP (STING), 1x10^3 U/ml human IFN-α or 1 ng/ml TNF-α. After 24h incubation, NF-κB and IRF activations were determined using QUANTI-Blue™ and by reading the optical density (OD) at 655 nm, or QUANTI-Luc™ and measuring relative light units (RLUs), respectively.



2- NF-κB and IRF responses of THP1-Dual™ and THP1-Dual™ KO-MyD cells to various PAMPs and cytokines. Cells were stimulated with 1 ng/ml Pam3CSK4 (TLR2), 3 µg/ml 2'3'-cGAMP (STING), 1 ng/ml IL-1β, 1 ng/ml TNF-α or 1x10^3 U/ml human IFN-α. After 24h incubation, NF-κB and IRF activations were determined using QUANTI-Blue™ and by reading the optical density (OD) at 655 nm, or QUANTI-Luc™ and measuring relative light units (RLUs), respectively.

RELATED PRODUCTS

PRODUCT	INFO
THP1-Dual™ cells	See page 66
CDS ligands	See page 82
RLR ligands	See page 81
STING ligands	See page 82-83

NF-κB and IRF responses of InvivoGen's reporter cells to PRR activation

Cell Lines	TLR2	TLR3	TLR4	TLR5	TLR7/8	TLR9	NOD1	NOD2	RIG-I/MDA5	CD5	STING	Dectin-1	Mincle
A549-Dual™ cells	+	+	-	+	-	-	-	-	+	+	+	-	-
B16-Blue™ ISG cells	-	+	-	-	-	-	-	-	+	+	+	-	-
HCT116-Dual™ cells	+	+	-	+	-	-	+	+	+	+	+	-	-
HEK-Blue™ ISG cells	-	-	-	-	-	-	-	-	-	-	+	-	-
J774-Dual™ cells	+	+	+	+	+	+		+/-	+	-	+	+/-	+
Jurkat-Dual™ cells	-	+	-	-	-	-	-	-	-	-	-	-	-
Jurkat-Lucia™ NFAT cells	-	-	-	-	-	-	-	-	-	-	-	-	-
RAW-Blue™ cells	+	+	+	-	+	+	+/-	+	+/-	+/-	-	+	+
RAW-Lucia™ ISG cells		+/-	+	-			-	-	+/-	+/-	+	-	-
Ramos-Blue™ cells	+	+	-	-	+	+	+	-	+/-	-	-	-	-
THP1-Blue™ NF-κB cells	+	-	+	+/-	+	-	+/-	+/-	+/-	+/-	+	-	-
THP1-Blue™ ISG cells	+	-	+/-	+/-	+/-	-	-	-	+/-	+	+	-	-
THP1-Dual™ cells	+	-	+	+	+	-	+	+	+/-	+	+	-	-

NF-κB response

IRF response

4 PRR LIGANDS

PAMPs Collection	75-83
TLR ligands	88-95
NLR ligands	96-98
RLR ligands	99
CLR ligands	99-101
CDS & STING ligands	102-103
Multi-PRR ligands	104-105
TLR & NOD Agonist Kits	106-107
PRR Ligand Screening Service	108-109

PRR LIGANDS

The innate immune system is an evolutionarily conserved system acting as a first-line of defense against invading microbial pathogens and other potential threats to the host. A range of pattern recognition receptors (PRRs) recognize specific pathogen-associated molecular patterns (PAMPs) exclusively present on microbes such as bacteria, fungi and viruses. The main PRRs families of the innate immune system are the Toll-Like receptors (TLRs), the NOD-Like receptors (NLRs), the C-type lectin receptors (CLRs), the RIG-I-Like receptors (RLRs), and cytosolic DNA sensors (CDS). InvivoGen offers a comprehensive choice of high quality PAMPs known to activate these PRRs.

TLR Agonists

TLR2 Agonists

TLR2 (also known as CD282) is a membrane surface receptor involved in the recognition of a wide array of microbial molecules. These molecules represent broad groups of species, such as Gram-positive and Gram-negative bacteria, as well as mycoplasma and yeast. They include peptidoglycan, lipoteichoic acid and lipoprotein from Gram-positive bacteria, lipoarabinomannan from mycobacteria, and zymosan from yeast cell walls.

The variety of TLR2 ligands is the greatest among all the TLRs and this is due to the heterodimerization needed for TLR2-mediated responses. TLR2 forms a heterodimer with TLR6 to recognize diacylated lipoproteins, whereas the TLR1/TLR2 heterodimer binds triacylated lipoproteins. Moreover, the CD14 co-receptor enhances pathogen recognition by TLR2.

TLR2 signaling is MyD88 (myeloid differentiation primary-response protein 88)-dependent and requires the adaptor molecule TIRAP (TIR-associated protein) to induce the production of pro-inflammatory cytokines following AP-1 (activator protein 1) and NF-κB (nuclear factor-κB) nuclear translocation.

TLR3 Agonists

TLR3 (also known as CD283) is an endosomal receptor that recognizes double-stranded RNA (dsRNA), a molecular pattern associated with viral infection. Polyinosine-polycytidylic acid (poly(I:C)), a synthetic analog of dsRNA, is the ligand of choice for TLR3. TLR3 signals mainly through a MyD88-independent pathway involving the adapter protein TRIF (TIR domain-containing adaptor protein-inducing IFN-β). Phosphorylation of IRF3 (IFN regulatory factor 3) leads to the production of IFN-β (interferon beta) with the subsequent activation of IFN-stimulated response elements (ISRE). Additionally, TLR3 signaling leads to the nuclear translocation of AP-1 and NF-κB and to the production of pro-inflammatory cytokines.

TLR4 Agonists

TLR4 is the receptor for Gram-negative lipopolysaccharide (LPS) and lipid A, its toxic moiety. In order to respond to LPS, TLR4 interacts with three different extracellular proteins: LPS binding protein (LBP), CD14 and myeloid differentiation protein 2 (MD-2). TLR4 signaling comprises two pathways: the MyD88-dependent and MyD88-independent pathways. The MyD88-dependent pathway involves AP-1 and NF-κB nuclear translocation and leads to the production of inflammatory cytokines. The MyD88-independent pathway activates IRF3 and involves another adaptor molecule, TRAM (TRIF-related adaptor molecule), and leads to the production of IFN-β and the expression of IFN-inducible genes through the activation of IRF3.

TLR5 Agonists

The cell surface receptor TLR5 recognizes flagellin, the major component of the bacterial flagellar filament, from both Gram-positive and Gram-negative bacteria. Activation of TLR5 stimulates the production of pro-inflammatory cytokines through signaling via the adaptor protein MyD88. As a homodimer, TLR5 can generate a pro-inflammatory signal, suggesting that it might be the only TLR participating in flagellin recognition.

TLR7/8 Agonists

TLR7 and TLR8 are endosomal receptors involved in the response to viral infection. They recognize GU-rich short single-stranded RNA, as well as small synthetic molecules, such as imidazoquinolines and nucleoside analogues. TLR7/TLR8 signaling is MyD88-dependent and is mediated by the nuclear translocation of AP-1 and NF-κB, in addition to the phosphorylation of IRF7 (IFN regulatory factor 7). IRF7 promotes the induction of ISRE and the subsequent expression of type I IFN genes, whereas AP-1 and NF-κB activation results in the production of pro-inflammatory cytokines.

TLR9 Agonists

The endosomal receptor TLR9 (also known as CD289) recognizes specific unmethylated CpG-ODN sequences that distinguish microbial DNA from mammalian DNA. Three types of stimulatory ODNs have been described: Class A (Type D), Class B (Type K) and Class C. Similar to most other TLRs, TLR9 signaling is MyD88-dependent, involving the transciption factors AP-1, NF-κB and IRF7. TLR9-induced pro-inflammatory cytokine production is mediated by AP-1 and NF-κB, whereas TLR9-induced IFN-α/β production is mediated by IRF7. The cytokine and IFN response is highly dependent on the class of CpG-ODN used to stimulate TLR9.

TLR13 Agonists

TLR13 is an endosomal TLR expressed in mice. Although its role and ligands remain unclear, 23S ribosomal RNA (rRNA) has been identified as a TLR13 ligand. TLR13 induces cytokine production in a MyD88- and UNC93B-dependent manner through the activation of AP-1 and NF-κB. TLR13 signaling also results in IFN-β production through the activation of IRF7. Humans lack TLR13 and probably rely on other pathogen receptors to detect pathogenic bacterial infection.

NLR Agonists

NOD1/2 Agonists

NOD1 (also known as CARD4) and NOD2 (also known as CARD15) are intracellular PRRs that sense bacterial peptidoglycans (PGNs). NOD1 senses the D-γ-glutamyl-meso-DAP dipeptide (iE-DAP), which is found in PGNs of all Gram-negative and certain Gram-positive bacteria, whereas NOD2 recognizes the muramyl dipeptide (MDP) structure found in almost all bacteria. Both iE-DAP and MDP must be delivered intracellularly either by bacteria that invade the cell or through other cellular uptake mechanisms. Ligand-bound NOD1 and NOD2 oligomerize and signal via the serine/threonine RIP2 kinase. Once activated, RIP2 mediates ubiquitination of NEMO/IKKγ leading to the activation of NF-κB and the production of inflammatory cytokines.

Inflammasome Inducers

Inflammasomes are multimeric protein complexes that are generally comprised of a member of the NLR family, such as NLRP3 and NLRC4, the adaptor protein ASC (apoptosis-associated speck-like protein with a CARD) and pro-caspase-1. They assemble in the cytoplasm of innate immune cells in response to cytosolic PAMPs or DAMPs (danger-associated molecular patterns). They promote the secretion of the pro-inflammatory cytokines IL-1β and IL-18 and cause a rapid and pro-inflammatory form of cell death called pyroptosis. The most intensely

studied inflammasome is the NLRP3 (NLR family, pyrin domain containing 3) inflammasome. It is activated by a broad variety of stimuli, including danger signals (e.g. ATP), crystalline substances (e.g. MSU) and microbial toxins (e.g. nigericin). The NLRC4 (NLR containing a caspase activating and recruitment domain 4) /NAIP (neuronal apoptosis inhibitory protein) inflammasome is triggered by cytosolic flagellin from various bacteria, such as *Salmonella typhimurium* and *Pseudomonas aeruginosa*. The NLRP1 inflammasome induces caspase-1 in response to diverse stimuli, including Bacillus anthracis lethal toxin, *Toxoplasma gondii*, muramyl dipeptide, and host intracellular ATP depletion.

RLR Agonists

RIG-I/MDA-5 Agonists

RIG-I and MDA-5 are cytoplasmic RNA helicases that recognize intracellular double-stranded RNA (dsRNA), a molecular pattern associated with viral infection. Despite their overall structural similarity, they detect distinct viral species. RIG-I participates in the recognition of Paramyxoviruses (Newcastle disease virus (NDV), Sendai virus (SeV)), Rhabdoviruses (vesicular stomatitis virus (VSV)), Flaviviruses (hepatitis C (HCV)) and Orthomyxoviruses (Influenza), whereas MDA-5 is essential for the recognition of Picornaviruses (encephalo-myocarditis virus (EMCV)) and poly(I:C), a synthetic analog of viral dsRNA. Although RIG-I and MDA-5 recognize different ligands, they share common signaling features. Upon recognition of dsRNA, they are recruited by the adaptor IPS-1 (also known as MAVS, CARDIF or VISA) to the outer membrane of the mitochondria, leading to the activation of several transcription factors including IRF3, IRF7 and NF-κB. IRF3 and IRF7 control expression of type I IFNs, while NF-κB regulates the production of pro-inflammatory cytokines.

CDS & STING Agonists

Cytosolic DNA Sensor (CDS) Agonists

CDSs detect damaged, mislocalized or pathogenic DNA, typically inducing type I IFN production through the TBK1-IRF3 pathway. DNA is normally compartmentalized in the nucleus or mitochondria; however, if it is not processed or transported correctly, then it can accumulate in the cytosol. Alternatively, viral or microbial DNA can enter the cytosol of host cells upon infection. Several CDSs have been identified, including the adenosine deaminase DAI, the helicase DDX41, the IFN-inducible IFI16 protein, and cGAS (cyclic GMP-AMP synthase). AIM2 is another cytosolic DNA sensor, which upon activation forms an inflammasome that leads to the secretion of IL-1β.

STING Agonists

STING, initially thought to serve solely as an adaptor protein for mediating signaling by CDSs, was found to be a direct sensor of cyclic dinucleotides (CDNs). CDNs are important messengers in bacteria, affecting numerous responses of the prokaryotic cell, as well as in mammalian cells, acting as drivers of the innate immune response. CDNs and xanthenone derivatives, such as DMXAA, bind to and activate STING, leading to a potent type I IFN response in a TBK1-IRF3-dependent manner.

CLR Agonists

Dectin-1 Agonists

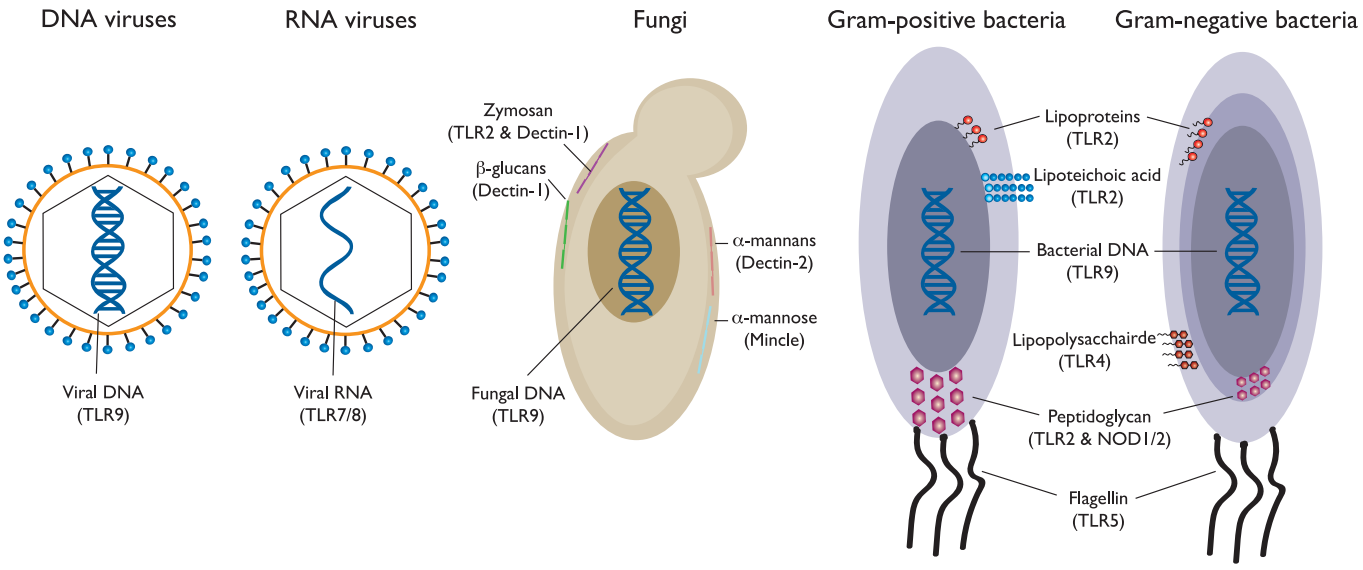
Dectin-1 is a member of the C-type lectin receptor (CLR) family and plays an important role in antifungal innate immunity. Dectin-1 is a specific transmembrane receptor of β-glucans, which are glucose polymers found in the cell walls of fungi, including the yeasts *Saccharomyces cerevisiae* and *Candida albicans*. Upon binding to its ligand, Dectin-1 triggers phagocytosis and signaling through the kinase Syk and the adaptors CARD9-Bcl10-Malt1, leading to the production of reactive oxygen species (ROS), the activation of NF-κB and the subsequent production of pro-inflammatory cytokines. Dectin-1 and TLR2 work in tandem to enhance their respective signaling responses.

Dectin-2 Agonists

Dectin-2 is a type II transmembrane CLR that binds high mannose-type carbohydrates and was shown to be the functional receptor for a-mannans. Upon binding to its ligand, Dectin-2 signals through the kinase Syk and the adaptors CARD9/Bcl-10/MALT1 triggering the activation of NF-κB and the subsequent production of pro-inflammatory cytokines. Dectin-2 is implicated in anti-bacterial immunity and allergy.

Mincle Agonists

Mincle is a multi-functional danger receptor that recognizes a wide variety of ligands such as damaged cells, fungus, yeast and mycobacteria. Exogenous ligands for Mincle include fungal a-mannose, and the mycobacterial glycolipid, trehalose-6'6'-dimycolate (TDM; also known as cord factor) the immunostimulatory component of *Mycobacterium tuberculosis*. Furthermore, Mincle senses damaged cells by recognizing endogenous DAMPs. One such DAMP identified is the spliceosome-associated protein 130 (SAP130), a soluble factor released by necrotic cells. Mincle triggers signaling through Syk leading to CARD9-dependent NF-κB activation. Syk induces also the mobilization of intracellular calcium (Ca^{2+}) and the activation of the calcineurin-NFAT pathway.



PAMPs COLLECTION

InvivoGen offers the most comprehensive choice of ligands known to activate specific PRRs, that can serve as controls in genetic and pharmaceutical studies on PRRs. InvivoGen strives to provide PRR ligands of the highest quality. We thoroughly validate our ligands to ensure high quality and lot-to-lot reproducibility.

- **Toll-Like Receptor (TLR) Ligands**

  Agonists for TLR2, TLR3, TLR4, TLR5, TLR7/8, TLR9 or TLR13, and antagonists for TLR4 or TLR9
- **NOD-Like Receptor (NLR) Ligands**

  Agonists for NOD1 and/or NOD2, and inducers for the NLRP3 inflammasome
- **RIG-I-Like Receptor (RLR) Ligands**

  Agonists for RIG-I and/or MDA-5, provided naked or precomplexed with a transfection reagent
- **Cytosolic DNA Sensor (CDS)/STING Ligands**

  Agonists for CDSs or STING, provided naked or precomplexed with a transfection reagent
- **C-type Lectin Receptor (CLR) Ligands**

  Agonists for Dectin-1, Dectin-2 and/or Mincle
- **Multi-PRR Ligands**

  Agonists for both TLR2 and TLR7 or TLR2 and NOD2

PRR Activity Validated

The activity of all PAMPs is tested using the appropriate reporter cell line chosen from InvivoGen's collection of reporter cells, such as HEK-Blue™ TLR, RAW-Blue™ or THP1-Blue™ ISG cells.

TLR2 & TLR4 Contaminant Activities Checked

The major contaminants of PRR ligands are lipoproteins that activate TLR2 and lipopolysaccharide (LPS, also known as endotoxin) that activate TLR4. The presence of these contaminants is determined by assessing the TLR2 and TLR4 activities of all InvivoGen's PAMPs using HEK-Blue™ TLR2 and HEK-Blue™ TLR4 cells, respectively. Endotoxin levels are also determined using a chromogenic LAL assay when possible. **PRR ligands containing undetectable levels of endotoxin are "EndoFit".**

Leading Supplier of PRR Ligands

InvivoGen's PRR ligands are the most cited in the literature. A selection of references of recent articles published in high-impact factor journals is listed on page 89.

PRODUCT	ORIGIN/DESCRIPTION	ENDOTOXIN LEVELS	WORKING CONCENTRATION	QTY	CATALOG CODE	INFO
TLR LIGANDS						
TLR2 Agonists						
CL419	Pam2C-spermine compound	EndoFit™	1 ng - 100 µg/ml	500 µg	tlrl-c419	p. 88
FSL-1	Synthetic diacylated lipoprotein - TLR2/6	EndoFit™	1 - 100 ng/ml	100 µg	tlrl-fsl	p. 88
HKEB	Heat Killed *Escherichia coli 0111:B4*	>1 EU/10^9 cells	10^5 - 10^7 cells/ml	10^{10} cells	tlrl-hkeb	p. 88
HKLM	Heat Killed *Listeria monocytogenes*	EndoFit™	10^7 - 10^8 cells/ml	10^{10} cells	tlrl-hklm	p. 88
HKLR	Heat Killed *Lactobacillus rhamnosus*	>1 EU/10^9 cells	10^8 - 10^9 cells/ml	10^{10} cells	tlrl-hklr	p. 88
HKMT	Heat Killed *Mycobacterium tuberculosis*	EndoFit™	100 ng - 10 µg/ml	10 mg 50 mg	tlrl-hkmt-1 tlrl-hkmt-5	p. 88
HKPA	Heat Killed *Pseudomonas aeruginosa*	>1 EU/10^8 cells	10^5 - 10^7 cells/ml	10^{10} cells	tlrl-hkpa	p. 88
HKPG	Heat Killed *Porphyromonas gingivalis*	EndoFit™	10^6 - 10^8 cells/ml	10^{10} cells	tlrl-hkpg	p. 88
HKSA	Heat Killed *Staphylococcus aureus*	>1 EU/10^9 cells	10^6 - 10^8 cells/ml	10^{10} cells	tlrl-hksa	p. 88
HKSE	Heat Killed *Staphylococcus epidermidis*	EndoFit™	10^7 - 10^9 cells/ml	10^{10} cells	tlrl-hkse	p. 88
HKSP	Heat Killed *Streptococcus pneumoniae*	EndoFit™	10^7 - 10^9 cells/ml	10^{10} cells	tlrl-hksp	p. 88
HKST	Heat Killed *Salmonella typhimurium*	>1 EU/10^8 cells	10^4 - 10^9 cells/ml	10^{10} cells	tlrl-hkst	p. 88
LAM-MS	Lipoarabinomannan from *M. smegmatis*	EndoFit™	100 ng - 10 µg/ml	500 µg	tlrl-lams	p. 88
LM-MS	Lipomannan from *Mycobacterium smegmatis*	<5 EU/mg	1 - 10 ng/ml	100 µg	tlrl-lmms2	p. 88
LPS-PG	Lipopolysaccharide from *P. gingivalis*	>10^4 EU/mg	10 ng - 10 µg/ml	1 mg	tlrl-pglps	p. 88
LTA-BS	Lipoteichoic acid from *Bacillus subtilis*	10 EU/mg	100 ng - 1 µg/ml	5 mg	tlrl-lta	p. 88
LTA-SA	Lipoteichoic acid from *S. aureus*	10 EU/mg	100 ng - 1 µg/ml	5 mg	tlrl-slta	p. 88
LTA-SA Purified	Purified lipoteichoic acid from *S. aureus*	EndoFit™	1 ng - 1 µg/ml	5 mg	tlrl-pslta	p. 88
Pam2CSK4	Synthetic diacylated lipoprotein - TLR2(6)	EndoFit™	1 - 100 ng/ml	1 mg	tlrl-pm2s-1	p. 88
Pam2CSK4 Biotin	Biotinylated Pam2CSK4	EndoFit™	1 - 100 ng/ml	50 µg	tlrl-bpam2	p. 88
Pam2CSK4 Rhodamine	Rhodamine-labeled Pam2CSK4	EndoFit™	1 - 100 ng/ml	50 µg	tlrl-rpam2	p. 88
Pam3CSK4	Synthetic triacylated lipoprotein - TLR1/2	EndoFit™	1 - 300 ng/ml	1 mg	tlrl-pms	p. 89
Pam3CSK4 Biotin	Biotinylated Pam3CSK4	EndoFit™	1 - 100 ng/ml	50 µg	tlrl-bpms	p. 89
Pam3CSK4 Rhodamine	Rhodamine-labeled Pam3CSK4	EndoFit™	1 - 300 ng/ml	50 µg	tlrl-rpms	p. 89
Pam3CSK4 VacciGrade™	Preclinical grade Pam3CSK4	EndoFit™	1 - 300 ng/ml	1 mg	vac-pms	p. 143
PamadiFectin™ (CL553)	TLR2/TLR7 agonist & nucleic acid carrier	EndoFit™	100 ng - 1 µg/ml	500 µg	tlrl-c553	p. 105
PGN-BS	Peptidoglycan from *B. subtilis*	EndoFit™	1 - 10 µg/ml	5 mg	tlrl-pgnbs	p. 89
PGN-EB	Peptidoglycan from *E. coli 0111:B4*	10^2 - 10^3 EU/mg	1 - 10 µg/ml	1 mg	tlrl-pgnec	p. 89
PGN-EK	Peptidoglycan from *E. coli K12*	10^2 - 10^3 EU/mg	1 - 10 µg/ml	1 mg	tlrl-pgnek	p. 89
PGN-SA	Peptidoglycan from *S. aureus*	1 EU/mg	1 - 10 µg/ml	5 mg	tlrl-pgnsa	p. 89
Zymosan	Cell wall preparation of *S. cerevisiae*	EndoFit™	10 µg/ml	100 mg	tlrl-zyn	p. 89
TLR3 Agonists						
Poly(A:U)	Polyadenylic–polyuridylic acid	<0.005 EU/µg	300 ng - 100 µg/ml	10 mg	tlrl-pau	p. 90
Poly(I:C) (HMW)	Polyinosine-polycytidylic acid High molecular weight (1.5-8 kb)	EndoFit™	10 ng - 10 µg/ml	10 mg 50 mg	tlrl-pic tlrl-pic-5	p. 90
Poly(I:C) (HMW) Biotin	Biotinylated poly(I:C) (HMW)	EndoFit™	10 ng - 10 µg/ml	10 µg	tlrl-picb	p. 90
Poly(I:C) (HMW) Fluorescein	Fluorescein-labeled poly(I:C) (HMW)	EndoFit™	10 ng - 10 µg/ml	10 µg	tlrl-picf	p. 90
Poly(I:C) (HMW) Rhodamine	Rhodamine-labeled poly(I:C) (HMW)	EndoFit™	10 ng - 10 µg/ml	10 µg	tlrl-picr	p. 90
Poly(I:C) (HMW) VacciGrade™	Preclinical grade poly(I:C) (HMW)	EndoFit™	10 ng - 10 µg/ml	10 mg	vac-pic	p. 143

PRODUCT	ORIGIN/DESCRIPTION	ENDOTOXIN LEVELS	WORKING CONCENTRATION	QTY	CATALOG CODE	INFO
TLR LIGANDS						
TLR3 Agonists						
Poly(I:C) (LMW)	Polyinosine-polycytidylic acid Low molecular weight (0.2-1 kb)	EndoFit™	30 ng - 10 µg/ml	25 mg 250 mg	tlrl-picw tlrl-picw-250	p. 90
Poly(I:C) (LMW) Rhodamine	Rhodamine-labeled poly(I:C) (LMW)	EndoFit™	10 ng - 10 µg/ml	10 µg	tlrl-piwr	p. 90
TLR4 Agonists						
LPS-B5	Standard lipopolysaccharide from *E. coli 055:B5*	>1 x 10^6 EU/mg	100 pg - 1 µg/ml	5 mg	tlrl-b5lps	p. 90
LPS-B5 Ultrapure	Ultrapure lipopolysaccharide from *E. coli 055:B5*	>1 x 10^6 EU/mg	100 pg - 1 µg/ml	5 mg	tlrl-pb5lps	p. 90
LPS-EB	Standard lipopolysaccharide from *E. coli 0111:B4*	>1 x 10^6 EU/mg	10 ng - 10 µg/ml	5 mg	tlrl-eblps	p. 90
LPS-EB Biotin	Biotinylated ultrapure LPS from *E. coli 0111:B4*	1 x 10^6 EU/mg	10 ng - 10 µg/ml	500 µg	tlrl-bblps	p. 90
LPS-EB Ultrapure	Ultrapure lipopolysaccharide from *E. coli 0111:B4*	>0.5 x 10^6 EU/mg	10 ng - 10 µg/ml	5x10^6 EU	tlrl-3pelps	p. 90
LPS-EB VacciGrade™	Preclinical grade ultrapure LPS from *E. coli 0111:B4*	>5 x 10^5 EU/mg	0.1 - 25 µg/mouse	5x10^6 EU	vac-3pelps	p. 143
LPS-EK	Standard lipopolysaccharide from *E. coli K12*	>1 x 10^5 EU/mg	1 ng - 10 µg/ml	5 mg	tlrl-eklps	p. 90
LPS-EK Ultrapure	Ultrapure lipopolysaccharide from *E. coli K12*	>5 x 10^5 EU/mg	1 ng - 10 µg/ml	1 mg	tlrl-peklps	p. 90
LPS-PG Ultrapure	Ultrapure lipopolysaccharide from *P. gingivalis*	>1 x 10^5 EU/mg	100 ng - 10 µg/ml	1 mg	tlrl-ppglps	p. 90
LPS-SM Ultrapure	Ultrapure lipopolysaccharide from *S. minnesota*	>1 x 10^5 EU/mg	10 ng - 10 µg/ml	5 mg	tlrl-smlps	p. 91
MPLA-SM	Monophosphoryl lipid A from *S. minnesota*	1 x 10^6 EU/mg	100 ng - 1 µg/ml	1 mg	tlrl-mpla	p. 91
MPLA-SM VacciGrade™	Preclinical grade detoxified MPLA	1 x 10^6 EU/mg	2 - 20 µg/mouse	1 mg	vac-mpla	p. 143
MPLAs	Synthetic monophosphoryl lipid A	1 x 10^6 EU/mg	10 ng - 10 µg/ml	1 mg	tlrl-mpls	p. 91
MPLAs VacciGrade™	Preclinical grade synthetic MPLA	1 x 10^6 EU/mg	2 - 20 µg/mouse	1 mg	vac-mpls	p. 143
TLR4 Antagonists						
LPS-RS	Lipopolysaccharide from *Rhodobacter sphaeroides*	>1 x 10^6 EU/mg	10 ng - 10 µg/ml	5 mg	tlrl-rslps	p. 91
LPS-RS Ultrapure	Ultrapure lipopolysaccharide from *R. sphaeroides*	>1 x 10^5 EU/mg	10 ng - 10 µg/ml	1 mg	tlrl-prslps	p. 91
TLR5 Agonists						
FLA-BS	Standard flagellin from *B. subtilis* - 10% pure	<0.1 EU/µg	10 ng - 10 µg/ml	100 µg	tlrl-bsfla	p. 91
FLA-BS Ultrapure	Ultrapure flagellin from *B. subtilis* - >95% pure	<0.05 EU/µg	1 ng - 1 µg/ml	50 µg	tlrl-pbsfla	p. 91
FLA-PA Ultrapure	Ultrapure flagellin from *P. aeruginosa* - >95% pure	<0.05 EU/µg	1 ng - 1 µg/ml	50 µg	tlrl-pafla	p. 91
FLA-ST	Standard flagellin from *S. typhimurium* - 10% pure	<10 EU/µg	10 ng - 10 µg/ml	100 µg	tlrl-stfla	p. 91
FLA-ST Ultrapure	Ultrapure flagellin from *S. typhimurium* - >95% pure	<0.05 EU/µg	10 - 100 ng/ml	10 µg 50 µg	tlrl-epstfla tlrl-epstfla-5	p. 91
RecFLA-ST	Recombinant flagellin from *S. typhimurium*	<0.05 EU/µg	10 - 100 ng/ml	10 µg 50 µg	tlrl-flic-10 tlrl-flic-50	p. 92
Flagellin Flic VacciGrade™	Preclinical grade RecFLA-ST	<0.05 EU/µg	1 - 10 µg/mouse	50 µg	vac-fla	p. 143
TLR7 Agonists						
CL264	Adenine analog	EndoFit™	50 ng - 10 µg/ml	500 µg 5 mg	tlrl-c264e tlrl-c264e-5	p. 92
CL264 FITC	FITC-labeled CL264	EndoFit™	1 - 10 µg/ml	100 µg	tlrl-fc264	p. 92
CL264 Rhodamine	Rhodamine-labeled CL264	EndoFit™	1 - 10 µg/ml	100 µg	tlrl-rc264	p. 92
CL307	Hydroxyadenine spermine compound	EndoFit™	5 ng - 1 µg/ml	500 µg	tlrl-c307	p. 92
Gardiquimod™	Imidazoquinoline compound	EndoFit™	0.1 - 3 µg/ml	500 µg 5 mg	tlrl-gdqs tlrl-gdq-5	p. 92
Gardiquimod™ VacciGrade™	Preclinical grade Gardiquimod™	EndoFit™	10 - 100 µg/mouse	5 mg	vac-gdq	p. 143
Imiquimod (R837)	Imidazoquinoline compound	EndoFit™	1 - 5 µg/ml	500 µg 5 mg	tlrl-imqs tlrl-imq	p. 92

PRODUCT	ORIGIN/DESCRIPTION	ENDOTOXIN LEVELS	WORKING CONCENTRATION	QTY	CATALOG CODE	INFO
TLR LIGANDS						
TLR7 Agonists						
Imiquimod VacciGrade™	Preclinical grade Imiquimod	EndoFit™	10 - 100 µg/mouse	5 mg	vac-imq	p. 143
Loxoribine	Guanosine analog	EndoFit™	300 µg/ml	50 mg	tlrl-lox	p. 92
TLR8 Agonists						
ORN02/LyoVec	ssRNA with 6 UUAU repeats / LyoVec™	EndoFit™	0.25 - 5 µg/ml	4 x 25 µg	tlrl-orn2	p. 92
ORN06/LyoVec	ssRNA with 6 UUGU repeats / LyoVec™	EndoFit™	0.25 - 5 µg/ml	4 x 25 µg	tlrl-orn6	p. 92
ssPolyU Naked	RNA homopolymer	EndoFit™	1 - 10 µg/ml	10 mg	tlrl-sspu	p. 93
ssPolyU/LyoVec	RNA homopolymer / LyoVec™	EndoFit™	1 - 10 µg/ml	4 x 25 µg	tlrl-lpu	p. 93
ssRNA40/LyoVec	HIV-1 LTR-derived ssRNA / LyoVec™	EndoFit™	0.25 - 5 µg/ml	4 x 25 µg	tlrl-lrna40	p. 93
ssRNA41/LyoVec	ssRNA40 control / LyoVec™	EndoFit™	0.25 - 5 µg/ml	4 x 25 µg	tlrl-lrna41	p. 93
ssRNA-DR/LyoVec	ssRNA with 2 GUCCUUCAA repeats / LyoVec™	EndoFit™	1 - 10 µg/ml	4 x 25 µg	tlrl-ssdr	p. 93
TL8-506	Benzoazepine analog	EndoFit™	10 - 100 ng/ml	500 µg	tlrl-tl8506	p. 93
TLR7/8 Agonists						
CL075	Thiazoquinoline compound	EndoFit™	100 ng - 5 µg/ml	500 µg 5 mg	tlrl-c75 tlrl-c75-5	p. 92
CL097	Imidazoquinoline compound	EndoFit™	50 ng - 5 µg/ml	500 µg 5 mg	tlrl-c97 tlrl-c97-5	p. 92
Poly(dT)	Thymidine homopolymer ODN (17 mer)	EndoFit™	10 µM	100 nmol	tlrl-pt17	p. 92
R848 (resiquimod)	Imidazoquinoline compound	EndoFit™	10 ng - 10 µg/ml	500 µg 5 mg	tlrl-r848 tlrl-r848-5	p. 92
R848 VacciGrade™	Preclinical grade R848	EndoFit™	10 - 100 µg/mouse	5 mg	vac-r848	p. 143
TLR9 Agonists						
E. coli ssDNA/LyoVec	*E. coli* single stranded DNA/LyoVec™ complexes	EndoFit™	1 - 10 µg/ml	200 µg	tlrl-ssec	p. 94
ODN 1585	Stimulatory CpG ODN Type A Mouse preferred	EndoFit™	1 - 5 µM	200 µg 1 mg 5 mg	tlrl-1585 tlrl-1585-1 tlrl-1585-5	p. 94
ODN 1585 control	Negative control for ODN 1585	EndoFit™	1 - 5 µM	200 µg 1 mg 5 mg	tlrl-1585c tlrl-1585c-1 tlrl-1585c-5	p. 94
ODN 1585 FITC	FITC-labeled CpG ODN - mouse preferred, type A	EndoFit™	10 ng - 10 µg/ml	50 µg	tlrl-1585f	p. 94
ODN 1585 VacciGrade™	Preclinical grade ODN 1585	EndoFit™	20 - 50 µg/mouse	1 mg	vac-1585-1	p. 143
ODN 1668	Stimulatory CpG ODN Type B Mouse preferred	EndoFit™	1 - 5 µM	200 µg 1 mg 5 mg	tlrl-1668 tlrl-1668-1 tlrl-1668-5	p. 94
ODN 1668 control	Negative control for ODN 1668	EndoFit™	1 - 5 µM	200 µg 1 mg 5 mg	tlrl-1668c tlrl-1668c-1 tlrl-1668c-5	p. 94
ODN 1668 FITC	FITC-labeled CpG ODN - mouse preferred, type B	EndoFit™	10 ng - 10 µg/ml	50 µg	tlrl-1668f	p. 94
ODN 1826	Stimulatory CpG ODN Type B Mouse preferred	EndoFit™	1 - 5 µM	200 µg 1 mg 5 mg	tlrl-1826 tlrl-1826-1 tlrl-1826-5	p. 94
ODN 1826 control (ODN 2138)	Negative control for ODN 1826	EndoFit™	1 - 5 µM	200 µg 1 mg 5 mg	tlrl-1826c tlrl-1826c-1 tlrl-1826c-5	p. 94
ODN 1826 Biotin	Biotinylated CpG ODN - mouse preferred, type B	EndoFit™	10 ng - 10 µg/ml	50 µg	tlrl-1826b	p. 94
ODN 1826 FITC	FITC-labeled CpG ODN - mouse preferred, type B	EndoFit™	10 ng - 10 µg/ml	50 µg	tlrl-1826f	p. 94
ODN 1826 VacciGrade™	Preclinical grade ODN 1826	EndoFit™	20 - 50 µg/mouse	1 mg	vac-1826-1	p. 143

PRODUCT	ORIGIN/DESCRIPTION	ENDOTOXIN LEVELS	WORKING CONCENTRATION	QTY	CATALOG CODE	INFO
TLR LIGANDS						
TLR9 Agonists						
ODN 2006 (ODN 7909)	Stimulatory CpG ODN Type B Human preferred	EndoFit™	1 - 5 µM	200 µg 1 mg 5 mg	tlrl-2006 tlrl-2006-1 tlrl-2006-5	p. 94
ODN 2006 control (ODN 2137)	Negative control for ODN 2006	EndoFit™	1 - 5 µM	200 µg 1 mg 5 mg	tlrl-2006c tlrl-2006c-1 tlrl-2006c-5	p. 94
ODN 2006 Biotin	Biotinylated CpG ODN - human preferred, type B	EndoFit™	10 ng - 10 µg/ml	50 µg	tlrl-2006b	p. 94
ODN 2006 FITC	FITC-labeled CpG ODN - human preferred, type B	EndoFit™	10 ng - 10 µg/ml	50 µg	tlrl-2006f	p. 94
ODN 2006-G5	Stimulatory CpG ODN Type B Human preferred	EndoFit™	1 - 5 µM	200 µg 1 mg 5 mg	tlrl-2006g5 tlrl-2006g5-1 tlrl-2006g5-5	p. 94
ODN 2006-G5 Control	Negative control for ODN 2006-G5	EndoFit™	1 - 5 µM	200 µg	tlrl-2006g5c	p. 94
ODN 2006 VacciGrade™	Preclinical grade ODN 2006	EndoFit™	20 - 50 µg/mouse	1 mg	vac-2006-1	p. 143
ODN 2007	Stimulatory CpG ODN Type B Multi-species	EndoFit™	1 - 5 µM	200 µg 1 mg 5 mg	tlrl-2007 tlrl-2007-1 tlrl-2007-5	p. 94
ODN 2007 control	Negative control for ODN 2007	EndoFit™	1 - 5 µM	200 µg 1 mg 5 mg	tlrl-2007c tlrl-2007c-1 tlrl-2007c-5	p. 94
ODN 2216	Stimulatory CpG ODN Type A Human preferred	EndoFit™	1 - 5 µM	200 µg 1 mg 5 mg	tlrl-2216 tlrl-2216-1 tlrl-2216-5	p. 94
ODN 2216 control (ODN 2243)	Negative control for ODN 2216	EndoFit™	1 - 5 µM	200 µg 1 mg 5 mg	tlrl-2243 tlrl-2243-1 tlrl-2243-5	p. 94
ODN 2216 Biotin	Biotinylated CpG ODN - human preferred, type A	EndoFit™	10 ng - 10 µg/ml	50 µg	tlrl-2216b	p. 94
ODN 2216 FITC	FITC-labeled CpG ODN - human preferred, type A	EndoFit™	10 ng - 10 µg/ml	50 µg	tlrl-2216f	p. 94
ODN 2336	Stimulatory CpG ODN Type A Human preferred	EndoFit™	1 - 5 µM	200 µg 1 mg 5 mg	tlrl-2336 tlrl-2336-1 tlrl-2336-5	p. 94
ODN 2336 control	Negative control for ODN 2336	EndoFit™	1 - 5 µM	200 µg 1 mg 5 mg	tlrl-2336c tlrl-2336c-1 tlrl-2336c-5	p. 94
ODN 2336 FITC	FITC-labeled CpG ODN - human preferred, type A	EndoFit™	10 ng - 10 µg/ml	50 µg	tlrl-2336f	p. 94
ODN 2395	Stimulatory CpG ODN Type C Human/mouse preferred	EndoFit™	1 - 5 µM	200 µg 1 mg 5 mg	tlrl-2395 tlrl-2395-1 tlrl-2395-5	p. 94
ODN 2395 control	Negative control for ODN 2395	EndoFit™	1 - 5 µM	200 µg 1 mg 5 mg	tlrl-2395c tlrl-2395c-1 tlrl-2395c-5	p. 94
ODN 2395 FITC	FITC-labeled CpG ODN - human preferred, type C	EndoFit™	10 ng - 10 µg/ml	50 µg	tlrl-2395f	p. 94
ODN 2395 VacciGrade™	Preclinical grade ODN 2395	EndoFit™	20 - 50 µg/mouse	1 mg	vac-2395-1	p. 143
ODN BW006 (ODN 684)	Class B CpG ODN, human/mouse preferred	EndoFit™	300 ng - 30 µg/ml	200 µg	tlrl-bw006	p. 94
ODN BW007	Negative control for ODN BW006	EndoFit™	1 - 5 µM	200 µg	tlrl-bw007	p. 94
ODN D-SL01	Class B CpG ODN, multispecies	EndoFit™	1 - 5 µM	200 µg	tlrl-dsl01	p. 94
ODN-D-SL03	Class C CpG ODN, multispecies	EndoFit™	1 - 5 µM	200 µg	tlrl-dsl03	p. 94
ODN M362	Stimulatory CpG ODN Type C Human/mouse preferred	EndoFit™	1 - 5 µM	200 µg 1 mg 5 mg	tlrl-m362 tlrl-m362-1 tlrl-m362-5	p. 94
ODN M362 control	Negative control for ODN M362	EndoFit™	1 - 5 µM	200 µg 1 mg 5 mg	tlrl-m362c tlrl-m362c-1 tlrl-m362c-5	p. 94
ODN M362 FITC	FITC-labeled CpG ODN - human preferred, type C	EndoFit™	10 ng - 10 µg/ml	50 µg	tlrl-m362f	p. 94

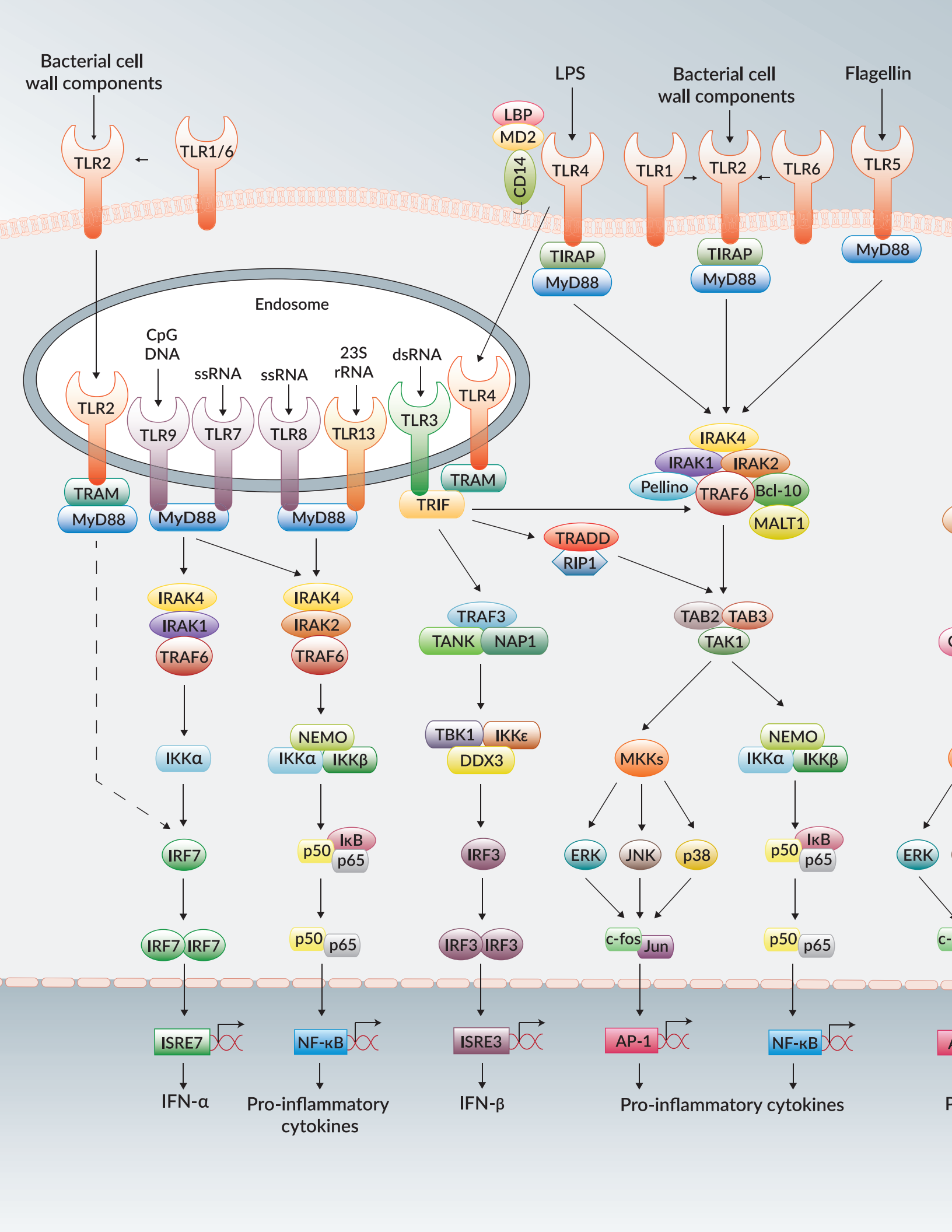
PRODUCT	ORIGIN/DESCRIPTION	ENDOTOXIN LEVELS	WORKING CONCENTRATION	QTY	CATALOG CODE	INFO
TLR LIGANDS						
TLR9 Antagonists						
ODN 2088	Inhibitory ODN, mouse preferred	EndoFit™	100 nM - 10 µM	200 µg 1 mg	tlrl-2088 tlrl-2088-1	p. 95
ODN 2088 control	Negative control for ODN 2088	EndoFit™	100 nM - 10 µM	200 µg 1 mg	tlrl-2088c tlrl-2088c-1	p. 95
ODN 4084-F	Class B inhibitory ODN	EndoFit™	100 nM - 10 µM	200 µg	tlrl-4084	p. 95
ODN INH-1	Class R inhibitory ODN	EndoFit™	100 nM - 10 µM	200 µg	tlrl-inh1	p. 95
ODN INH-18	Inhibitory ODN, human/mouse preferred	EndoFit™	100 nM - 10 µM	200 µg	tlrl-inh18	p. 95
ODN INH-47	Negative control for ODN INH-1	EndoFit™	100 nM - 10 µM	200 µg	tlrl-inh47	p. 95
ODN TTAGGG (A151)	Inhibitory ODN, human preferred	EndoFit™	100 nM - 10 µM	200 µg 1 mg	tlrl-ttag151 tlrl-ttag151-1	p. 95
ODN TTAGGG control	Negative control for ODN TTAGGG	EndoFit™	100 nM - 10 µM	200 µg 1 mg	tlrl-ttagc tlrl-ttagc-1	p. 95
G-ODN	Inhibitory guanosine-rich ODN	EndoFit™	100 nM - 10 µM	200 µg	tlrl-godn	p. 95
G-ODN control	Negative control for G-ODN	EndoFit™	100 nM - 10 µM	200 µg	tlrl-godnc	p. 95
TLR13 Agonists						
ORN Sa19	S. aureus 23S rRNA-derived oligoribonucleotide	EndoFit™	0.02 - 2 µg/ml	200 µg	tlrl-orn19	p. 95
ORN Sa19 Control	Control oligoribonucleotide for ORN Sa19	EndoFit™	0.02 - 2 µg/ml	200 µg	tlrl-orn19c	p. 95
NLR LIGANDS						
NOD1 Agonists						
C12-iE-DAP	Acylated derivative of iE-DAP	EndoFit™	1 ng - 1 µg/ml	1 mg	tlrl-c12dap	p. 96
iE-DAP	D-γ-Glu-mDAP	EndoFit™	1 - 100 µg/ml	5 mg	tlrl-dap	p. 96
iE-Lys	iE-DAP negative control	EndoFit™	1 - 100 µg/ml	5 mg	tlrl-lys	p. 96
Tri-DAP	L-Ala-γ-D-Glu-mDAP	EndoFit™	100 ng - 10 µg/ml	1 mg	tlrl-tdap	p. 96
Tri-Lys	Tri-DAP negative control	EndoFit™	100 ng - 10 µg/ml	1 mg	tlrl-tlys	p. 96
NOD2 Agonists						
L18-MDP	Muramyldipeptide with a C18 fatty acid chain	EndoFit™	1 - 100 ng/ml	1 mg	tlrl-lmdp	p. 97
MDP	Muramyldipeptide (L-D isoform, active)	EndoFit™	10 ng - 10 µg/ml	5 mg	tlrl-mdp	p. 97
MDP control	Muramyldipeptide (D-D isoform, inactive)	EndoFit™	10 ng - 10 µg/ml	5 mg	tlrl-mdpc	p. 97
MDP Biotin	Biotinylated Muramyldipeptide	EndoFit™	100 ng - 10 µg/ml	500 µg	tlrl-bmdp	p. 97
MDP FITC	FITC-labeled Muramyldipeptide	EndoFit™	10 ng - 10 µg/ml	500 µg	tlrl-fmdp	p. 97
MDP Rhodamine	Rhodamine-labeled Muramyldipeptide	EndoFit™	100 ng - 10 µg/ml	500 µg	tlrl-rmdp	p. 97
M-TriLYS	Synthetic muramyl tripeptide	EndoFit™	100 ng - 10 µg/ml	1 mg	tlrl-mtl	p. 97
Murabutide	Synthetic derivative of muramyldipeptide	EndoFit™	10 ng - 1 µg/ml	5 mg	tlrl-mbt	p. 97
Murabutide control	Murabutide analog (D isoform, inactive)	EndoFit™	10 ng - 1 µg/ml	5 mg	tlrl-mbtc	p. 97
N-Glycolyl-MDP	N-glycolylated muramyldipeptide	EndoFit™	100 ng - 10 µg/ml	5 mg	tlrl-gmdp	p. 97
N-Glycolyl-MDP VacciGrade™	Preclinical grade N-glycolylated muramyldipeptide	EndoFit™	5 -30 µg/mouse	5 mg	vac-gmdp	p. 143
NOD1/2 Agonists						
M-TriDAP	MurNAc-L-Ala-γ-D-Glu-mDAP	EndoFit™	1 - 100 µg/ml	1 mg	tlrl-mtd	p. 96
PGN-ECndi ultrapure	Insoluble peptidoglycan from *E. coli K12*	EndoFit™	1 - 5 µg/ml	5 mg	tlrl-kipgn	p. 96
PGN-ECndss ultrapure	Soluble sonicated peptidoglycan from *E. coli K12*	EndoFit™	1 - 5 µg/ml	1 mg	tlrl-ksspgn	p. 96
PGN-SAndi ultrapure	Insoluble peptidoglycan from *S. aureus*	EndoFit™	1 - 5 µg/ml	5 mg	tlrl-sipgn	p. 96

PRODUCT	ORIGIN/DESCRIPTION	ENDOTOXIN LEVELS	WORKING CONCENTRATION	QTY	CATALOG CODE	INFO
NLR LIGANDS						
NLRP3 Inflammasome Inducers						
Alum Crystals	Aluminium potassium sulfate	EndoFit™	10 - 200 µg/ml	1 g	tlrl-alk	p. 97
ATP	Adenosine 5'-triphosphate disodium salt	EndoFit™	5 mM	1 g	tlrl-atp	p. 97
Chitosan Ultrapure	Deacetylated derivative of chitin	EndoFit™	10 - 100 µg/ml	100 mg	tlrl-cht	p. 98
Chitosan VacciGrade™	Preclinical grade deacetylated derivative of chitin	EndoFit™	100 µg/mouse	100 mg	vac-cht	p. 143
CPPD Crystals	Calcium pyrophosphate dihydrate	EndoFit™	50 - 200 µg/ml	5 mg	tlrl-cppd	p. 98
Curdlan AL	Beta-1,3-glucan from *Alcaligenes faecalis*	*100 ng/ml	100 µg/ml	100 mg	tlrl-cura	p. 99
Hemozoin	Synthetic heme crystal	EndoFit™	50 - 400 µg/ml	5 mg	tlrl-hz	p. 98
MSU Crystals	Monosodium urate (uric acid)	EndoFit™	50 - 200 µg/ml	5 mg 25 mg	tlrl-msu tlrl-msu-25	p. 98
Nano-SiO2	Nanoparticles of silica dioxide	EndoFit™	10 - 200 µg/ml	10 mg	tlrl-sio	p. 98
Nigericin	Nigericin, sodium salt	EndoFit™	1 mM	10 mg 50 mg	tlrl-nig tlrl-nig-5	p. 98
Zymosan	Cell wall preparation of *S. cerevisiae*	EndoFit™	10 µg/ml	100 mg	tlrl-zyn	p. 100
RLR LIGANDS						
RIG-I Agonists						
5'ppp-dsRNA	5'Triphosphate blunt-end double-stranded RNA	EndoFit™	30 ng - 1 µg/ml	25 µg 100 µg	tlrl-3prna tlrl-3prna-100	p. 99
5'ppp-dsRNA/LyoVec™	5'ppp-dsRNA/LyoVec™ complexes	EndoFit™	100 ng - 1 µg/ml	25 µg 100 µg	tlrl-3prnalv tlrl-3prnalv-100	p. 99
5'ppp-dsRNA Control	Blunt-end double-stranded RNA, control	EndoFit™	30 ng - 1 µg/ml	25 µg 100 µg	tlrl-3prnac tlrl-3prnac-100	p. 99
5'ppp-dsRNA Control/LyoVec™	5'ppp-dsRNA Control/LyoVec™ complexes	EndoFit™	100 ng - 1 µg/ml	25 µg 100 µg	tlrl-3prnaclv tlrl-3prnaclv-100	p. 99
RIG-I/MDA-5 Agonists						
Poly(I:C) (HMW)/LyoVec	Poly(I:C) (HMW)/LyoVec™ complexes	EndoFit™	100 ng - 1 µg/ml	100 µg	tlrl-piclv	p. 99
Poly(I:C) (LMW)/LyoVec	Poly(I:C) (LMW)/LyoVec™ complexes	EndoFit™	100 ng - 1 µg/m	100 µg	tlrl-picwlv	p. 99
CLR LIGANDS						
Dectin-I Agonists						
Beta-Glucan Peptide	Beta-glucan from *Trametes versicolor*	EndoFit™	10 - 100 µg/ml	50 mg	tlrl-bgp	p. 99
Curdlan AL	Beta-1,3-glucan from *Alcaligenes faecalis*	*100 ng/ml	100 µg/ml	100 mg	tlrl-cura	p. 99
HKCA	Heat-killed *Candida albicans*	EndoFit™	10^8 cells/ml	10^9 cells	tlrl-hkca	p. 100
HKSC	Heat-killed *Saccharomyces cerevisiae*	EndoFit™	10^8 cells/ml	10^9 cells	tlrl-hksc	p. 100
Laminarin	Soluble beta-glucan from *Laminaria digitata*	*10 µg/ml	1 - 100 µg/ml	100 mg	tlrl-lam	p. 100
Pustulan	Beta-glucan from *Lasallia pustulata*	*1 µg/ml	1 - 100 µg/ml	100 mg	tlrl-pst	p. 100
Schizophyllan	Beta-glucan from *Schizophyllum commune*	*1 µg/ml	1 - 100 µg/ml	100 mg	tlrl-spg	p. 100
Scleroglucan	Beta-glucan from *Sclerotium rolfsii*	*100 ng/ml	1 - 100 µg/ml	100 mg	tlrl-scg	p. 100
WGP Dispersible	Whole Glucan Particles, insoluble	*100 µg/ml	1 - 200 µg/ml	50 mg	tlrl-wgp	p. 100
WGP Soluble	Whole Glucan Particles, soluble	EndoFit™	1 ng - 1 µg/ml	50 mg	tlrl-wgps	p. 100
Zymosan	Cell wall preparation from *S. cerevisiae*	*10 ng/ml	1 - 100 µg/ml	100 mg	tlrl-zyn	p. 100
Zymosan Depleted	Hot alkali treated zymosan	EndoFit™	100 µg/ml	10 mg	tlrl-dzn	p. 100
Dectin-2 Agonist						
Furfurman	Malassezia furfur cell wall preparation		100 ng - 10 µg/ml	10 mg	tlrl-ffm	p. 101

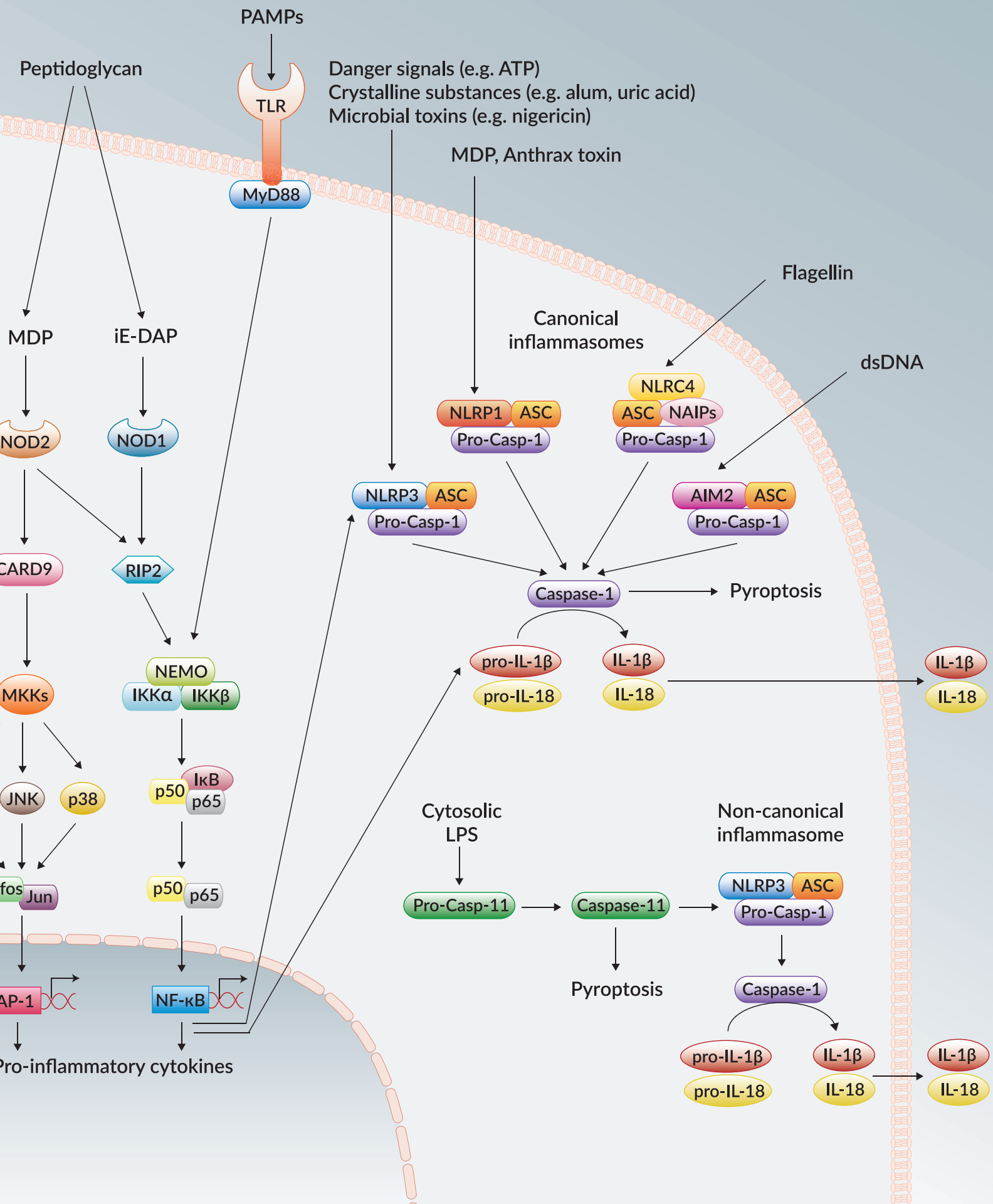
PRODUCT	ORIGIN/DESCRIPTION	ENDOTOXIN LEVELS	WORKING CONCENTRATION	QTY	CATALOG CODE	INFO
CLR LIGANDS						
Mincle Agonists						
HKMT	Heat Killed *Mycobacterium tuberculosis*	EndoFit™	10 - 100 µg/ml	10 mg 50 mg	tlrl-hkmt-1 tlrl-hkmt-5	p. 101
TDB	Synthetic analog of the cord factor	*50 µg/ml	1 - 100 µg/ml	2 mg	tlrl-tdb	p. 101
TDB VacciGrade™	Preclinical grade TDB	*50 µg/ml	50 µg/mouse	2 mg	vac-tdb	p. 144
TDB HS-15	Formulated TDB	*50 µg/ml	1 - 100 µg/ml	2 mg	tlrl-stdb	p. 101
CDS & STING LIGANDS						
CDS Agonists						
dsDNA-EC	*E. coli K12* genomic DNA	EndoFit™	30 ng - 1 µg/ml	200 µg	tlrl-ecdna	p. 102
HSV-60 Naked	HSV1-derived 60 bp oligonucleotide	EndoFit™	30 ng - 10 µg/ml	200 µg	tlrl-hsv60n	p. 102
HSV-60/LyoVec	Precomplexed HSV1-derived 60 bp ODN	EndoFit™	300 ng - 10 µg/ml	100 µg	tlrl-hsv60c	p. 102
HSV-60c Naked	Control for HSV-60 ODN	EndoFit™	30 ng - 10 µg/ml	200 µg	tlrl-hsv60cn	p. 102
HSV-60c/LyoVec	Precomplexed control for HSV-60 ODN	EndoFit™	300 ng - 10 µg/ml	100 µg	tlrl-hsv60cc	p. 102
ISD Naked	Interferon stimulatory DNA	EndoFit™	100 ng - 10 µg/ml	200 µg	tlrl-isdn	p. 102
ISD/LyoVec	Precomplexed interferon stimulatory DNA	EndoFit™	300 ng - 10 µg/ml	100 µg	tlrl-isdc	p. 102
ISD Control Naked	Non-immunostimulatory ODN	EndoFit™	100 ng - 10 µg/ml	200 µg	tlrl-isdcn	p. 102
ISD Control/LyoVec	Precomplexed non-immunostimulatory ODN	EndoFit™	300 ng - 10 µg/ml	100 µg	tlrl-isdcc	p. 102
Poly(dA)	Polydeoxyadenylic acid	EndoFit™	30 ng - 10 µg/ml	200 µg	tlrl-pan	p. 102
Poly(dA)/LyoVec	Precomplexed polydeoxyadenylic acid	EndoFit™	100 ng - 10 µg/ml	100 µg	tlrl-pac	p. 102
Poly(dA:dT) Naked	Poly(dA-dT)•poly(dT-dA)	EndoFit™	1 - 5 µg/ml	200 µg 1 mg	tlrl-patn tlrl-patn-1	p. 102
Poly(dA:dT)/LyoVec	Poly(dA-dT)•poly(dT-dA)/LyoVec™ complexes	EndoFit™	1 - 5 µg/ml	100 µg	tlrl-patc	p. 102
Poly(dA:dT)/Rhodamine	Rhodamine labeled poly(dA-dT)•poly(dT-dA)	EndoFit™	100 ng - 10 µg/ml	10 µg	tlrl-patrh	p. 102
Poly(dG:dC) Naked	Poly(dG-dC)•poly(dG-dC)	EndoFit™	1 - 5 µg/ml	200 µg	tlrl-pgcn	p. 102
Poly(dG:dC)/LyoVec	Poly(dG-dC)•poly(dG-dC)/LyoVec™ complexes	EndoFit™	1 - 5 µg/ml	100 µg	tlrl-pgcc	p. 102
VACV-70 Naked	Vaccinia virus-derived 70 bp oligonucleotide	EndoFit™	30 ng - 10 µg/ml	200 µg	tlrl-vav70n	p. 102
VACV-70/LyoVec	Precomplexed vaccinia virus-derived 70 bp ODN	EndoFit™	300 ng - 10 µg/ml	100 µg	tlrl-vav70c	p. 102
VACV-70c Naked	Control for VACV-70 ODN	EndoFit™	30 ng - 10 µg/ml	200 µg	tlrl-vav70cn	p. 102
VACV-70c/LyoVec	Precomplexed control for VACV-70 ODN	EndoFit™	300 ng - 10 µg/ml	100 µg	tlrl-vav70cc	p. 102
STING Agonists						
2'2'-cGAMP	2'5'-2'5' cyclic GMP-AMP	EndoFit™	0.1 - 100 µg/ml	500 µg 1 mg	tlrl-nacga22 tlrl-nacga22-1	p. 102
2'3'-cGAMP	2'5'-3'5' cyclic GMP-AMP	EndoFit™	0.1 - 100 µg/ml	500 µg 1 mg 5 mg	tlrl-nacga23 tlrl-nacga23-1 tlrl-nacga23-5	p. 102
2'3'-cGAMP Control	Linear dinucleotide analog of 2'3'-cGAMP	EndoFit™	0.1 - 100 µg/ml	1 mg	tlrl-nagpap	p. 102
2'3'-cGAMP VacciGrade™	Preclinical grade 2'5'-3'5' cyclic GMP-AMP	EndoFit™	5 - 50 µg/mouse	1 mg	vac-nacga23	p. 142
2'3'-cGAM(PS)2 (Rp/Sp)	Bis-phosphorothioate analog of 2'3'-cGAMP	EndoFit™	0.1 - 100 µg/ml	250 µg	tlrl-nacga2srs	p. 102
3'3'-cGAMP	3'5'-3'5' cyclic GMP-AMP	EndoFit™	0.1 - 100 µg/ml	500 µg 1 mg	tlrl-nacga tlrl-nacga-1	p. 103
3'3'-cGAMP Control	Linear dinucleotide analog of 3'3'-cGAMP	EndoFit™	0.1 - 100 µg/ml	1 mg	tlrl-napgpa	p. 103
3'3'-cGAMP Fluorinated	Fluorinated 3'5'-3'5' cyclic GMP-AMP	EndoFit™	0.1 - 100 µg/ml	100 µg	tlrl-nacgaf	p. 103
3'3'-cGAMP VacciGrade™	Preclinical grade 3'5'-3'5' cyclic GMP-AMP	EndoFit™	5 - 50 µg/mouse	1 mg	vac-nacga	p. 142

PRODUCT	ORIGIN/DESCRIPTION	ENDOTOXIN LEVELS	WORKING CONCENTRATION	QTY	CATALOG CODE	INFO
CDS & STING LIGANDS						
STING Agonists						
c-di-AMP	3'5' Cyclic di-AMP	EndoFit™	1 - 100 µg/ml	1 mg	tlrl-nacda	p. 103
c-di-AMP Control	Linear dinucleotide analog of c-di-AMP - pApA	EndoFit™	1 - 100 µg/ml	1 mg	tlrl-napapa	p. 103
c-di-AMP Fluorinated	Fluorinated cyclic di-AMP	EndoFit™	1 - 100 µg/ml	100 µg	tlrl-nacdaf	p. 103
c-di-AMP VacciGrade™	Sterile 3'5' cyclic di-AMP	EndoFit™	5 - 50 µg/mouse	1 mg	vac-cda	p. 142
2'3'-c-di-AMP	2'5'-3'5' Cyclic di-AMP - c-di-AMP analog	EndoFit™	1 - 100 µg/ml	500 µg	tlrl-nacda23	p. 103
2'3'-c-di-AM(PS)2 (Rp,Rp)	Bis-phosphorothioate analog of 2'3'-c-di-AMP	EndoFit™	1 - 100 µg/ml	100 µg 500 µg	tlrl-nacda2r-01 tlrl-nacda2r	p. 103
c-di-GMP	3'5' Cyclic di-GMP	EndoFit™	10 - 100 µg/ml	1 mg	tlrl-cdg	p. 103
c-di-GMP Control	Linear dinucleotide analog of c-di-GMP - pGpG	EndoFit™	10 - 100 µg/ml	1 mg	tlrl-napgpg	p. 103
c-di-GMP Fluorinated	Fluorinated cyclic di-GMP	EndoFit™	10 - 100 µg/ml	100 µg	tlrl-nacdgf	p. 103
c-di-GMP VacciGrade™	Sterile 3'5' cyclic di-GMP	EndoFit™	5 - 50 µg/mouse	1 mg	vac-cdg	p. 142
2'3'-c-di-GMP	2'5'-3'5' Cyclic di-GMP - c-di-GMP analog	EndoFit™	10 - 100 µg/ml	500 µg	tlrl-nacdg23	p. 103
c-di-IMP	3'5' Cyclic di-IMP	EndoFit™	10 - 100 µg/ml	1 mg	tlrl-cdi	p. 103
DMXAA	5,6-dimethylxanthenone-4-acetic acid	EndoFit™	10 - 100 µg/ml	5 mg	tlrl-dmx	p. 103
MULTI-PRR LIGANDS						
TLR2/TLR7 Agonists						
CL347 - AdiFectin™	Hydroxyadenine spermine compound	EndoFit™	300 ng - 3 µg/ml	500 µg	tlrl-c347	p. 104
CL401	PAM2C-conjugated hydroxyadenine compound	EndoFit™	1 ng - 10 µg/ml	500 µg	tlrl-c401	p. 104
CL401 VacciGrade™	Preclinical grade CL401	EndoFit™	20 µg/mouse	1 mg	vac-c401	p. 143
CL413 - Adilipoline™	PAM2CK4-conjugated hydroxyadenine compound	EndoFit™	50 pg - 10 µg/ml	500 µg	tlrl-c413	p. 104
CL413 VacciGrade™	Preclinical grade CL413	EndoFit™	20 µg/mouse	1 mg	vac-c413	p. 143
CL419	Pam2C-spermine compound	EndoFit™	1 ng - 100 µg/ml	500 µg	tlrl-c419	p. 105
CL531	PAM2CK4-conjugated hydroxyadenine compound	EndoFit™	5 pg - 10 µg/ml	500 µg	tlrl-c531	p. 104
CL553 - PamadiFectin™	Hydroxyadenine spermine compound	EndoFit™	100 ng - 1 µg/ml	500 µg	tlrl-c553	p. 105
CL572	Monoacyl-ethyl-cystein hydroxyadenine compound	EndoFit™	0.5 ng - 1 µg/ml	500 µg	tlrl-c572	p. 104
TLR2/NOD2 Agonist						
CL429	Pam2C-conjugated murabutide	EndoFit™	1 ng - 10 µg/ml	5 mg	tlrl-c429	p. 97
CL429 VacciGrade™	Preclinical grade Pam2C-conjugated murabutide	EndoFit™	20 -50 µg/mouse	5 mg	vac-c429	p. 143

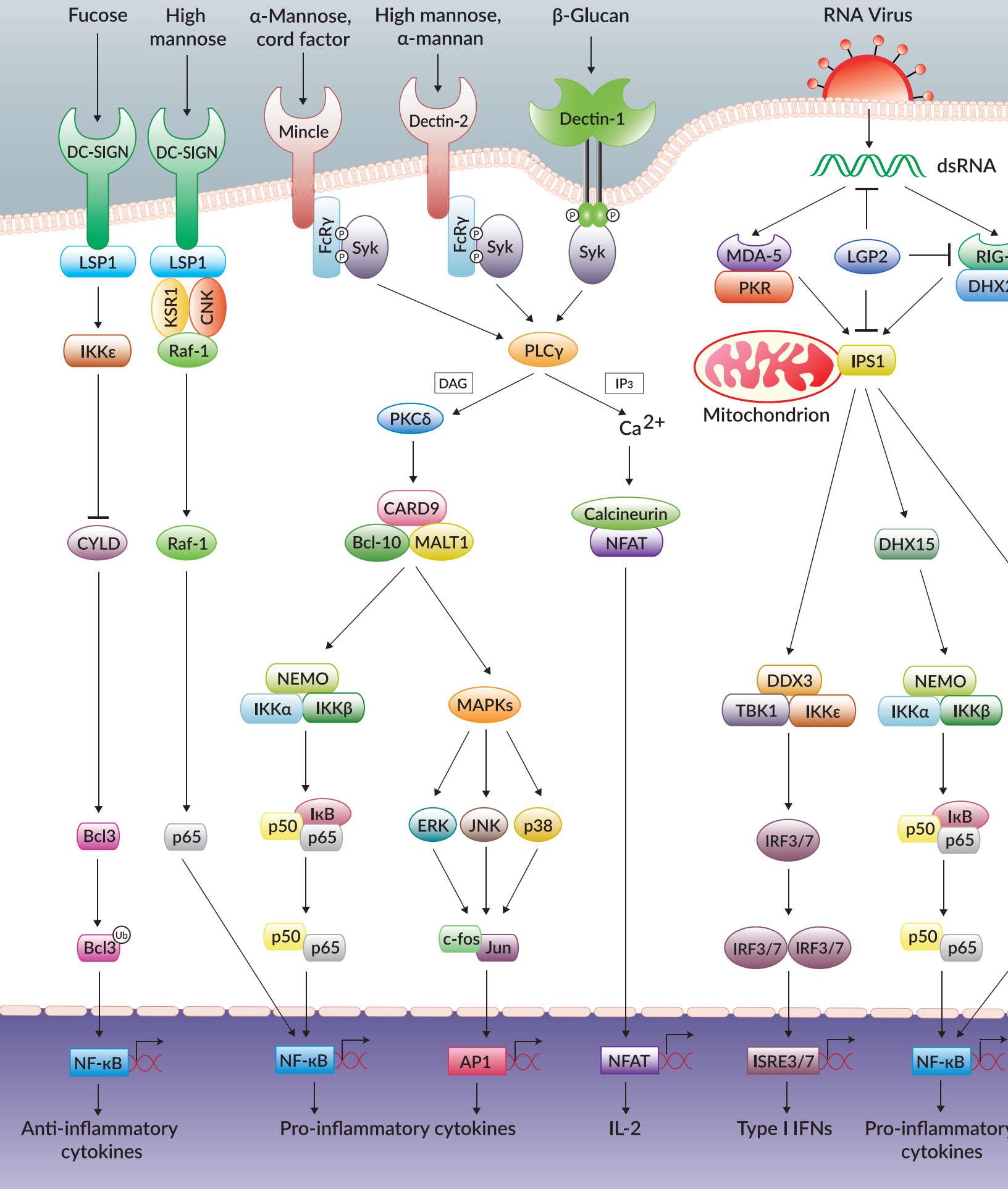
* The levels of endotoxin in certain PAMPs, such as cristals and β-glucans, cannot been determined using the chromogenic LAL assay. For these products, the value listed in the table corresponds to the highest concentration tested that does not activate the HEK-Blue™ TLR4 cell line.

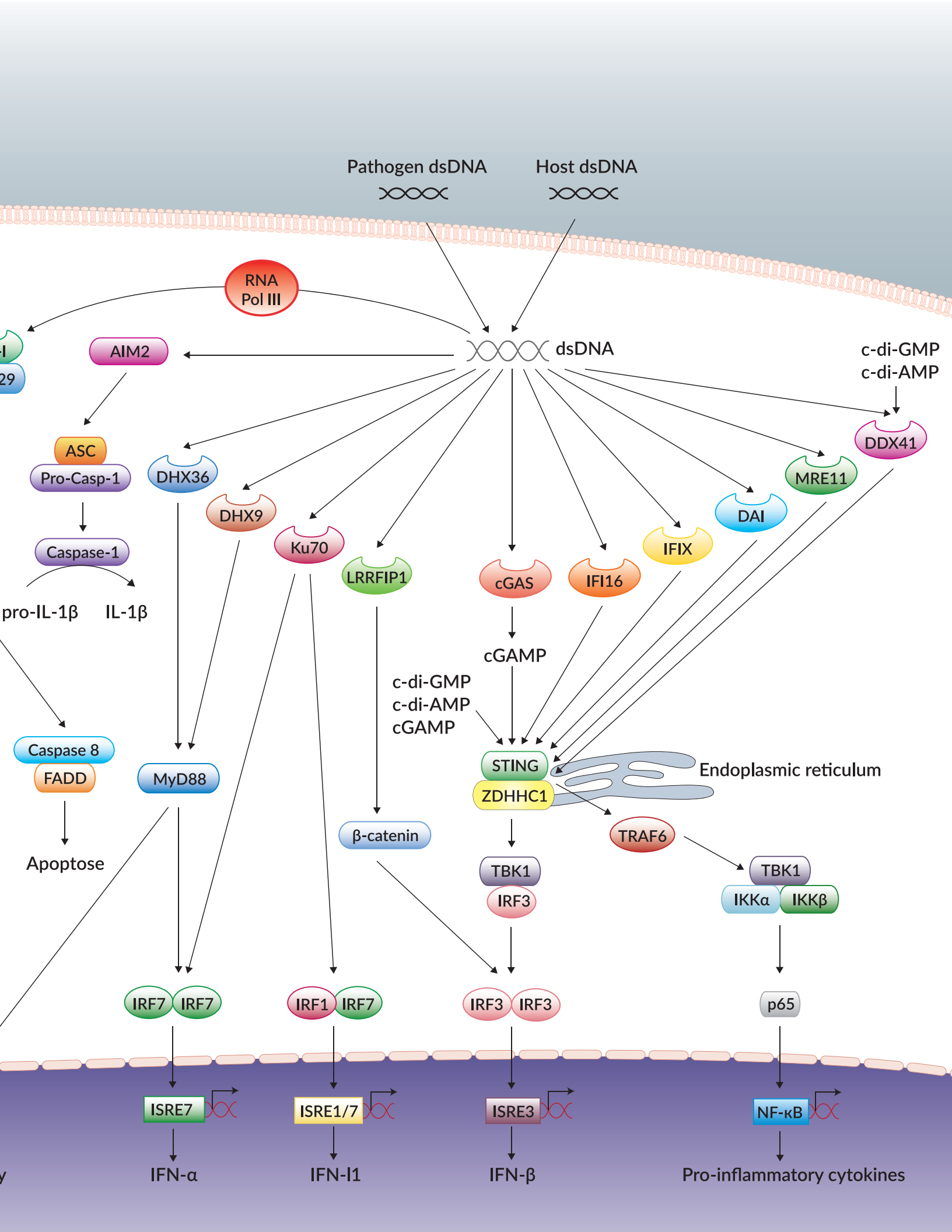

Bacterial cell wall components
TLR2
TLR1/6
LPS
LBP
MD2
CD14
TLR4
Bacterial cell wall components
TLR1
TLR2
TLR6
Flagellin
TLR5
TIRAP
MyD88
TIRAP
MyD88
MyD88
Endosome
CpG DNA
ssRNA
ssRNA
23S rRNA
dsRNA
TLR2
TLR9
TLR7
TLR8
TLR13
TLR3
TLR4
TRAM
MyD88
MyD88
MyD88
TRAM
TRIF
IRAK4
IRAK1
IRAK2
Pellino
TRAF6
Bcl-10
MALT1
TRADD
RIP1
IRAK4
IRAK1
TRAF6
IRAK4
IRAK2
TRAF6
TRAF3
TANK
NAP1
TAB2
TAB3
TAK1
IKKα
NEMO
IKKα
IKKβ
TBK1
IKKε
DDX3
MKKs
NEMO
IKKα
IKKβ
IRF7
p50
IκB
p65
IRF3
ERK
JNK
p38
p50
IκB
p65
ERK
IRF7
IRF7
p50
p65
IRF3
IRF3
c-fos
Jun
p50
p65
ISRE7
NF-κB
ISRE3
AP-1
NF-κB
IFN-α
Pro-inflammatory cytokines
IFN-β
Pro-inflammatory cytokines

TLR & NLR Signaling Pathways



CLR, RLR & CDS Signaling Pathways




Pathogen dsDNA
Host dsDNA
RNA Pol III
AIM2
dsDNA
c-di-GMP
c-di-AMP
DDX41
MRE11
DAI
IFIX
IFI16
cGAS
LRRFIP1
Ku70
DHX9
DHX36
ASC
Pro-Casp-1
Caspase-1
pro-IL-1β
IL-1β
cGAMP
c-di-GMP
c-di-AMP
cGAMP
Caspase 8
FADD
Apoptose
MyD88
STING
ZDHHC1
Endoplasmic reticulum
β-catenin
TRAF6
TBK1
IRF3
TBK1
IKKα
IKKβ
IRF7
IRF7
IRF1
IRF7
IRF3
IRF3
p65
ISRE7
ISRE1/7
ISRE3
NF-κB
IFN-α
IFN-l1
IFN-β
Pro-inflammatory cytokines

TLR LIGANDS

TLR2 Agonists

CL419 - TLR2 Agonist
CL419 (S-(2,3-bis(palmitoyloxy)-(2RS)propyl)-(R)-cysteinyl spermine) is a polyamine TLR2 agonist derived from Pam2CSK4 by replacement of Ser-(Lys)4 by a cationic sperminyl group. CL419 forms positively charged liposomes which allows it to complex nucleic acids and transport them into the cytosol and the nucleus.

FSL-1 - TLR2/6 Agonist
FSL-1 (Pam2CGDPKHPKSF) is a synthetic lipoprotein derived from *Mycoplasma salivarium*, *which is* similar to MALP-2, a *M. fermentans* derived lipopeptide (LP)[1,2]. Mycoplasmal LPs, such as FSL-1, contain a diacylated cysteine residue, whereas bacterial LP contain a triacylated one. FSL-1 is recognized by TLR2 and TLR6, whereas bacterial LPs are recognized by TLR2 and TLR1[3].

HKEB *(Escherichia coli)*
HKEB is a heat-killed preparation of the Gram-negative bacterium, *E.coli O111:B4*. Cell wall components from this bacterium, such as peptidoglycan (PGN) and lipopolysaccharide (LPS), are recognized by TLR2 and TLR4[4]. HKEB can stimulate TLR2 and induce the production of NF-κB and pro-inflammatory cytokines, such as IL-8[5]. HKEB is a potent stimulator of TLR2, and has a weak stimulatory effect on TLR4.

HKLM *(Listeria monocytogenes)*
HKLM is a freeze-dried heat-killed preparation of *Listeria monocytogenes* (LM), a facultative intracellular Gram-positive bacterium. Infection with LM induces the secretion of inflammatory cytokines, such as TNF-α, IL-12, and several chemokines, allowing the recruitment and activation of immune cells. This response is mediated mainly by the interaction between MyD88 and TLR2[6, 7].

HKLR *(Lactobacillus rhamnosus)*
Lactobacillus rhamnosus is a nonpathogenic Gram-positive inhabitant of the human microflora. It is used as a natural preservative in yogurt and other dairy products to extend their shelf life. *L. rhamnosus* is known to have health beneficial effects, such as the nonspecific enhancement of the immune system. Indeed, heat-killed *L. rhamnosus* (HKLR) has been shown to be a potent inducer of TNF-α from mouse mononuclear cells. This immune response is dependent on TLR2 and CD14[8].

HKMT *(Mycobacterium tuberculosis)*
HKMT is a heat-killed preparation of the avirulent strain of *Mycobacterium tuberculosis* H37 Ra. HKMT is sensed by Mincle, which recognizes the mycobacterial cell wall glycolipid, trehalose 6,6'-dimycolate (TDM, also known as cord factor)[9] (see p. 101). HKMT also possesses a large repertoire of TLR2 ligands, such as lipoproteins and lipomannan[10]. Upon HKMT sensing, both Mincle and TLR2 receptors lead to the activation of NF-κB.

HKPA *(Pseudomonas aeruginosa)*
Pseudomonas aeruginosa is a virulent Gram-negative pathogen that infects patients through the respiratory tract, in particular patients with cystic fibrosis. Heat-killed *Pseudomonas aeruginosa* (HKPA) initiates host inflammatory responses through TLR2 and TLR5 but not TLR4[11, 12].

HKPG *(Porphyromonas gingivalis)*
HKPG is a freeze-dried heat-killed preparation of the periodontopathic Gram-negative bacteria *Porphyromonas gingivalis*. In CHO cells expressing TLR2 and CD14, exposure to HKPG induces the activation of NF-κB through TLR2. Expression of TLR4 fails to enhance the response to HKPG suggesting that either the whole bacterial components of *P. gingivalis* are not recognized by TLR4 or some components of these bacteria inhibit TLR4-mediated activation[13].

HKSA *(Staphylococcus aureus)*
HKSA is a lyophilized heat-killed preparation of *Staphyloccocus aureus*, a Gram-positive extra-cellular growing bacterium. HKSA is recognized mainly by TLR2[14]. HKSA induces tolerance to a secondary HKSA stimulation but causes priming to LPS, suggesting a differential regulation of cytokines and chemokines in Gram-positive- and Gram-negative-induced inflammatory events[15].

HKSE *(Staphylococcus epidermidis)*
HKSE is a heat killed preparation of *Staphylococcus epidermidis*, a Gram positive bacterium. *S. epidermidis* is a ubiquitous skin commensal and a major cause of nosocomial bacteremia. The recognition and clearance of *S. epidermidis* bacteremia is mediated by TLR2[16]. *In vitro* studies demonstrate that cell wall components from this bacterium, such as peptidoglycan (PGN), are recognized by TLR2[17].

HKSP *(Streptococcus pneumoniae)*
Streptococcus pneumoniae, a Gram-positive bacterium, is the principal etiologic agent of bacterial meningitis in adults. Heat-killed *Streptococcus pneumoniae* (HKSP) induce activation of NF-κB in a TLR2- and CD14-dependent manner[18]. TLR2 has been shown to play an important role in the protein- and polysaccharide-specific type 1 IgG isotype response following immunization with HKSP[19].

HKST *(Salmonella typhimurium)*
HKST is a heat killed preparation of the flagellated Gram-negative bacterium, *S. typhimurium*. Recognition of HKST is largely mediated by TLR2, TLR4 and TLR5[20, 21]. TLR2 and TLR4 recognize cell wall components from HKST, such as peptidoglycan (PGN) and lipopolysaccharide (LPS), resulting in the production of pro-inflammatory cytokines, such as IL-6 and TNF-α[20] TLR5 recognizes extracellular flagellin present on HKST resulting in NF-κB activation[21].

LM-MS & LAM-MS *(Mycobacterium smegmatis)*
Lipoarabinomannans (LAM) and lipomannans (LM) are lipoglycans found in mycobacterial cell walls. LM and LAM derived from the non-pathogenic *Mycobacterium smegmatis* are proinflammatory molecules[22]. Both LM-MS and LAM-MS activate macrophages in a TLR2-dependent manner[23].

LPS-PG *(Porphyromonas gingivalis)*
Recognition of lipopolysaccharide from *Porphyromonas gingivalis* (LPS-PG), a Gram-negative bacterium, was first believed to be mediated by TLR2 since this LPS, which contains a unique lipid A motif, was able to induce a septic shock in TLR4-deficient C3H/HeJ mice[24]. Now, the TLR2 activity of this LPS is ascribed to a contaminant lipoprotein.

LTA-BS & LTA-SA *(B. subtilis and S. aureus)*
Lipoteichoic acid (LTA) is a major immunostimulatory component of Gram-positive bacteria. Like LPS, LTA is an amphiphile formed by a hydrophilic polyphosphate polymer linked to a neutral glycolipid. LTA stimulates immune cells through TLR2 to produce TNF-α and other inflammatory cytokines[25]. Recognition of LTA also involves LPS-binding protein (LBP) and CD14[26].
InvivoGen provides LTA from *B. subtilis* (LTA-BS) and *S. aureus* (LTA-SA), as well as a purified form of LTA-SA, which is EndoFit™ (<0.001 EU/mg).

Pam2CSK4 - TLR2 Agonist
Pam2CSK4 is a synthetic diacylated lipopeptide (LP) and a strong inducer of NF-κB. Unlike FSL-1, another diacylated LP, it induces signaling in a TLR2-dependent but TLR6-independent manner[27], suggesting that both the lipid and peptide part of lipoproteins take part in the specificity of recognition by TLR2 heterodimers.
Pam2CSK4 Biotin and **Pam2CSK4 Rhodamine** are available.

Pam3CSK4 - TLR1/2 Agonist

Pam3CSK4 is a synthetic triacylated lipopeptide (LP) that mimics the acylated amino terminus of bacterial LPs. Pam3CSK4 is a potent activator of the proinflammatory transcription factor NF-κB[28]. Activation is mediated by the interaction between TLR2 and TLR1 which recognize lipopeptides with three fatty acids, a structural characteristic of bacterial LPs[29].

Pam3CSK4 Biotin and **Pam3CSK4 Rhodamine** are available.

PGN-BS, PGN-EB, PGN-EK & PGN-SA *(B. subtilis, E. coli and S. aureus)*

Peptidoglycan (PGN) is a major surface component of Gram-positive bacteria. It is embedded in a relatively thick cell wall and is usually covalently attached to other polymers, such as lipoproteins and LTAs. PGN is known to be a potent activator of NF-κB and TNF-α through TLR2[5]. However, other pattern recognition proteins have been reported to mediate the immunostimulatory activity of PGN[30-32]. This discrepancy is attributed to the method of purification.

PGN-BS, PGN-EB, PGN-EK and PGN-SA are purified by detergent lysis, enzymatic treatment, LiCl/EDTA and acetone cleaning. These preparations of PGN induce the production of NF-κB through TLR2.

Zymosan - TLR2 & Dectin-1 Agonist

Zymosan, an insoluble preparation of cell wall from *Saccharomyces cerevisiae*, activates macrophages via TLR2. TLR2 cooperates with TLR6 and CD14 in response to zymosan[29]. Zymosan is also recognized by Dectin-1, a phagocytic receptor expressed on macrophages and dendritic cells, which collaborates with TLR2 and TLR6 enhancing the immune responses triggered by the recognition of Zymosan by each receptor[33].

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RELATED PRODUCTS

PRODUCT	INFO
HEK-Blue TLR2 cells	See page 42
Anti-TLR2 antibodies	See page 133

TLR3 Agonists

Poly(A:U)

Polyadenylic–polyuridylic acid (poly(A:U)) is a synthetic double stranded RNA molecule that signals only through TLR3. Recognition of poly(A:U) by TLR3 induces the activation of dendritic cells and T lymphocytes. When combined with an antigen in mice, poly(A:U) has been shown to promote antigen-specific Th1-immune responses and boost antibody production[3]. The potent adjuvant activity of poly(A:U) has been exploited in the treatment of breast cancers that express TLR3[4].

Poly(I:C) (HMW) & Poly(I:C) (LMW)

Polyinosine-polycytidylic acid (poly(I:C)) is a synthetic analog of double-stranded RNA (dsRNA), a molecular pattern associated with viral infection. Poly(I:C) is recognized by TLR3 inducing the activation of NF-κB and the production of cytokines through distinct mechanisms that are MyD88-dependent or MyD88-independent[1,2].
InvivoGen provides poly(I:C) with a high molecular weight (HMW) or a low molecular weight (LMW) that might activate the immune system differently:
Poly(I:C) (HMW) has an average size of 1.5-8 kb. It is available labeled with biotin, fluorescein or rhodamine.
Poly(I:C) (LMW) has an average size of 0.2-1 kb. It is available labeled with rhodamine.

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RELATED PRODUCTS

PRODUCT	INFO
HEK-Blue TLR3 cells	See page 43

4

TLR4 Agonists & Antagonists

Bacterial lipopolysaccharide (LPS) is the major structural component of the outer wall of all Gram-negative bacteria and a potent activator of the immune system. LPS is recognized by Toll-like receptor 4 (TLR4) which interacts with three different extracellular proteins: LPS binding protein (LBP), CD14 and, myeloid differentiation protein 2 (MD-2), to induce a signaling cascade leading to the activation of NF-κB and the production of proinflammatory cytokines.
LPS molecules consist of three components: the polysaccharide chain named the O-antigen, the core oligosaccharide and lipid A. The latter, also known as endotoxin, is responsible for the immunostimulatory activity of LPS. The most active form of lipid A contains six fatty acyl groups and is found in pathogenic bacteria such as Escherichia coli and Salmonella species[1]. Underacylated lipid A structures, containing four or five fatty acids, induce markedly less host defense responses and can inhibit in a dose-dependent manner the strong endotoxic response triggered by hexa-acylated LPS[2]. In certain bacteria, the O-antigen may be absent giving rise to a so-called rough LPS in contrast to the typical phenotype of smooth LPS. The lack of the O-antigen modulates the process of LPS recognition by cells of the immune system.

LPS-B5 Standard & Ultrapure (*E. coli 055:B5*)

LPS-B5 is a smooth (S)-form lipopolysaccharide (LPS) extracted from *E coli* 055:B5, often used as an endotoxin standard in Limulus amebocyte lysate (LAL) assays. LPS-B5 is extracted by successive enzymatic hydrolysis steps and purified by a phenol-TEA-DOC extraction method[4] and provided as two grades of purity. The standard grade of LPS-B5 contains other bacterial components, such as lipopeptides, and therefore stimulates both TLR4 and TLR2. The ultrapure grade of LPS-PG only activates TLR4.

LPS-EB Standard & Ultrapure (*E. coli O111:B4*)

LPS-EB is an S-form LPS extracted from *E. coli O111:B4*. This LPS is widely used to study the immune response to LPS. LPS-EB is prepared similarly to LPS-B5. LPS-EB standard activates both TLR4 and TLR2, while LPS-EB ultrapure only activates TLR4.
LPS-EB (Ultrapure) Biotin is available.

LPS-EK Standard & Ultrapure (*E. coli K12*)

LPS-EK is a rough (R)-form LPS extracted from *E. coli K12*. LPS from *E coli* K12 is structurally heterogeneous and characterized by the occurence of heptose in the outer core and rhamnose in the inner core[5]. LPS-EK is prepared similarly to LPS-B5. LPS-EK standard activates both TLR4 and TLR2, while LPS-EK ultrapure only activates TLR4.

LPS-PG Standard & Ultrapure (*Porphyromonas gingivalis*)

LPS from *P. gingivalis* (LPS-PG), an important virulence factor in the mechanisms of periodontal diseases, presents a unique and heterogenous chemical structure, which differs from traditionally recognized enteric bacterium-derived LPS. The fact that LPS-PG exhibits activity in C3H/HeJ mice, which are deficient for TLR4, led to the common belief that this LPS is a TLR2 ligand[6,7]. However, structural and functional studies of LPS-PG have revealed that it activates cells through TLR4. The TLR2 activity of this LPS has been ascribed to a contaminant lipoprotein[8]. InvivoGen provides LPS-PG with two grades of purity. LPS-PG Standard contains the lipoprotein and thus activates both TLR4 and TLR2, while LPS-PG Ultrapure, which underwent an enzymatic treatment, contains no detectable lipoprotein and activates only TLR4.

LPS-RS Standard & Utrapure (*Rhodobacter sphaeroides*)

LPS from the photosynthetic bacterium *Rhodobacter sphaeroides* (LPS-RS) is a potent antagonist of LPS from pathogenic bacteria[1]. Complete competitive inhibition of LPS activity is possible at a 100 fold excess of the antagonist. LPS-RS does not induce TLR4 signaling but is detected by the LAL assay, the standard endotoxin detection assay. LPS-RS is a standard preparation that possesses TLR2 activity. LPS-RS Ultrapure is a purified preparation by phenol extraction of LPS-RS. It displays strong TLR4 antagonist activity while lacking TLR2 activity.

LPS-SM Ultrapure (*S. minnesota*)

LPS-SM ultrapure is a R-form LPS isolated and purified from *Salmonella minnesota* R595 (Re mutant). This LPS is solely composed of lipid A and 3-deoxy-D-manno-octulosonic acid (2-keto-3-deoxyoctonate, KDO). LPS-SM is extracted by successive enzymatic hydrolysis steps and purified by a phenol-TEA-DOC extraction method[4]. LPS-SM strongly activates TLR4 and has little to no activity on TLR2.

MPLA-SM (*S. minnesota*)

MPLA (monophosphoryl lipid A) is the less toxic form of lipid A, which contains two phosphate groups. MPLA-SM is prepared from the LPS produced by the Re mutant of *Salmonella minnesota* R595. MPLA-SM is extracted using treatment with acid and heat followed by chromatography. It strongly activates TLR4 and has little to no activity on TLR2.

MPLAs (synthetic)

MPLAs is a synthetic monophosphoryl lipid A from *E. coli* with 6 fatty acyl groups. It is structurally very similar to natural MPLA except that natural MPLA contains a mixture of 5, 6, and 7 acyl Lipid A. This synthetic *E. coli* MPLA is a potent inducer of TLR4 but has no activity on TLR2 even at high concentrations reflecting its high purity.

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RELATED PRODUCTS

PRODUCT	INFO
HEK-Blue TLR4 cells	See page 42
Anti-TLR4 antibodies	See page 133

TLR5 Agonists

Flagellin is the major component of the bacterial flagellar filament, which confers motility on a wide range of bacterial species. Extracellular flagellin is recognized by TLR5, which promotes the activation of NF-κB and the subsequent production of cytokines[1]. Flagellin monomers are also translocated into the host cell cytosol by a mechanism that requires bacterial secretion systems. This triggers the formation of a NAIP-NLRC4 inflammasome, leading to the secretion of IL-1β[2].

FLA-BS Standard & Ultrapure (*B. subtilis*)

FLA-BS is a flagellin isolated from the Gram-positive bacterium *Bacillus subtilis*. It is purified by acid hydrolysis, heating and ultrafiltration according to Ibrahim GF. *et al.*[3]. The purity of FLA-BS is estimated at 10%.
FLA-BS Ultrapure is a high purity grade of flagellin. It is extracted by violent agitation and purified by several different separation techniques resulting in the depolymerized protein. This flagellin is >95% pure and migrates on SDS-PAGE at ~30 kDa.

FLA-PA Ultrapure (*P. aeruginosa*)

Pseudomonas aeruginosa is a virulent Gram$^-$ bacterial pathogen that infects the respiratory tracts. FLA-PA Ultrapure is a flagellin isolated from *P. aeruginosa* by acid hydrolysis and purified by ultrafiltration and chromatography with a purity of >95%. FLA-PA Ultrapure migrates on SDS-PAGE at ~52 kDa and strongly activates TLR5.

FLA-ST Standard & Ultrapure (*S. typhimurium*)

FLA-ST is a flagellin isolated from the Gram-negative bacteria *S. typhimurium*. It is purified by acid hydrolysis, heating and ultrafiltration according to Ibrahim GF. *et al.*[3]. The purity of FLA-ST is estimated at 10%.
FLA-ST ultrapure is further purified by monoclonal anti-FliC affinity chromatography. The purity of FLA-ST ultrapure is >95%.

RecFLA-ST (*S. typhimurium*)
RecFLA-ST is a recombinant flagellin purified from mammalian cells transfected with the FliC gene which encodes flagellin in *S. typhimurium*. RecFLA-ST is endotoxin-free according to the gel clot LAL Assay. It activates TLR5 but does not activate TLR2 nor TLR4.

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RELATED PRODUCTS

PRODUCT	INFO
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RECENT CITATIONS

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TLR7/8 Agonists

CL075 - TLR7/8 Agonist
CL075 (3M002) is a thiazoloquinolone derivative that stimulates TLR8 in human PBMC. It activates NF-κB and triggers preferentially the production of TNF-α and IL-12[1]. CL075 also seems to induce the secretion of IFN-α through TLR7 but to a lesser extent. It induces the activation of NF-κB at 0.4 µM (0.1 µg/ml) in TLR8-transfected HEK293 cells, and ~ 10 times more CL075 is required to activate NF-κB in TLR7-transfected HEK293 cells.

CL097 - TLR7/8 Agonist
CL097 is a highly water-soluble derivative of the imidazoquinoline compound R848 (≥ 20 mg/ml). Similarly to R848, CL097 is a TLR7 and TLR8 ligand[2, 3]. CL097 induces the production of pro-inflammatory cytokines in macrophages and plasmacytoid dendritic cells[4] and has been demonstrated to enhance both cellular and humoral immunity making it a promising candidate adjuvant[5].

CL264 - TLR7 Agonist
CL264 is a novel 9-substituted-8 hydroxyadenine derivative. Similarly to SM360320, CL264 induces the activation of NF-κB and the secretion of IFN-α in TLR7-expressing cells[6]. CL264 is a TLR7-specific ligand, it does not stimulate TLR8 even at high concentrations (> 10 mg/ml). In TLR7-transfected HEK293 cells, CL264 triggers NF-κB activation at a concentration of 0.1 µM which is 5-10 times less than imiquimod.
CL264 is available labeled with FITC or rhodamine.

CL307 - TLR7 Agonist
CL307 (N1-glycinyl[4-((6-amino-2-(butylamino)-8-hydroxy-9H-purin-9-yl)methyl)benzoyl] spermine) was generated by covalently linking a spermine to the hydroxyadenine compound CL264[7]. CL307 is a very potent TLR7 agonist. Titration experiments have showed that CL307 induces robust NF-κB activation even at concentrations as low as 20 nM (10 ng/ml).

Gardiquimod - TLR7 Agonist
Gardiquimod is an imidazoquinoline compound developed and manufactured by InvivoGen. Gardiquimod induces the activation of NF-κB in HEK293 cells expressing human or mouse TLR7 and it is more potent than its first member of the imidazoquinlone analog imiquimod. Gardiquimod is specific for TLR7 when used at less than 1 µg/ml.

Imiquimod - TLR7 Agonist
Imiquimod (R837), an imidazoquinoline amine analogue to guanosine, is an immune response modifier with potent indirect antiviral activity. This low molecular weight synthetic molecule induces the production of cytokines such as IFN-α through the activation of TLR7[9]. This activation is MyD88-dependent and leads to the induction of the transcription factor NF-κB[10].

Loxoribine - TLR7 Agonist
Loxoribine is a guanosine analog derivatized at position N7 and C8. This L-nucleoside is a strong stimulator of the immune system[11]. It signals through TLR7 leading to the activation of NF-κB[9, 12]. Similarly to the imidazoquinoline compound imiquimod, loxoribine recognition is restricted to TLR7.

ORN02 & ORN06 - TLR8 Agonists
ORN06 is a 20 mer oligoribonucleotide (ORN) containing 6 repeats of the UUGU sequence motif, identified as the minimal motif responsible for ssRNA40 immunoactivity[21]. ORN02 is also a 20 mer ORN, which derives from ORN06 by substitution of G to A. ORN02 and ORN06 are provided pre-complexed with the cationic lipid LyoVec™ to facilitate its uptake.

Poly(dT) - TLR7/8 Modulator
Poly(dT), a thymidine homopolymer phosphorothioate ODN, is a modulator of human TLR7 and TLR8. In combination with an imidazoquinoline, such as R848 and CL075, it increases TLR8-mediated signaling but abolishes TLR7-mediated signaling[13, 14]. Alone poly(dT) has no significant effect on either of these TLRs. Furthermore, co-incubation of poly(dT) and an imidazoquinoline was shown to induce NF-kB activation in HEK293 cells transfected with murine TLR8- and primary TLR8-expressing mouse cells, demonstrating that murine TLR8 is functional[15].

R848 - TLR7/8 Agonist
R848 is an imidazoquinoline compound with potent anti-viral activity. This low molecular weight synthetic molecule activates immune cells via the TLR7/TLR8 MyD88-dependent signaling pathway[10, 13]. Recently, R848 was shown to trigger NF-κB activation in cells expressing murine TLR8 when combined with poly(dT)[15]. Unlike other commercially available R848 preparations, InvivoGen's R848 is water soluble (~1 mg/ml).

ssPolyU - TLR8 Agonist

ssPolyU is a single-stranded poly-uridine (polyU) oligonucleotide, which can substitute for viral RNAs in inducing IFN-α production in plasmacytoid dendritic cells[18]. ssPolyU is provided naked or complexed with the cationic lipid LyoVec™, to protect it from degradation and facilitate its uptake.

ssRNA40 - TLR8 Agonist

ssRNA40 is a 20-mer phosphorothioate protected single-stranded RNA oligonucleotide containing a GU-rich sequence. ssRNA40 is complexed with the cationic lipid LyoVec™, to protect it from degradation and facilitate its uptake. When complexed with cationic lipids, ssRNA40 can substitute for viral RNAs in inducing TNF-α and IFN-α production in peripheral blood mononuclear cells[18, 19].

ssRNA41 - TLR8 Agonist Negative Control

ssRNA41 is a 20-mer phosphorothioate protected ssRNA derived from ssRNA40 by replacement of all U nucleotides with adenosine[2]. ssRNA41 is complexed with the cationic lipid LyoVec™, to protect it from degradation and facilitate its uptake, and lyophilized to generate ssRNA41/LyoVec. Unlike ssRNA40, ssRNA41 is unable to induce the production of type I IFNs, and therefore can be used as a negative control for ssRNA40[19, 20].

ssRNA-DR - TLR8 Agonist

ssRNA-DR is a short single-stranded RNA (<30 bp) that contains two copies of the 9 mer sequence GUCCUUCAA. This sequence is a putative immunostimulatory motif recognized by human TLR8 and mouse TLR7 that induces type I interferons[21]. ssRNA-DR is provided pre-complexed with the cationic lipid LyoVec™ to facilitate its uptake.

TL8-506 - TLR8 Agonist

TL8-506 is a benzoazepine compound, analog of the TLR8 agonist VTX-2337[16, 17]. TL8-506 activates TLR8 more potently than R848 and CL075. TL8-506 is ~50x and ~25x more potent in inducing NF-kB activation in TLR8-transfected HEK293 cells than R848 and CL075, respectively. TL8-506 is a selective agonist of TLR8, it is ~500x more active on TLR8-transfected HEK293 than TLR7-transfected HEK293 cells. TL8-506 has been formulated to increase its solubility in water.

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R848 - Shang Y. *et al.*, 2016. The transcriptional repressor Hes1 attenuates inflammation by regulating transcription elongation. Nat Immunol. [Ahead of print].

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RELATED PRODUCTS

PRODUCT	INFO
HEK-Blue TLR7 cells	See page 42
HEK-Blue TLR8 cells	See page 42

TLR9 Agonists

CpG ODNs

Bacterial DNA contains unmethylated "CpG motifs" that are recognized Toll-like receptor (TLR) 9 and induce strong immunostimulatory effects in mammals. Synthetic oligodeoxynucleotides containing such CpG motifs (CpG ODNs) stimulate B cells, natural killer (NK) cells and professional antigen-presenting cells to proliferate and/or secrete a variety of cytokines, chemokines and immunoglobulins. Three major classes of stimulatory CpG ODNs have been identified based on structural characteristics and activity on human peripheral blood mononuclear cells (PBMCs), in particular B cells and plasmacytoid dendritic cells (pDCs). These three classes are Class A (Type D), Class B (Type K) and Class C.

- **Class A CpG ODNs** are characterized by a PO central CpG-containing palindromic motif and a PS-modified 3' poly-G string. They induce high IFN-α production from pDCs but are weak stimulators of TLR9-dependent NF-κB signaling and pro-inflammatory cytokine production.
- **Class B CpG ODNs** contain a full PS backbone with one or more CpG dinucleotides. They strongly activate B cells and TLR9-dependent NF-κB signaling but weakly stimulate IFN-α secretion.
- **Class C CpG ODNs** combine features of both classes A and B. They contain a complete PS backbone and a CpG-containing palindromic motif. C-Class CpG ODNs induce strong IFN-α production from pDC as well as B cell stimulation.

These stimulatory CpG ODNs differentially induce the stimulation of human and murine immune cells *in vitro*. This species-specificity is also observed with nonresponsive cells such as HEK293 cells transfected with human or mouse TLR9.

Class A Stimulatory ODNs

ODN 1585 (mouse preferred)[1]	5'-ggGGTCAACGTTGAgggggg-3'
ODN 2216 (human preferred)[2]	5'-ggGGGACGA:TCGTCgggggg-3'
ODN 2336 (human preferred)[3]	5'-gggGACGAC:GTCGTGgggggg-3'

Class A Control ODNs

ODN 1585 control	5'-ggGGTCAAGCTTGAgggggg-3'
ODN 2243 (2216 control)	5'-ggGGGAGCA:TGCTGgggggg-3'
ODN 2336 control	5'-gggGAGCAG:CTGCTGgggggg-3'

Class B Stimulatory ODNs

ODN 1668 (mouse preferred)[4]	5'-tccatgacgttcctgatgct-3'
ODN 1826 (mouse preferred)[5]	5'-tccatgacgttcctgacgtt-3
ODN 2006 (human preferred)[3, 6]	5'-tcgtcgttttgtcgttttgtcgtt-3'
ODN 2007 (bovine/porcine)[7, 8]	5'-tcgtcgttgtcgttttgtcgtt-3
ODN BW006 (human/mouse)[9, 10]	5'-tcgacgttcgtcgttcgtcgttc-3'
ODN D-SL01 (multispecies)[11]	5'-tcgcgacgttcgcccgacgttcggta-3'
ODN 2006-G5[12]	5'-TCGTCGTTTTGTCGTTTTGTCGTTGGGGG-3'

Class B Control ODNs

ODN 1668 control	5'-tccatgagcttcctgatgct-3'
ODN 1826 control	5'-tccatgagcttcctgagctt-3'
ODN 2006 control	5'-tgctgctttgtgctttgtgctt-3'
ODN 2007 control	5'-tgctgcttgtgctttgtgctt-3'
ODN BW007	5'-tgcagcttgctgcttgctgcttc-3'

Class C Stimulatory ODNs

ODN 2395 (human/mouse)[3, 6]	5'-tcgtcgttttcggcgc:gcgccg-3'
ODN M362 (human/mouse)[13]	5'-tcgtcgtcgttc:gaacgacgttgat-3'
ODN D-SL03 (multispecies)[11]	5'-tcgcgaac:gttcgccgcgttc:gaacgcgg-3'

Class C Control ODNs

ODN 2395 control	5'-tgctgctttgggggggcccccc-3'
ODN M362 control	5'-tgctgctgcttg:caagcagcttgat-3'

Bases in capital letters are phosphodiester, bases in lower case are phosphorothioate. Palindrome is underlined.

ODN 1585, ODN 1668, ODN 1826, ODN 2006, ODN 2216, ODN 2336, ODN2395 and ODN M362 are available labeled with FITC. ODN 1826, ODN 2006 and ODN 2216 are available labeled with biotin.

E. coli ssDNA/LyoVec - TLR9 Agonist

E. coli ssDNA/LyoVec™ as sheared single-stranded DNA fragments produced by treating genomic *E. coli* DNA with ultrasound followed by heat denaturation. These fragments are complexed with LyoVec™, a lipid-based transfection reagent, to allow penetration of the DNA in the cells. *E. coli* ssDNA/LyoVec™ activate TLR9 similarly to CpG-ODNs in a species-independent manner.

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ODN 1826 - Hubbard N. *et al.*, 2016. Targeted gene editing restores regulated CD40L function in X-linked hyper-IgM syndrome. Blood. 127(21):2513-22.

ODN 2006 - DeKosky BJ. *et al.*, 2016. Large-scale sequence and structural comparisons of human naive and antigen-experienced antibody repertoires. PNAS 113(19):E2636-45.

ODN 2216 - Fang F. *et al.*, 2016. TLR9 signaling is augmented in systemic sclerosis and elicits TGF-β-dependent fibroblast activation. Arthritis Rheumatol. [Ahead of print].

ODN 2395 - Brennan TV. *et al.*, 2016. Heparan sulfate mimetic PG545-mediated antilymphoma effects require TLR9-dependent NK cell activation. J Clin Invest. 126(1):207-19.

RELATED PRODUCTS

PRODUCT	INFO
HEK-Blue TLR9 cells	See page 42

TLR9 Antagonists

Inhibitory ODNs

Recent studies suggest the existence of DNA sequences that can neutralize the stimulatory effect of CpG ODNs. The most potent inhibitory sequences are (TTAGGG)4 found in mammalian telomeres[1] and ODN 2088, which derives from a murine stimulatory CpG ODN by replacement of 3 bases[2]. Other inhibitory ODNs have been described. G-ODN is a guanosine-rich ODN suppressive in murine DC and macrophages, as well as in human plasmacytoid DC[3]. ODN 4084-F, ODN INH-1 and ODN INH-18 contain an inhibitory DNA motif consisting of two nucleotide triplets, a proximal CCT and a more distal GGG, spaced from each other by four nucleotides[4,5]. Inhibitory ODNs seem to act by disrupting the colocalization of CpG ODNs with TLR9 in endosomal vesicles without affecting cellular binding and uptake. Inhibitory ODNs are often utilized to demonstrate a TLR9 dependence in murine systems.

ODN 2088 (mouse preferred)[2]	5'-tcctggcggggaagt-3'
ODN 2088 control	5'-tcctgagcttgaagt-3'
ODN TTAGGG (human preferred)[1]	5'-tttagggttagggttagggttaggg-3'
ODN TTAGGG control	5'-gctagatgttagcgt-3'
G-ODN[3]	5'-ctcctattgggggtttcctat-3'
G-ODN control	5'-ctcctattgtgtgtttcctat-3'
ODN 4084-F (human/mouse)[4, 5]	5'-cctggatgggaa-3'
ODN INH-1 (human/mouse)[4, 5]	5'-cctggatgggaa:ttcccatccagg-3'
ODN INH-18 (human/mouse)[4, 5]	5'-cctggatgggaacttaccgctgca-3'
ODN INH-47 (control)	5'-tatggattttaa:ttaaaatccata-3'

Bases are phosphorothioate.

1. Stunz LL. *et al.*, 2002. Inhibitory oligonucleotides specifically block effects of stimulatory CpG oligonucleotides in B cells. Eur J Immunol. 32(5):1212-22. **2. Gursel L. *et al.*, 2003.** Repetitive elements in mammalian telomeres suppress bacterial DNA-induced immune activation. J Immunol. 171(3):1393-400. **3. Peter M. *et al.*, 2008.** Characterization of suppressive oligodeoxynucleotides that inhibit Toll-like receptor-9-mediated activation of innate immunity. Immunology. 123(1):118-28. **4. Lenert P, *et al.*, 2003.** Structural characterization of the inhibitory DNA motif for the type A (D)-CpG-induced cytokine secretion and NK-cell lytic activity in mouse spleen cells. DNA Cell Biol. 22(10):621-31. **5. Lenert P, *et al.*, 2009.** DNA-like class R inhibitory oligonucleotides (INH-ODNs) preferentially block autoantigen-induced B-cell and dendritic cell activation in vitro and autoantibody production in lupus-prone MRL-Fas(lpr/lpr) mice in vivo. Arthritis Res Ther. 11(3):R79.

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ODN TTAGGG - Flierl U. *et al.*, 2015. Phosphorothioate backbone modifications of nucleotide-based drugs are potent platelet activators. J Exp Med. 212(2):129-37.

TLR13 Agonists

ORN Sa19 & ORN Sa19 Control

ORN Sa19 is a 19 mer *S. aureus* 23S rRNA derived oligoribonucleotide[1]. This ORN, which contains an A in its center to mirror *S. aureus* A2085, is highly stimulatory in TLR13-expressing cells in contrast to ORN Sa19 Control, which carries a G in place of the central A. ORN Sa19 and ORN Sa19 Control are stabilized by phosphorothioate modification.

ORN Sa19	5'-GGACGG**A**AAGACCCCGUGG-3'
ORN Sa19 Control	5'-GGACGG**G**AAGACCCCGUGG-3'

1. Oldenburg M. *et al.*, 2012. TLR13 recognizes bacterial 23S rRNA devoid of erythromycin resistance-forming modification. Science. 337(6098).

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ORN Sa19- Kameyama T. &, Takaoka A., 2014. Characterization of innate immune signalings stimulated by ligands for pattern recognition receptors. Methods Mol Biol. 2014;1142:19-32.

RELATED PRODUCTS

PRODUCT	INFO
HEK-Blue TLR13 cells	See page 42

NLR LIGANDS

NOD1 Agonists

C12-iE-DAP - NOD1 Agonist
C12-iE-DAP is an acylated derivative of iE-DAP. It was generated by addition of a lauroyl (C12) group to the glutamic residue of iE-DAP. C12-iE-DAP specifically stimulates NOD1 at concentrations 100- to 1000-fold lower than the original iE-DAP.

iE-DAP - NOD1 Agonist
iE-DAP (D-γ-Glu-mDAP) is a dipeptide present in the PGN of a subset of bacteria that include Gram-negative bacilli and particular Gram-positive bacteria such as *Bacillus subtilis* and *Listeria monocytogenes*[1]. iE-DAP is the minimal motif recognized by NOD1.
iE-Lys is a peptide found in the PGN of Gram-positive bacteria. It is not recognized by NOD1 and thus can be used as negative control for iE-DAP.

Tri-DAP - NOD1 Agonists
Tri-DAP (L-Ala-γ-D-Glu-mDAP) comprises the iE-DAP dipeptide and an L-Ala residue. Similarly to iE-DAP, this tripeptide is specifically recognized by NOD1 but exhibits a ~3-fold higher ability to activate NF-κB than iE-DAP[2].
Tri-Lys is a peptide found in the PGN of Gram-positive bacteria. It is not recognized by NOD1 and thus can be used as negative control for Tri-DAP.

1. **Chamaillard M. *et al.*, 2003.** An essential role for NOD1 in host recognition of bacterial peptidoglycan containing diaminopimelic acid. Nat. Immunol.4(7):702-7.
2. **Girardin SE. *et al.*, 2003.** Peptidoglycan molecular requirements allowing detection by Nod1 and Nod2. J Biol Chem. 278(43):41702-8.

RECENT CITATIONS

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Tri-DAP - Hedl M. & Abraham C., 2015. A TPL2 (MAP3K8) disease-risk polymorphism increases TPL2 expression thereby leading to increased pattern recognition receptor-initiated caspase-1 and caspase-8 activation, signalling and cytokine secretion. Gut. [Ahead of print]

RELATED PRODUCTS

PRODUCT	INFO
HEK-Blue NOD1 cells	See page 44

NOD1/2 Agonists

M-TriDAP - NOD1/ NOD2 Agonist
M-TriDAP (MurNAc-L-Ala-D-g-Glu-mDAP), also called DAP-containing muramyl tripeptide is a peptidoglycan (PGN) degradation product found mostly in Gram-negative bacteria. M-TriDAP is recognized by NOD1 and to a lesser extent NOD2. M-TriDAP induces the activation of NF-κB at similar levels to Tri-DAP[1].

PGN-ECndi Ultrapure - NOD1/2 Agonist
PGN-ECndi from *E. coli K12* is an insoluble preparation of PGN purified by detergent lysis and hydrolysis under basic conditions to eliminate lipophilic constituents. This PGN preparation has lost its ability to activate TLR2-transfected HEK293 cells but still activates NOD2-transfected cells. PGN-ECndi activates also NOD1-transfected cells.

PGN-ECndss Ultrapure - NOD1/2 Agonist
PGN-ECndss from *E. coli K12* is obtained after sonication of insoluble PGNs. At the working concentrations, it activates HEK293 cells transfected with either NOD1 or NOD2 but not cells transfected with TLR2 or TLR4.

PGN-SAndi ultrapure
PGN-SAndi is an insoluble PGN preparation extracted from *S. aureus* purified by detergent lysis and hydrolysis under basic conditions to eliminate lipophilic constituents, responsible for the TLR2-dependent activity of PGN. PGN-SAndi activates NOD2-transfected cells but not NOD1- or TLR2-transfected cells.

1. **Girardin SE. *et al.*, 2003.** Peptidoglycan molecular requirements allowing detection by Nod1 and Nod2. J Biol Chem. 278(43):41702-8.

RECENT CITATIONS

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PGN-ECndi & PGN-ECndss ultrapure - Read CB. ***et al.*, 2015.** Cutting Edge: identification of neutrophil PGLYRP1 as a ligand for TREM-1. J Immunol. 194(4):1417-21.

RELATED PRODUCTS

PRODUCT	INFO
HEK-Blue NOD2 cells	See page 44

NOD2 Agonists

CL429 - TLR2 & NOD2 Agonist

CL429 is a novel chimeric compound that stimulates both TLR2 and NOD2. This compound is composed of murabutide (NOD2 ligand) covalently linked to Pam2C (TLR2 ligand) via a spacer[1]. CL429 is able to induce both receptors in RAW-derived macrophages significantly more than murabutide and Pam2C separately.

MDP & L18-MDP

MDP (MurNAc-L-Ala-D-isoGln, also known as muramyl dipeptide), is the minimal bioactive peptidoglycan motif common to all bacteria and the essential structure required for adjuvant activity in vaccines. MDP has been shown to be recognized by NOD2, but not TLR2, nor TLR2/1 or TLR2/6 associations[2, 3]. This recognition is highly stereospecific of the L-D isomer, excluding any reaction to the D-D or L-L analogs[3, 4]. NOD2 mutants associated with susceptibility to Crohn's disease have been found to be deficient in their recognition of MDP[2, 3]. The potent adjuvant activity of MDP may also be linked to an activation of the CIAS1/NALP3/Cryopyrin inflammasome[5].

L18-MDP is a synthetic derivative of MDP. It has been shown to display a higher adjuvant activity than MDP[6].

MDP control (MurNAc-D-Ala-D-isoGln), the D-D isomer of muramyl dipeptide, is unable to induce NOD2 signaling.

MDP is available labeled with biotin, FITC or rhodamine.

M-TriLYS

M-TriLYS (MurNAc-Ala-D-isoGln-Lys) is a muropeptide released by *Lactobacillus salivarus* after digestion of their PGN. This chemically synthesized muropeptide is sensed by NOD2 and induces the activation of NF-κB[7]. M-TriLYS has been shown to produce the anti-inflammatory cytokine IL-10 and protect mice from colitis.

Murabutide

Murabutide (MurNAc-L-Ala-D-GlnOBu) is a synthetic immuno-modulator derived from MDP. In contrast to MDP, murabutide is devoid of pyrogenic activity[8] and lacks somnogenic activity[9]. Murabutide is recognized by the intracellular receptor NOD2 inducing the activation of NF-κB.

Murabutide control contains D-alanine instead of L-alanine and is inactive on NOD2.

N-glycolyl-MDP

The cell wall of mycobacteria is known to be extremely immunogenic. This potent activity is attributed to their MDP which is N-glycolylated in contrast to the MDP of most bacteria which is N-acetylated. N-glycolyl-MDP has been reported to display a stronger NOD2-mediated activity than N-acetyl-MDP and thus to be a more potent vaccine adjuvant than N-acetyl-MDP[10].

1. Pavot V. et al., 2014. Cutting edge: New chimeric NOD2/TLR2 adjuvant drastically increases vaccine immunogenicity. J Immunol. 193(12):5781-5. **2. Girardin SE. *et al.*, 2003.** Nod2 is a general sensor of peptidoglycan through muramyl dipeptide (MDP) detection. J Biol Chem. 278(11):8869-72. **3. Inohara N. *et al.*, 2003.** Host recognition of bacterial muramyl dipeptide mediated through NOD2. Implications for Crohn's disease. J Biol Chem. 278(8):5509-12. **4. Traub S. *et al.*, 2004.** Structural requirements of synthetic muropeptides to synergize with lipopolysaccharide in cytokine induction. J Biol Chem. 279(10):8694-700. **5. Martinon F. *et al.*, 2004.** Identification of bacterial muramyl dipeptide as activator of the NALP3/cryopyrin inflammasome. Curr Biol. 14(21):1929-34. **6. Ishihara C. *et al.* 1985.** Effect of muramyl dipeptide and its stearoyl derivatives on resistance to Sendai virus infection in mice. Vaccine. 3(5):370-4. **7. Macho Fernandez E. *et al.*, 2011.** Anti-inflammatory capacity of selected lactobacilli in experimental colitis is driven by NOD2-mediated recognition of a specific peptidoglycan-derived muropeptide, Gut, 60: 1050 - 1059. **8. Chedid LA. *et al.*, 1982.** Biological activity of a new synthetic muramyl peptide adjuvant devoid of pyrogenicity. Infect Immun. 35(2):417-24. **9. Krueger JM. *et al.*, 1984.** Muramyl peptides. Variation of somnogenic activity with structure. J Exp Med. 159(1):68-76. **10. Coulombe F. *et al.*, 2009.** Increased NOD2-mediated recognition of N-glycolyl muramyl dipeptide. J Exp Med. 206(8):1709-16.

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MDP - Reynolds JM. et al., 2015. Interleukin-17B Antagonizes Interleukin-25-Mediated Mucosal Inflammation. Immunity. 42(4):692-703.

L18-MDP - Nachbur U. et al., 2015. A RIPK2 inhibitor delays NOD signalling events yet prevents inflammatory cytokine production. Nat Commun. 6:6442.

M-Tri$_{LYS}$ - Jacquier N. et al., 2015. Disassembly of a Medial Transenvelope Structure by Antibiotics during Intracellular Division. Chem Biol. 22(9):1217-27.

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NLRP3 Inflammasome Inducers

Alum Crystals

Aluminum hydroxide and potassium salts (alum) are commonly used vaccine adjuvants. Adjuvants are vaccine additives that stimulate the immune system without having any specific antigenic effect. Alum has been demonstrated to activate caspase-1 and trigger IL-1β and IL-18 secretion[1]. All alum preparations contain crystals. The alum-induced release of IL-1β in macrophages is dependent on NLRP3 and ASC, indicating that alum triggers inflammation through activation of the NLRP3 inflammasome[2]. Alum has been shown to trigger NLRP3 activation through lysosomal destabilization[2].

ATP

Adenosine triphosphate (ATP), a potassium efflux agent, can trigger the activation of NLRP3 inflammasome in response to PAMPs, such as lipopolysaccharide (LPS). It stimulates the caspase-1-dependent cleavage and secretion of IL-1β from LPS-stimulated cells[3]. ATP triggers the opening of the non-selective cation channel of the purinergic P2X7 receptor, followed by the gradual opening of a larger pore. The larger pore is attributed to pannexin-1, which is recruited upon P2X7 receptor activation[4]. Activation of the P2X7 receptor results in potassium efflux which is necessary for the post-translational maturation of IL-1β[5].

Chitosan Ultrapure

Chitosan is the deacetylated derivative of chitin, a polysaccharide found in the exoskeletons of crustaceans and in the cell walls of fungi. Chitosan is well known for its use in drug delivery, where it is used to enhance the absorption of proteins across mucosal surfaces. In addition to its role in drug delivery, chitosan is an immunostimulator[6]: it activates the NLRP3 inflammasome, leading to potent IL-1β production[7, 8]. Chitosan is of interest for immunotherapy and vaccination due to its immunomodulatory effects.

Hemozoin

Hemozoin is a dark-brown heme crystal produced by the intraerythrocytic parasite *Plasmodium*, the causative agent of malaria. Hemozoin is taken up by macrophages initiating signals that lead to the production of IL-1β. Hemozoin-induced IL-1β production is dependent on the activation of the NLRP3 inflammasome[9, 10]. Synthetic hemozoin has been shown to possess adjuvant properties that differ depending on the method of synthesis[11]. InvivoGen provides a chemically synthesized hemozoin using an acidic method.

MSU & CPPD Crystals

Crystals of monosodium urate (MSU) and calcium pyrophosphate dihydrate (CPPD) are the aetiological agents of the inflammatory joint diseases gout and pseudo-gout, respectively. Both pathogenic crystals have been recently shown to be potent activators of caspase-1 through the NLRP3 inflammasome[12]. Involvement of the inflammasome is suggested by the finding that macrophages from mice deficient in various components of the inflammasome are defective in crystal-induced IL-1β induction. NLRP3 activation requires the phagocytosis of crystals that leads to lysosomal damage which appears to be the signal recognized by the inflammasome resulting in its activation[2].

Nano-SiO2

SiO2 nanoparticles (Nano-SIO2) are single particles of silica dioxide, an inorganic metal oxide, with a diameter less than 100 nm. Nano-SiO2 was recently shown to trigger caspase-1 cleavage and IL-1β secretion in human macrophages and keratinocytes[13]. The pro-inflammatory activity of nano-SiO2 is mediated by the NLRP3 inflammasome. THP1 cells stably infectected with shRNAs against caspase-1 or NLRP3 did not secrete IL-1β in response to nano-SiO2.

Nigericin

Nigericin is a microbial toxin derived from *Streptomyces hygroscopicus*. Nigericin acts as a potassium ionophore. The release of IL-1β in response to nigericin has been demonstrated to be NLRP3-dependent[3]. Similar to ATP, nigericin induces a net decrease in intracellular levels of potassium which is crucial for the activation of caspase-1[5]. Nigericin requires signaling through pannexin-1 to induce caspase-1 maturation and IL-1β processing and release[14].

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RELATED PRODUCTS

PRODUCT	INFO
Inflammasome test cells	See page 46
Inflammasome inhibitors	See page 117-118

RLR LIGANDS

RIG-I/MDA-5 Agonists

5'ppp-dsRNA - RIG-I Agonist

5'ppp-dsRNA is a short (19 mer) blunt-end double-stranded RNA with a 5' triphosphate. Transfected 5'ppp-dsRNA is a ligand for RIG-I[1]. This dsRNA sensor is specifically activated by the uncapped 5' triphosphate moiety on viral RNA. This triphosphate occurs during viral replication and is absent from most cytosolic self-RNA. A synthetic approach to the exact structure requirement to RIG-I recognition demonstrated that a short blunt double-stranded conformation containing a triphosphate at the 5' end is required[2].

5'ppp-dsRNA is available naked or complexed with the cationic lipid LyoVec™, to protect it from degradation and facilitate its uptake.

5'ppp-dsRNA Control is a 19 mer blunt-end dsRNA without a 5' triphosphate.

5'ppp-dsRNA	5'- pppGCAUGCGACCUCUGUUUGA -3' 3'- CGUACGCUGGAGACAAACU -5'
5'ppp-dsRNA Control	5'- GCAUGCGACCUCUGUUUGA -3' 3'- CGUACGCUGGAGACAAACU -5'

Poly(I:C)/LyoVec - RIG-I/MDA-5 Agonist

Unlike naked poly(I:C) which is recognized by TLR3, transfected poly(I:C) is sensed by RIG-I/MDA-5 in a cell-type-specific manner[3, 4]. Poly(I:C) (HMW)/LyoVec and poly(I:C) (LMW)/LyoVec are preformed complexes between poly(I:C) (HMW) or poly(I:C) (LMW) and the transfection reagent LyoVec™. These complexes induce the activation of the RIG-I/MDA-5 signaling pathway at concentrations ranging from 10 ng to 1 µg/ml in A549-Dual, a human lung carcinoma reporter cell line (see p. 64).

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RELATED PRODUCTS

PRODUCT	INFO
A549-Dual™ cells	See page 64

CLR LIGANDS

Dectin-1 Agonists

Beta-glucan peptide (BGP) - β(1→4,1→3, 1→6)-glucan

Beta-glucan peptide (BGP) is a high molecular weight (~100 kDa) polysaccharide extracted from the fungus *Trametes versicolor*. BGP consists of a highly ramified glucan portion, comprising a beta 1-4 main chain and beta 1-3 side chain, with beta 1-6 side chains covalently linked to a polypeptide portion rich in aspartic, glutamic and other amino acids. BGP activates murine macrophages and HEK-Blue™ Dectin-1 cells.

Curdlan AL - β(1→3)-glucan

Curdlan is a high molecular weight linear polymer consisting of β-(1 ->3)-linked glucose residues, produced as a water-insoluble polysaccharide by the soil bacterium, *Alcaligenes faecalis*. It is recognized by the membrane bound Dectin-1 receptor leading to the CARD9-dependent activation of NF-κB and MAP kinases[1]. Furthermore, Dectin-1 signaling activates the NFAT transcription factor. Recent data suggest that Curdlan is also recognized by the cytosolic NLRP3 inflammasome complex which cooperates with Dectin-1 resulting in robust activation of IL-1β–mediated inflammatory response[2].

HKCA (*Candida albicans*)

HKCA is a heat-killed preparation of *Candida albicans*, an opportunistic yeast that causes serious infections in immunocompromised patients. Beta-glucans represent 40% of the cell wall of *C. albicans*. Heat killing of this yeast results in the exposure of the beta-glucans on the surface of the cell wall and their subsequent recognition by the beta-glucan receptor, Dectin-1[3]. HKCA derives from the strain ATCC 10231.

HKSC (*Saccharomyces cerevisiae*)

HKSC is a heat-killed preparation of the yeast *Saccharomyces cerevisiae*. The cell wall of *S. cerevisiae* consists mainly of equal amounts of α-mannans and β-glucans[4]. Early studies have suggested that the phagocytosis of unopsonized HKSC is mediated by both mannose and β-glucans receptors. However, recent data show that the β-glucan receptor, Dectin-1, is the predominant receptor involved in this process[5].

Laminarin - β(1→3, 1→6)-glucan

Laminarin from the brown seaweed *Laminaria digitata* is a linear β(1-3)-glucan with β(1-6)-linkages. Laminarin is a low molecular weight (6 kDa), water-soluble β-glucan. It can bind to Dectin-1 without stimulating downstream signaling[6] and is able to block binding to Dectin-1 of particulate β(1-3)-glucans, such as zymosan[5].

Pustulan - β(1→6)-glucan

Pustulan is a median molecular weight (20 kDa), linear (1-6) linked β-D-glucan from lichen *Lasallia pustulata*. Pustulan is recognized by Dectin-1[7] and activates HEK-Blue™ Dectin-1 and RAW-Blue™ cells.

Schizophyllan - β(1→3, 1→6)-glucan

Schizophyllan (SPG) is a gel-forming β-glucan from the fungus *Schizophyllum commune*. SPG is a high molecular weight (450 kDa) (1-3)-β-D-glucan that has a 1,6-b-monoglucosyl branch in every three 1,3-β-glucosyl residues on the main chain. SPG binds to Dectin-1[8] and triggers a signaling cascade leading to NF-κB activation.

Scleroglucan - β(1→3, 1→6)-glucan

Scleroglucan is a high molecular weight (>1000 kDa) polysaccharide produced by fermentation of the filamentous fungus *Sclerotium rolfsii*. Scleroglucan consists of a linear β(1-3) D-glucose backbone with one β(1-6) D-glucose side chain every three main residues. Scleroglucan is recognized by Dectin-1[9] and strongly activates HEK-Blue™ Dectin-1.

WGP Dispersible - β(1→3)-glucan

WGP Dispersible (Wellmune WGP® Dispersible, Biothera), whole glucan particles, is a particulate *Saccharomyces cerevisiae* β-glucan preparation. It consists of hollow yeast cell wall "ghosts" composed primarily of long polymers of β-1,3 glucose obtained after a series of alkaline and acid extractions from *S. cerevisiae* cell wall[10]. In contrast to Zymosan, WGP lacks TLR-stimulating activity[11]. Similarly to Zymosan, WGP induces Dectin-1-dependent responses, including phagocytosis and induction of TNF-α, IL-6 and ROS by macrophages and dendritic cells.

WGP Soluble - β(1→3)-glucan

WGP Soluble (Wellmune WGP® Soluble, Biothera) is a pure soluble WGP preparation. Similarly to WGP Dispersable, WGP Soluble binds efficiently Dectin-1, however it is uncapable of activating the receptor[11]. Furthermore, WGP Control is able to significantly block the binding of WGP to macrophages and its immunostimulatory effect[10].

Zymosan - β(1→3)-glucan

Zymosan is an insoluble preparation of *Saccharomyces cerevisiae* cell wall. It primarily contains β-glucans as well as other components, such as mannans, mannoproteins and chitin. Zymosan activates macrophages via TLR2 which cooperates with TLR6 and CD14 to mediate the activation of NF-κB[12]. Zymosan is also recognized by Dectin-1, which collaborates with TLR2 and TLR6 enhancing the immune responses triggered by the recognition of Zymosan by each receptor[13]. Furthermore, zymosan was shown to induce the production of IL-1β in dendritic cells and macrophages through the activation of the NLRP3 inflammasome[14].

Zymosan Depleted - β(1→3)-glucan

Zymosan depleted is a *S. cerevisiae* cell wall preparation treated with hot alkali to remove all its TLR-stimulating properties[13]. Zymosan depleted activates Dectin-1 but not TLR2.

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RELATED PRODUCTS

PRODUCT	INFO
HEK-Blue™ Dectin-1 cells	See page 45
Anti-Dectin-1 antibodies	See page 132

Dectin-2 Agonists

Furfurman (Mallassezia furfur)
Furfurman is a cell wall preparation of Malassezia furfur, an opportunistic skin fungal pathogen that is recognized by the C-type lectin receptor Dectin-2[1]. This receptor binds to mannose carbohydrates, such as the mannose-capped lipoarabinomannan of mycobacteria[2] and α-mannans found in Candida albicans[3]. Furfurman selectively activates Dectin-2, although at high concentrations it can also activate Dectin-1.

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RELATED PRODUCTS

PRODUCT	INFO
HEK-Blue™ Dectin-2 cells	See page 45
Anti-Dectin-2 antibodies	See page 132

Mincle Agonists

HKMT - Heat-killed *Mycobacterium tuberculosis*
HKMT is a heat-killed preparation of the avirulent strain of *Mycobacterium tuberculosis* H37 Ra. HKMT is sensed by Mincle which recognizes the mycobacterial cell wall glycolipid TDM (trehalose 6,6'-dimycolate, also known as cord factor)[1]. HKMT also possesses a large repertoire of TLR2 ligands, such as lipoproteins and lipomannan[2]. Upon HKMT sensing, both Mincle and TLR2 lead to the activation of NF-κB.

TDB - Mincle Agonist
Trehalose-6,6-dibehenate (TDB) is a synthetic analog of trehalose-6,6-dimycolate (TDM, also known as cord factor), which is the most studied immunostimulatory component of *Mycobacterium tuberculosis*[1]. TDB binds the C-Type lectin, Mincle (macrophage-inducible C-type lectin)[1,3]. Upon TDB recognition Mincle interacts with the Fc receptor common γ-chain (FcRγ), which triggers intracellular signaling through Syk leading to CARD9-dependent NF-κB activation.

TDB-HS15 - Synthetic cord factor analog
Trehalose-6,6-dibehenate (TDB) is a non-toxic synthetic analogue of TDM and an effective adjuvant for Th1/Th17 vaccination. TDB was found to rely on Mincle, Syk and Card9 for its adjuvant activity[3]. TDB is a poorly soluble compound and thus was formulated with Kolliphor® HS 15, a potent low-toxicity non-ionic solubilizer, to generate TDB-HS15, which is particularly suitable for *in vivo* studies.

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RELATED PRODUCTS

PRODUCT	INFO
HEK-Blue™ Mincle cells	See page 45
Anti-Mincle antibodies	See page 133

CDS & STING LIGANDS

CDS Agonists

dsDNA-EC
dsDNA-EC is an ultrapure, endotoxin-free preparation of *E. coli* K12 double-stranded DNA. Intracellular dsDNA-EC is recognized by the endosomal receptor, TLR9 and mutliple cytosolic DNA sensors (CDSs), leading to the production of type I IFNs[1]. In HEK293 cells transfected with TLR9, dsDNA-EC induces the activation of NF-κB.

HSV60
HSV-60 is a 60 bp oligonucleotide (ODN) containing viral DNA motifs[2]. It derives from the herpes simplex virus 1 genome. Transfected HSV-60 was shown to potently induce IFN-β in a TLR-, DAI- and RNA Pol III-independent, but STING-, TBK1- and IRF3-dependent manner. HSV-60 is recognized by the CDSs, DDX41[3] and IFI16[2].
HSV-60c (control) is a single-stranded oligonucleotide which, unlike its double-stranded counterpart does not induce type I IFNs[2].
HSV-60 and HSV-60c are also available complexed with the cationic lipid transfection reagent LyoVec™ to facilitate their uptake.

ISD
ISD is a 45-bp non-CpG oligomer from the *Listeria monocytogenes* genome. When transfected into various cell types, including plasmacytoid and conventional DCs, macrophages and murine embryonic fibroblasts, ISD strongly enhances the expression of IFN-β[4]. This ISD-induced response is mediated by the STING-TBK1-IRF3 signaling axis[4, 5].
ISD Control is a non-immunostimulatory single-stranded ODN with the same sequence as ISD, its double-stranded counterpart.
ISD and ISD Control are also available complexed with the cationic lipid transfection reagent LyoVec™ to facilitate their uptake.

Poly(dA)
Poly(dA) is a repetitive synthetic single-stranded DNA sequence of polydeoxyadenylic acid with no IFN stimulatory property. Poly(dA) is a control for poly(dA:dT). Poly(dA) is available naked or complexed with the cationic lipid transfection reagent LyoVec™ to facilitate its uptake.

Poly(dA:dT)
Poly(dA:dT) is a repetitive synthetic double-stranded DNA sequence of poly(dA-dT)•poly(dT-dA) and a synthetic analog of B-DNA. Poly(dA:dT) is recognized by several sensors, including DAI, LRRFIP1 and AIM2[3-5]. It has also been shown to be transcribed by RNA polymerase III into dsRNA with a 5'-triphosphate moiety which is a ligand for RIG-I[6]. Poly(dA:dT) is available naked or complexed with the cationic lipid LyoVec™ to facilitate their uptake, and labeled with rhodamine.

Poly(dG:dC)
Poly(dG:dC) is a repetitive synthetic double-stranded DNA sequence of poly(dG-dC)•poly(dC-dG). Intracellular poly(dG:dC) is detected by several cytosolic DNA sensors (CDS), such as cGAS, DAI, DDX41, IFI16 and LRRFIP1, triggering the production of type I interferons (IFNs). Poly(dG:dC) is available naked or complexed with the cationic lipid LyoVec™ to facilitate their uptake.

VACV-70
VACV-70 is a 70 bp oligonucleotide containing a sequence conserved in various poxviral genomes including the vaccinia virus (VACV)[2]. Transfection of VACV-70 was shown to induce a strong IFN-β response dependent on STING, TBK1 and IRF3. The cytosolic DNA sensor IFI16 appears to be involved in the recognition of VACV-70[2].
VACV-70c (control), is the single-stranded form of VACV-70 that does not induce the production IFN-β[2].
VACV-70 and VACV-70c are also available complexed with the cationic lipid transfection reagent LyoVec™ to facilitate its uptake.

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STING Agonists

2'3'-cGAMP - Mammalian cGAMP
2'3'-cGAMP (cyclic [G(2',5')pA(3',5')p]) is the only isomer of cGAMP produced by the mammalian DNA sensor cGAMP synthase (cGAS) in response to cytosolic DNA[1]. This isomer contains two distinct phosphodiester linkages, a noncanonical (2',5') linkage at the GpA step and a canonical (3',5') linkage at the ApG step[1-3]. Mammalian 2'3'-cGAMP binds to (stimulator of IFN genes) STING with high affinity and is a potent inducer of IFN-β[3].
2'3'-cGAMP Control, also known as 2'5'-GpAp, is a linear dinucleotide analog of 2'3'-cGAMP. This compound is intended to serve as a negative control for 2'3'-cGAMP in type I IFN induction assays.

2'3'-cGAM(PS)$_2$ (Rp/Sp)
2'3'-cGAM(PS)$_2$ (Rp/Sp) is the Rp,Sp-isomer of the bis-phosphorothioate analog of 2'3'-cGAMP. This analog is resistant to the enzyme ENPP1, which cleaves 2'3'-cGAMP. 2'3'-cGAM(PS)$_2$ is more stable and more potent than 2'3'-cGAMP[4].

2'2'-cGAMP
2'2' cGAMP (cyclic [G(2',5')pA(2',5')p]) is a synthetic analog of 2'3'-cGAMP. It contains two noncanonical (2',5') phosphodiester linkages. Compared to 2'3'-cGAMP, it binds with lower affinity to STING[3] but induces similar IFN-β response in cell-based assays[3, 5].

3'3'-cGAMP - Bacterial cGAMP

3'3'-cGAMP (cyclic [G(3',5')pA(3',5')p]) is a cyclic dinucleotide (CDN) produced by bacteria. It is also referred to as "canonical" cGAMP due the presence of two conventional (3',5') phosphodiester linkages. 3'3'-cGAMP is differentially recognized by STING variants and induces mainly the type I IFN pathway[5].

3'3'-cGAMP Control, also known as 3'5'-pGpA, is a linear dinucleotide analog of 3'3'-cGAMP[6]. This compound is intended to serve as a negative control for 3'3'-cGAMP in type I IFN induction assays.

3'3'-cGAMP Fluorinated (c-[2'FdGMP]-[2'FdAMP]) is a synthetic analog of 3'3-cGAMP with a fluorine atom at 2' position of the nucleosides. When used at low concentrations in various cellular assays, 3'3'-cGAMP Fluorinated induces higher levels of type I IFNs than does 3'3'-cGAMP.

c-di-AMP - Bacterial CDN

Bis-(3'-5')-cyclic dimeric adenosine monophosphate (c-di-AMP) is a second messenger molecule produced in bacteria but not in mammals. c-di-AMP induces the production of type I interferons through a signaling axis that involves STING, TBK1 and IRF3[7, 8].

c-di-AMP Control, also known as pApA, is a linear dinucleotide analog of c-di-AMP[9]. This compound is intended to serve as a negative control for c-di-AMP in type I IFN induction assays.

c-di-AMP Fluorinated (c-di[2'FdAMP]) is a synthetic analog of c-di-AMP with a fluorine atom at 2' position of the nucleosides. When used at low concentrations in various cellular assays, c-di-AMP Fluorinated induces higher levels of type I IFNs than does c-di-AMP.

2'3'-c-di-AMP & 2'3'-c-di-GMP

2'3'-c-di-AMP and 2'3'-c-di-GMP are synthetic analogs of the bacterial CDNS, c-di-AMP and c-di-GMP, respectively. They contain a 2'-5', 3'-5' mixed linkage, as found in endogenous human CDNs produced by cGAS. When used at low concentrations in various cellular assays, 2'3'-c-di-AMP and 2'3'-c-di-GMP were found to induce higher levels of type I IFNs than their bacterial counterpart.

2'3'-c-di-AM(PS)2 (Rp, Rp)

2'3'-c-di-AM(PS)$_2$ (Rp,Rp) is the Rp,Rp-isomer of the 2'3' bis-phosphorothioate analog of c-di-AMP. It has a higher affinity for STING than c-di-AMP due to the presence of a 2'-5', 3'-5' mixed linkage, as found in endogenous human CDNs produced by cGAS[10]. It activates all known human STING alleles as well as mouse STING. In addition, this analog contains two phosphorothioate diester linkages to protect it against degradation by phosphodiesterases that are present in host cells or in the systemic circulation[11]. The Rp, Rp dithio diastereoisomer was found to induce higher type I IFN production compared to the Rp/Sp dithio diastereoisomers or c-di-AMP *in vitro*, and to confer increased anti-tumor activity over 2'3'-cGAMP in mice[10]. This compound, named MIW815, has been selected for clinical development (NCT02675439).

c-di-GMP - Bacterial CDN

Bis-(3'-5')-cyclic dimeric guanosine monophosphate (c-di-GMP) is a second messenger produced in bacteria involved in complex biological processes, such as biofilm formation, virulence and photosynthesis. c-di-GMP is recognized by STING, initiating a response characterized by the production of type I IFNs through the TBK1/IRF3 axis[8]. Recent studies have demonstrated that c-di-GMP exhibits potent adjuvant properties[12].

c-di-GMP Control, also known as 5'-pGpG, is a linear dinucleotide analog that can be obtained after hydrolysis of cyclic diguanylate monophosphate (c-di-GMP) by phosphodiesterases[12].This compound is intended to serve as a negative control for c-di-GMP in type I IFN induction assays.

c-di-IMP

Cyclic di-inosine monophosphate (c-di-IMP) is a synthetic analog of c-di-AMP and c-di-GMP. It displays comparable capacities to c-di-GMP in promoting the activation and maturation of antigen presenting cells[13]. Furthermore, c-di-IMP possesses strong adjuvant properties when co-administered with an antigen by the mucosal route[13].

DMXAA - Xanthenone Analog

DMXAA (5,6-dimethylxanthenone-4-acetic acid, also known as Vadimezan or ASA404) was initially identified as a potent tumor vascular disrupting agent in mice through the induction of cytokines, notably IFN-β. DMXAA was shown to target the STING pathway[15]. However, its activity is restricted to mice as it has no effect on human STING[15,16].

1. Gao P. *et al.*, 2013. Cyclic [G(2',5')pA(3',5')p] is the metazoan second messenger produced by DNA-activated cyclic GMP-AMP synthase. Cell. 153(5):1094-107. **2. Ablasser A. *et al.*, 2013.** cGAS produces a 2'-5'-linked cyclic dinucleotide second messenger that activates STING. Nature. 498(7454):380-4. **3. Zhang X. *et al.*, 2013.** Cyclic GMP-AMP containing mixed phosphodiester linkages is an endogenous high-affinity ligand for STING. Mol Cell. 2013 Jul 25;51(2):226-35. **4. Li L. *et al.*, 2014.** Hydrolysis of 2'3'-cGAMP by ENPP1 and design of nonhydrolyzable analogs. Nat Chem Biol. 10(12):1043-8. **5. Diner E. *et al.*, 2013.** The Innate Immune DNA Sensor cGAS Produces a Noncanonical Cyclic Dinucleotide that Activates Human STING. Cell Rep. 3(5):1355-61. **6. Gao J. et al., 2015.** Identification and characterization of phosphodiesterases that specifically degrade 3'3'-cyclic GMP-AMP. Cell Res. 25(5):539-50. **7. Jin L. et al., 2011.** MPYS is required for IFN response factor 3 activation and type I IFN production in the response of cultured phagocytes to bacterial second messengers cyclic-di-AMP and cyclic-di-GMP. J Immunol. 187(5):2595-601. **8. Burdette DL. *et al.*, 2011.** STING is a direct innate immune sensor of cyclic di-GMP. Nature. 478(7370):515-8. **9. Huynh TN. & Woodward JJ, 2016.** Too much of a good thing: regulated depletion of c-di-AMP in the bacterial cytoplasm. Curr Opin Microbiol. 30:22-29. **10. Corrales L *et al.*, 2015.** Direct activation of STING in the tumor microenvironment leads to potent and systemic tumor regression and immunity. Cell Rep. 11(7):1018-30. **11. Yan H. *et al.*, 2008.** Synthesis and immunostimulatory properties of the phosphorothioate analogues of cdiGMP. Bioorg. Med. Chem. Lett. 18, 5631–5634. **12. Römling U. *et al.*, 2013.** Cyclic di-GMP: the First 25 Years of a Universal Bacterial Second Messenger. Microbiol Mol Biol Rev. 77(1): 1–52. **13. Libanova R. et al., 2010.** The member of the cyclic di-nucleotide family bis-(3', 5')-cyclic dimeric inosine monophosphate exer ts potent activity as mucosal adjuvant. Vaccine. 28(10):2249-58. **14. Prantner D. *et al.*, 2012.** 5,6-Dimethylxanthenone-4-acetic acid (DMXAA) activates stimulator of interferon gene (STING)-dependent innate immune pathways and is regulated by mitochondrial membrane potential. J Biol Chem. 287(47):39776-88. **15. Conlon J. *et al.*, 2013.** Mouse, but not human STING, binds and signals in response to the vascular disrupting agent 5,6-dimethylxanthenone-4-acetic acid. J Immunol. 190(10):5216-25. **16. Kim S. *et al.*, 2013.** Anticancer Flavonoids Are Mouse-Selective STING Agonists. ACS Chem Biol. 8(7): 1396-1401.

RECENT CITATIONS

2'3'-cGAMP - Mukai K. *et al.*, 2016. Activation of STING requires palmitoylation at the Golgi. Nat Commun. 7:11932.

2'3'-cGAMP, 2'2'-cGAMP & c-di-IMP- Tang CH. *et al.*, 2016. Agonist-Mediated Activation of STING Induces Apoptosis in Malignant B Cells. Cancer Res. 76(8):2137-52.

2'3'-cGAMP & 3'3'-cGAMP - Zhang G. *et al.*, 2016. Cytoplasmic isoforms of Kaposi sarcoma herpesvirus LANA recruit and antagonize the innate immune DNA sensor cGAS. PNAS 113(8):E1034-43.

c-di-AMP, c-di-GMP & DMXAA - Surpris G. *et al.*, 2016. Cutting Edge: Novel Tmem173 Allele Reveals Importance of STING N Terminus in Trafficking and Type I IFN Production. J Immunol. 196(2):547-52.

c-di-GMP - York AG. *et al.*, 2015. Limiting Cholesterol Biosynthetic Flux Spontaneously Engages Type I IFN Signaling. JCell. 163(7):1716-29.

RELATED PRODUCTS

PRODUCT	INFO
THP1-Dual™ cells	See page 66
STING KO cell lines	See page 67-69

MULTI-PRR LIGANDS

InvivoGen has developed a series of novel molecules designed to induce potent immune responses through the combined activation of several pattern recognition receptors (PRRs) that trigger different innate immune signaling pathways. These molecules are agonists for TLR2, TLR7 or both. In addition, some of these ligands have the ability to form complexes with nucleic acids and facilitate their penetration into the cell resulting in their recognition by additional PRRs that sense nucleic acids, such as the cytosolic DNA sensors cGAS and IFI16 and the dsRNA receptors TLR3 and RIG-I/MDA-5.

TLR2 & TLR7 AGONISTS

CL401 - TLR2 & TLR7 Ligand

CL401 (S-(2,3-bis(palmitoyloxy)-(2RS)propyl)-(R)-cysteinyl 4-((6-amino-2(butyl amino)-8-hydroxy-9H-purin-9-yl)methyl) aniline) is a small lipophilic molecule comprising an 8-hydroxyadenine compound conjugated with a Pam2C group. This bipartite structure confers to CL401 the ability to efficiently stimulate both TLR7 and TLR2, respectively (fig. 1, 2, 3). Intratumoral injection of CL401 leads to a significant antitumor activity (fig. 6).

CL413 - Adilipoline™ - TLR2 & TLR7 Ligand

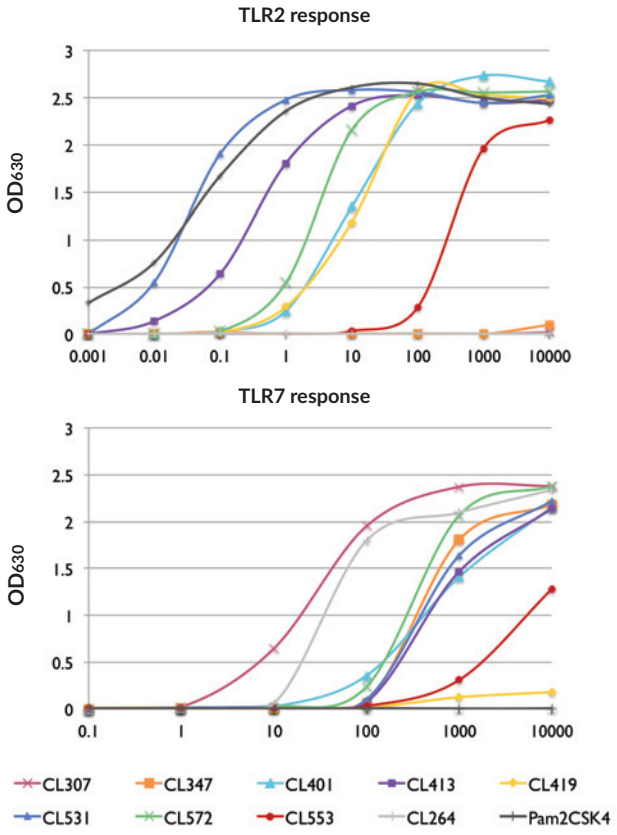
Adilipoline™ (S-(2,3-bis(palmitoyloxy)-(2RS)propyl)-(R)-cysteinyl-(S)-seryl-(S)-lysyl-(S)-lysyl-(S)-lysyl-(S)-lysyl 4-((6-amino-2-(butylamino)-8-hydroxy-9H-purin-9-yl)methyl) aniline) was generated by conjugation of an 8-hydroxyadenine moiety to the terminal acid function of Pam2CSK4. Adilipoline™ is a good ligand for both TLR2 and TLR7 (fig. 1, 2, 3). *In vivo* tumor studies have demonstrated that Adilipoline™ is a potent antitumor agent (fig. 6). Intratumoral injection of Adilipoline™ in established B16 tumors resulted in tumor regression. However, in contrast to AdiFectin™ (CL347), no protection after tumor rechallenge was observed.

CL531 - TLR2 & TLR7 Ligand

CL531 (S-(2,3-bis(palmitoyloxy)-(2RS)propyl)-(R)-cysteinyl-(S)-seryl-(S)-lysyl-Ne-(4-((6-amino-2-(butylamino)-8-hydroxy-9H-purin-9-yl)methyl) benzylamido)(S)-lysyl-(S)-lysyl-(S)-lysine) is an 8-hydroxyadenine derivative conjugated to the lateral chain of the second lysine of Pam2CSK4. CL531 is a very potent TLR2 agonist and a good TLR7 agonist (fig. 1 & 2). TLR2-mediated activation of NF-κB is achieved with concentrations as low as 5 pM (0.01 ng/ml).

CL572 - TLR2 (human) & TLR7 Ligand

CL572 (S-(2-myristoyloxy ethyl)-(R)-cysteinyl 4-((6-amino-2-(butylamino)-8-hydroxy-9H-purin-9-yl)methyl) aniline) is a 8-hydroxy-adenine compound conjugated to a monoacyl-ethyl-cystein group via a glutamic acid derivative. Monoacy-ethyl-cystein-containing dipeptides have been recently shown to specifically activate human TLR2. Indeed, CL572 is a robust inducer of human TLR2 (fig. 1) but is unable to stimulate mouse TLR2. CL572 is also a potent inducer of TLR7 (fig. 2).

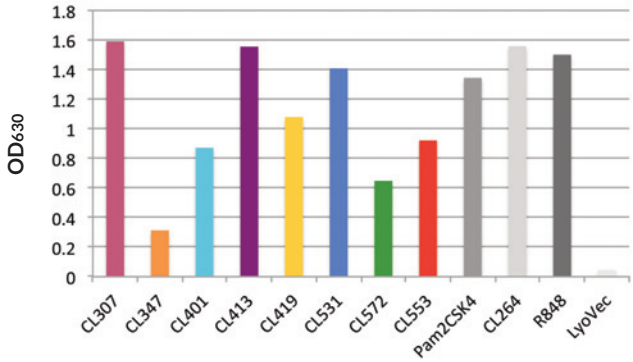


1 & 2- TLR2 and TLR7 responses. HEK-Blue™ hTLR2 cells and HEK-Blue™ hTLR7 cells, which stably express an NF-κB-inducible SEAP reporter gene and human TLR2 or TLR7, respectively, were incubated in HEK-Blue™ Detection medium and stimulated with increasing concentrations of the agonists indicated in the graph. After 24h incubation, the OD_{630} was read to determine the levels of NF-κB-induced SEAP.

TLR2/TLR7 AGONISTS & NUCLEIC ACID CARRIERS

CL347 - AdiFectin™ - TLR7 Ligand

AdiFectin™ (bis(phytanyl) N4-{N1-[(4-((6-amino-2-(butylamino)-8-hydroxy-9H-purin-9-yl)methyl)benzoyl)glycinyl]sperminyl}propyl phosphonate) is derived from CL307 by conjugation with a bis(phytanyl) phosphonate group. Addition of this lipid confers to the molecule the ability to form positively charged liposomes, which can encapsulate DNA (or RNA). AdiFectin™ is a weaker TLR7 agonist than CL307 (fig. 2 & 3), but in contrast to CL307, is able to efficiently complex nucleic acids resulting in a strong IFN response (fig. 4) and transgene expression when the nucleic acid is a plasmid DNA carrying an expression cassette



3- NF-κB response. RAW-Blue™ cells, which stably express an NF-κB-inducible SEAP reporter gene, were stimulated with 0.6 µg/ml of multi-PRR ligands complexed with 0.1 µg/ml HSV-60 (dsDNA). After 24h incubation, the levels of NF-κB-induced SEAP were determined using QUANTI-Blue™.

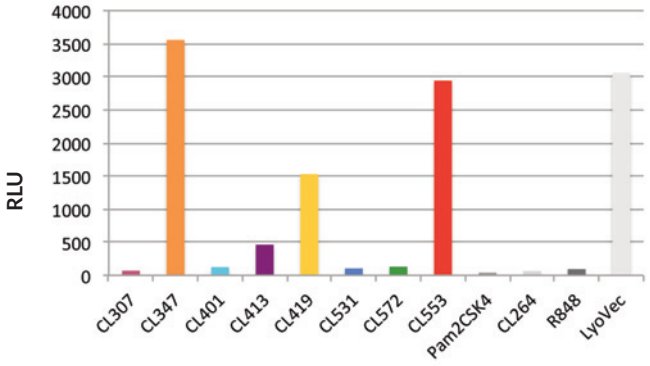
(fig. 5). Repeated *in vivo* studies have showed that pDNA/AdiFectin™ complexes display robust anti-tumor activity (fig. 7). Notably, mice that achieved long-term clearance of tumor following AdiFectin™ treatment were protected from subsequent tumor rechallenge suggesting the generation of a tumor-specific memory immune response.

CL419 - TLR2 Ligand

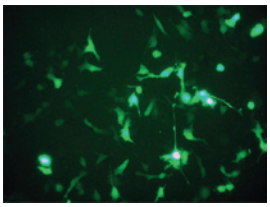
CL419 (S-(2,3-bis(palmitoyloxy)-(2RS)propyl)-(R)-cysteinyl spermine) is a polyamine TLR2 agonist derived from Pam2CSK4 by replacement of Ser-(Lys)4 by a cationic sperminyl group. CL419 forms positively charged liposomes which allows it to complex nucleic acids and transport them into the cytosol and the nucleus. CL419/nucleic acid complexes are recognized by TLR2 and nucleic acid sensors leading to the significant activation of the NF-kB and IRF pathways (fig. 1, 3, 4). *In vivo*, CL419 complexed with a plasmid DNA (pDNA) and injected intratumorally induces a modest reduction of the tumor growth (fig. 7).

CL553 - PamadiFectin™ - TLR2 & TLR7 Ligand

PamadiFectin™ (N4-(S-((2,3-bis(palmitoyloxy))-(2RS)propyl)-(R)-cysteinyl) N1-(4-(((6-amino-2-(butylamino)-8-hydroxy-9H-purin-9-yl)methyl) benzoyl) glycinyl) spermine) was generated by conjugation of CL307 to a Pam2C group. PamadiFectin™ induces NF-κB activation through stimulation of both TLR2 and TLR7 (fig. 1, 2, 3). In addition, at physiological pH, PamadiFectin™ is able to form complexes with nucleic acids and carry them in the cytosol and nucleus leading to a strong induction of the IRF pathway (fig. 4). Intratumoral administration of pDNA/PamadiFectin™ complexes leads to spectacular reduction of tumor growth and improved long-term survival in B16-F1 tumor-bearing mice (fig. 7). Tumor rechallenge experiments have not yet been performed.



4- IRF response. RAW-Lucia™ ISG cells, which stably express an IRF-inducible Lucia luciferase reporter gene, were stimulated with 6 µg/ml of multi-PRR ligands complexed with 1 µg/ml HSV-60 (dsDNA). After 24h incubation, the levels of IRF-induced Lucia™ were determined using QUANTI-Luc™, a Lucia luciferase detection reagent.



5- Transfection efficiency of AdiFectin™ B16-F1 cells were incubated with pDNA-GFP/CL347 complexes at a 1/6 ratio (w/w). After 48h incubation, GFP expression was detected using fluorescence microscopy. Similar results were obtained with pDNA-GFP complexed with CL419 or CL553. No fluorescence was observed with pDNA-GFP mixed with other multi-PRR ligands, such as CL307 or CL531.

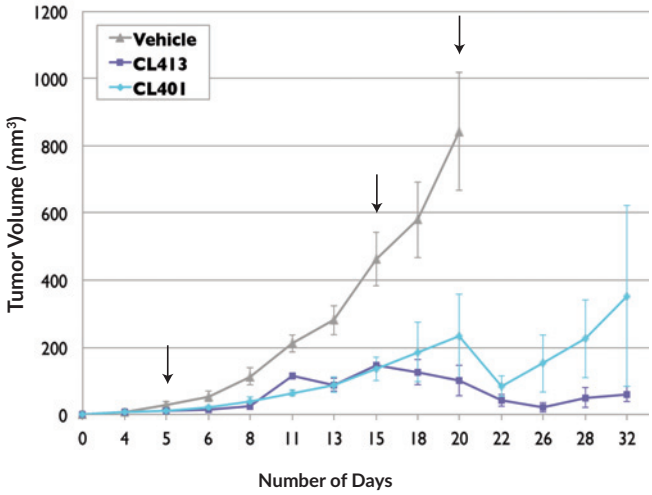


Figure 6: Tumor growth after CL413 or CL401 treatment. C57BL/6 mice were inoculated subcutaneously with $5x10^5$ B16-F1 mouse melanoma cells. CL413, CL401 or vehicle were injected intratumorally (50 µg/mouse/50 µl) on days 5, 15 and 20. Each group contained 8 mice. Black arrows represent the days of injection. Tumor growth was monitored and measured with calipers after day 5 of grafting tumor cells into mice and then every 2 days thereafter. Tumor volume in mm^3 was determined according to the formula $V = W^2 \times L/2$, where L = length (mm) and W = width (mm).

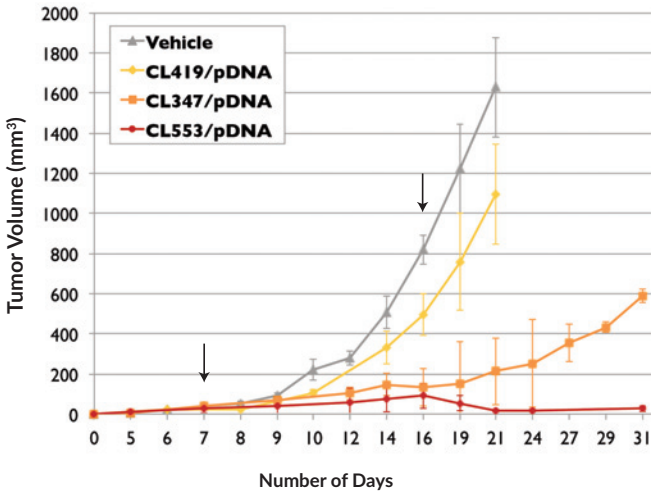


Figure 7: Tumor growth after treatment with CL419, CL347 or CL553 complexed with plasmid DNA (pDNA). C57BL/6 mice were inoculated subcutaneously with 5.10^5 B16-F1 cells. Complexes of pDNA/CL419, pDNA/CL347 or pDNA/CL553 were injected intratumorally at a 10:40 (w:w) ratio (10 µg:40 µg/mouse/100 µl) on days 7 and 16. A fourth group received intratumoral injections of the vehicle. Each group contained 8 mice. Black arrows represent the days of injection. Tumor growth was monitored and measured with calipers after day 5 of grafting tumor cells into mice and then every 2 days thereafter. Tumor volume in mm^3 was determined according to the formula $V = W^2 \times L/2$, where L = length (mm) and W = width (mm).

TLR & NOD AGONIST KITS

The TLR and NOD agonist kits provide convenient and economical tools to study the stimulation of the TLRs and NOD1/NOD2, respectively. Each kit contains one or several known agonists for a given TLR or NOD and allows to perform 100 tests (100 µl in a 96-well plate).

Human TLR1-9 Agonist Kit

This kit contains 10 agonists known to activate TLR1 to TLR9. It can be used to determine the TLR profile of human cell lines.
- **Pam3CSK4** - TLR1/2 agonist
- **FSL-1** - TLR2/6 agonist
- **HKLM** - TLR2 agonist
- **Poly(I:C) (HMW) and Poly(I:C) (LMW)** - TLR3 agonists
- **LPS-EK standard** - TLR4 agonist
- **FLA-ST standard** - TLR5 agonist
- **Imiquimod** - TLR7 agonist
- **ssRNA40/LyoVec** - TLR8 agonist
- **ODN 2006** - TLR9 agonist

Mouse TLR1-9 Agonist Kit

This kit contains 9 agonists known to activate TLR1 to TLR9. It can be used to determine the TLR profile of mouse cell lines.
- **Pam3CSK4** - TLR1/2 agonist
- **FSL-1** - TLR2/6 agonist
- **HKLM** - TLR2 agonist
- **Poly(I:C) (HMW) and Poly(I:C) (LMW)** - TLR3 agonists
- **LPS-EK standard** - TLR4 agonist
- **FLA-ST standard** - TLR5 agonist
- **ssRNA40/LyoVec** - TLR7 agonist
- **ODN 1826** - TLR9 agonist

Human TLR3/7/8/9 Agonist Kit

This kit contains 14 agonists known to activate the endosomal TLRs, that are TLR3, TLR7, TLR8 and TLR9. These agonists are mainly nucleic acids or their analogs. This kit is designed for studies in human cells.
- **Poly(A:U), Poly(I:C) (HMW) and Poly(I:C) (LMW)** - TLR3 agonists
- **Imiquimod** - TLR7 agonist
- **R848 and CL075** - TLR7/8 agonists
- **ssRNA40/LyoVec and ssRNA41/LyoVec** - TLR8 agonists
- **ODN 2006 and ODN 2006 control** - B class, TLR9 agonists
- **ODN 2216 and ODN 2216 control** - A class, TLR9 agonists
- **ODN 2395 and ODN 2395 control** - C class, TLR9 agonists

Human/mouse TLR2 Agonist Kit

This kit contains 7 agonists, that all activate TLR2 in a TLR1/6-dependent or independent manner. It can be used in human or mouse studies.
- **Pam2CSK4** - TLR2/6 agonist
- **Pam3CSK4** - TLR1/2 agonist
- **FSL-1** - TLR2/6 agonist
- **HKLM** - TLR2 agonist
- **LPS-PG** - TLR2 agonist
- **LTA-SA standard** - TLR2 agonist
- **PGN-SA** - TLR2 agonist

Human TLR9 Agonist Kit

This kit contains 6 agonists. It features prototype CpG ODNs of the A, B or C class that function best in human. These CpG ODNs are often cited in the literature. This kit will help you choose the most appropriate class of CpG ODNs for studies in human cells.
- **ODN 2006 and ODN 2006 control** - B class
- **ODN 2216 and ODN 2216 control** - A class
- **ODN 2395 and ODN 2395 control** - C class

PRODUCT	QUANTITY	CAT. CODE
Human TLR1-9 Agonist Kit		
- Pam3CSK4	10 µg	
- FSL-1	10 µg	
- HKLM	10^9 cells	
- Poly(I:C) (HMW)	500 µg	
- Poly(I:C) (LMW)	500 µg	tlrl-kit1hw
- LPS-EK standard	100 µg	
- FLA-ST standard	10 µg	
- Imiquimod	25 µg	
- ssRNA40/LyoVec	25 µg	
- ODN 2006	100 µg	
Mouse TLR1-9 Agonist Kit		
- Pam3CSK4	10 µg	
- FSL-1	10 µg	
- HKLM	10^9 cells	
- Poly(I:C) (HMW)	500 µg	tlrl-kit1mw
- Poly(I:C) (LMW)	500 µg	
- LPS-EK standard	100 µg	
- FLA-ST standard	10 µg	
- ssRNA40/LyoVec	25 µg	
- ODN 1826	100 µg	
Human TLR3/7/8/9 Agonist Kit		
- Poly(A:U)	500 µg	
- Poly(I:C) (HMW)	500 µg	
- Poly(I:C) (LMW)	500 µg	
- Imiquimod	25 µg	
- R848	25 µg	
- CL075	25 µg	
- ssRNA40/LyoVec	25 µg	tlrl-kit3hw3
- ssRNA41/LyoVec (control)	25 µg	
- ODN 2006	100 µg	
- ODN 2006 control (ODN 2137)	100 µg	
- ODN 2216	100 µg	
- ODN 2216 control (ODN 2243)	100 µg	
- ODN 2395	100 µg	
- ODN 2395 control	100 µg	
Human/mouse TLR2 Agonist Kit		
- Pam2CSK4	10 µg	
- Pam3CSK4	10 µg	
- FSL-1	10 µg	tlrl-kit2hm
- HKLM	10^9 cells	
- LPS-PG	100 µg	
- LTA-SA standard	100 µg	
- PGN-SA	100 µg	
Human TLR9 Agonist Kit		
- ODN 2006	100 µg	
- ODN 2006 control (ODN 2137)	100 µg	
- ODN 2216	100 µg	
- ODN 2216 control (ODN 2243)	100 µg	tlrl-kit9h
- ODN 2395	100 µg	
- ODN 2395 control	100 µg	

Mouse TLR9 Agonist Kit

This kit contains prototype CpG ODNs of the A, B or C class described to work well in mice studies. This kit allows to compare their effectiveness and select the most suitable one for a given application in murine cells or mice.

- **ODN 1585 and ODN 1585 control** - A class
- **ODN 1826 and ODN 1826 control** - B class
- **ODN 2395 and ODN 2395 control** - C class

B-Class TLR9 Agonist Kit - Multispecies

CpG ODNs of this kit belong exclusively to the B class and are active in human and/or mouse and other species. The kit contains prototype CpG ODNs as well as less popular but worth testing CpG ODNs.

- **ODN 1668 and ODN 1826** - B class, mouse preferred
- **ODN 2006** - B class, human preferred
- **ODN 2007** - B class, bovine/porcine
- **ODN BW006** - B class, human/mouse
- **ODN D-SL01** - B class, multispecies

A&C-Classes TLR9 Agonist Kit - Multispecies

This kit contains CpG ODNs that belong to the A or C class. They are active in several species.

- **ODN 1585** - A class, mouse preferred
- **ODN 2216 and ODN 2336** - A class, human preferred
- **ODN 2395 and ODN M362** - C class, human/mouse
- **ODN D-SL03** - C class multispecies

TLR9 Antagonist Kit - Multispecies

This kit contains inhibitory ODNs, that are active in human and/or mouse, and a control ODN, that can be used with each inhibitory ODN.

- **ODN 2088** - Mouse preferred
- **ODN 4084-F** - Human/mouse
- **ODN INH-1** - Human/mouse
- **ODN INH-18** - Human/mouse
- **ODN TTAGGG** - Human preferred
- **Neutral ODN** - Control ODN

Human/mouse NOD1/2 Agonist Kit

This kit contains 10 agonists, that activate NOD1 and/or NOD2. It can be used in human or mouse studies.

- **C12-iE-DAP, iE-DAP and Tri-DAP** - NOD1 agonists
- **MDP, L18-MDP and murabutide** - NOD2 agonists
- **M-TriDAP** - NOD1/2 agonist
- **M-TriLYS** - NOD2 agonist
- **PGN-ECndi ultrapure and PGN-SAndi ultrapure** - NOD1/2 agonists

Contents

All agonists are provided in a powdered form. Products are shipped at room temperature and should be stored at 4°C or -20°C according to the product label.

PRODUCT	QUANTITY	CAT. CODE
Mouse TLR9 Agonist Kit - ODN 1585 - ODN 1585 control - ODN 1826 - ODN 1826 control (ODN 2138) - ODN 2395 - ODN 2395 control	 100 µg 100 µg 100 µg 100 µg 100 µg 100 µg	tlrl-kit9m
B-Class TLR9 Agonist Kit - ODN 1668 - ODN 1826 - ODN 2006 - ODN 2007 - ODN BW006 - ODN D-SL01	 100 µg 100 µg 100 µg 100 µg 100 µg 100 µg	tlrl-kit9b
A&C-Class TLR9 Agonist Kit - ODN 1585 - ODN 2216 - ODN 2336 - ODN 2395 - ODN D-SL03 - ODN M362	 100 µg 100 µg 100 µg 100 µg 100 µg 100 µg	tlrl-kit9ac
TLR9 Antagonist Kit - ODN 2088 - ODN 4084-F - ODN INH-1 - ODN INH-18 - ODN TTAGGG (ODN A151) - Neutral ODN	 100 µg 100 µg 100 µg 100 µg 100 µg 100 µg	tlrl-kit9i
Human/mouse NOD1/2 Agonist Kit - iE-DAP - C12-iE-DAP - Tri-DAP - MDP - L18-MDP - Murabutide - M-TriDAP - M-TriLYS - PGN-ECndi ultrapure - PGN-SAndi ultrapure	 100 µg 25 µg 25 µg 100 µg 25 µg 100 µg 25 µg 25 µg 100 µg 100 µg	tlrl-nodkit2

RECENT CITATIONS

Human TLR1-9 Agonist Kit - Cram ED. *et al.*, 2016. Enhanced Direct Major Histocompatibility Complex Class I Self-Antigen Presentation Induced by Chlamydia Infection. Infect Immun. 84(2):480-90.

Kato E. *et al.*, 2016. Effects of Cytokines and TLR Ligands on the Production of PlGF and sVEGFR1 in Primary Trophoblasts. Gynecol Obstet Invest. [Ahead of print].

Mouse TLR1-9 Agonist Kit - Deng M. *et al.*, 2015. Shedding of the tumor necrosis factor (TNF) receptor from the surface of hepatocytes during sepsis limits inflammation through cGMP signaling. Sci Signal. 8(361):ra11.

Lyu JH. *et al.*, 2016. Regulation of PHLDA1 Expression by JAK2-ERK1/2-STAT3 Signaling Pathway. J Cell Biochem. 117(2):483-90.

RELATED PRODUCTS

PRODUCT	INFO
HEK-Blue™ TLR cells	See page 42
HEK-Blue™ NOD cells	See page 44

PRR LIGAND SCREENING SERVICE

TLR, NOD, RLR AND CLR SCREENING SERVICE

There is a growing interest in the targeting of Toll-like receptors (TLRs) and other pattern recognition receptors (PRRs) for drug discovery research. As a recognized industry leader in innate immunity, InvivoGen provides a high quality immunomodulatory compound screening service to assist our clients' drug discovery and development needs.

- **Short turnaround time -** Screening turnaround: ONLY 3 weeks
- **Screening flexibility -** Screening parameters can be selected and/or modified based on customer requirements
- **Cost effective -** A set-up charge applies for the first compound. Subsequent compounds are heavily discounted
- **Reliable -** Screening service utilized consistently by leading Biotech and Pharmaceutical companies and academic institutes for many years

Description

Over the past several years, InvivoGen has developed a large collection of cellular assays to detect compounds that activate or block the immune system through activation of PRRs with an emphasis on TLRs, NOD1/2 and CLRs. These sensitive cellular assays feature engineered HEK293-derived cells which utilize an NF-κB-inducible SEAP (secreted embryonic alkaline phosphatase) reporter gene as the read-out. Ligand-induced activation of the NF-κB or IRF pathways is monitored using proprietary detection assays designed to provide rapid and reliable results.

TLR Ligand Screening

The TLR ligand screening service is performed using a panel of HEK-Blue™ TLR cells (see page 42), engineered to express only a single specific TLR and a SEAP–reporter plasmid activated with NF-κB. The TLRs expressed are human or mouse TLR2, 3, 4, 5, 7, 8 or 9. The customer's compound can be tested on all human and/or mouse TLRs to determine if one or several TLRs recognize the compound. TLR ligands are typically recognized by a single TLR or potentially two (TLR7 and TLR8). Recognition by TLR4 usually reflects the presence of endotoxins in the sample.
Antagonist assays can also be performed.

NOD1/2 Ligand Screening

The NOD1/2 ligand screening service utilizes the HEK-Blue NOD cells (see page 44). These cells stably express an NF-κB-inducible SEAP reporter gene and the NOD1 or NOD2 gene. The customer's compound can be tested on human and/or mouse NOD1 and NOD2.

Dectin-1 Ligand Screening

The Dectin-1 ligand screening service is performed on the HEK-Blue™ hDectin-1b cell line, which expresses the human dectin-1b isoform (see page 45). This cell line is activated specifically by particulate Dectin-1 ligands, such as zymosan depleted. It does not respond to soluble b-glucans, such as laminarin and WGP soluble, in accordance with the literature.
Antagonist assays can also be performed.

Mincle Ligand Screening

The Mincle ligand screening service utilizes the HEK-Blue™ hMincle reporter cell line, which co-expresses the human mincle gene, genes of the Mincle-NF-κB signaling pathway and an NF-κB-inducible SEAP reporter gene. This cell line responds specifically to Mincle ligands, such as trehalose-6,6-dibehenate (TDB) and heat-killed *M. tuberculosis* (HKMT).

Screening Services

Two choices of services are offered, Compound Profiling and Compound Dose Response, that can be performed sequentially or individually.

Compound Profiling (level 1): Single dose testing on a set of PRRs.
Screening is performed at a single concentration, typically a 1/10 dilution of the original compound solution provided, or as specified by customer.

Compound Dose Response (level 2): Dose response on one or several PRRs.
Three concentrations of the compound(s), typically 1/10, 1/100 and 1/1000 dilutions of the original compound solution, are tested on the PRR(s) recognizing the compound(s) as determined in level 1 or specified by the customer.

Personalized Service

Our staff will help choose the most appropriate screening parameters to ensure that your company's needs are met in the most reliable, timely, and cost-effective manner possible.

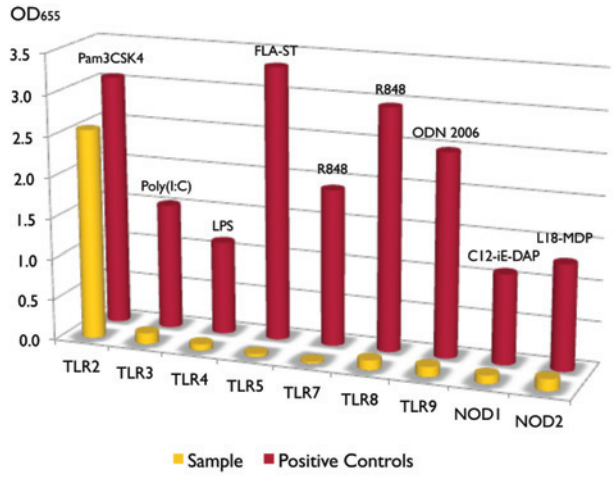
A detailed report is prepared and provided to the customer electronically and in hard copy. All procedures are performed according to strict guidelines. Confidentiality is guaranteed.

PRODUCT	CAT. CODE
Compound Profiling	tlrl-test1
Compound Dose Response	tlrl-test2

Question 1: Is my compound a TLR or NOD agonist?

To answer this question, all 7 HEK-Blue™ TLR or NOD cells will be stimulated with a single dose of your compound as well as a set of known TLR agonists that serve as positive controls. After overnight incubation, the production of SEAP is assessed using QUANTI-Blue™, a SEAP detection medium. The OD is measured and analyzed.

Example 1: As seen in graph 1, only HEK-Blue™ hTLR2 cells produce SEAP in response to the compound tested, suggesting that this compound activates TLR2 and no other TLR. Thus, the compound tested is a TLR2 agonist.

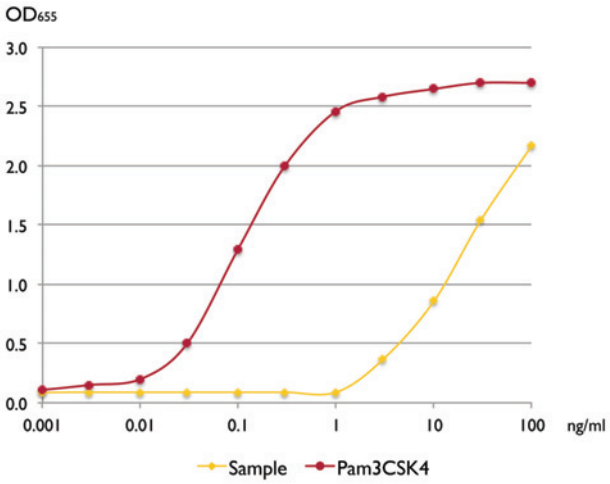


Graph 1. HEK-Blue™ TLR (human TLR2, 3, 4, 5, 7, 8, 9) and HEK-Blue™ NOD (human NOD1 or NOD2) cells were stimulated with 1/10 dilution of the sample solution provided and a fixed concentration of the positive controls: 100 ng/ml Pam3CSK4 (TLR2), 1 µg/ml poly(I:C) (TLR3), 100 ng/ml LPS-EK (TLR4), 100 ng/ml FLA-ST UP (TLR5), 10 µg/ml R848 (TLR7/8), 10 µg/ml ODN 2006 (TLR9), 10 µg/ml C12-iE-DAP (NOD1) and 10 µg/ml L18-MDP (NOD2). After 24h incubation, TLR-induced NF-κB activation was assessed by measuring the levels of SEAP in the supernatants of HEK-Blue™ cells using QUANTI-Blue™.

Question 2: Is my compound a potent TLR2 agonist?

To determine the potency of the compound tested as a TLR2 agonist, a dose response of the compound and a TLR2 positive control is performed on the HEK-Blue™ hTLR2 cells assayed using QUANTI-Blue™. The TLR2 positive control used is Pam3CSK4, a synthetic lipopeptide, that activates TLR2 at sub-nanogram per ml concentrations.

Example 2: As seen in graph 2, the compound tested is a good TLR2 agonist although not as potent as the positive control. The linear range of TLR2 activation is 5-100 ng/ml for the sample and 0.05-0.5 ng/ml for Pam3CSK4.



Graph 2. HEK-Blue™ hTLR2 cells were stimulated with increasing concentrations of the sample provided or the TLR2 positive control, Pam3CSK4. After 24h incubation, TLR2-induced NF-κB activation was assessed by measuring the levels of SEAP in the supernatants of HEK-Blue™ hTLR2 cells using the QUANTI-Blue™ assay.

RECENT CITATIONS

Siefert AL. *et al.*, 2016. Artificial bacterial biomimetic nanoparticles synergize pathogen-associated molecular patterns for vaccine efficacy. Biomaterials. 97:85-96..

Dimier-Poisson I. *et al.*, 2015. Porous nanoparticles as delivery system of complex antigens for an effective vaccine against acute and chronic Toxoplasma gondii infection. Biomaterials. 50:164-75.

Jonas A. *et al.*, 2014. Axonally derived matrilin-2 induces proinflammatory responses that exacerbate autoimmune neuroinflammation. J Clin Invest. 124(11):5042-56.

Contact us for more information at info@invivogen.com

5 INHIBITORS

Inhibitors of JAK/STAT Activation 114

Inhibitors of NF-κB & MAPK Activation 115-116

Inhibitors of PRR Signaling 117-119

Inhibitors of mTOR & Calcineurin Signaling 120-121

Inhibitors of DNA Synthesis & Chromatin Remodeling 122

Inhibitors of Hsp90 123

INHIBITORS

InvivoGen offers an expanding collection of inhibitors that block key cellular processes, such as replication and transcription, or key signaling pathways such as those leading to the activation of the transcription factors NF-κB or STAT. Many of these inhibitors are small molecules that are being tested or are currently used in the treatment of a variety of human diseases, including cancer, diabetes, malaria and rheumatoid arthritis.

InvivoGen is continuously expanding its collection of inhibitors.

- Inhibitors of JAK/STAT activation
- Inhibitors of NF-κB & MAPK activation
- Inhibitors of PRR signaling
- Inhibitors of mTOR & calcineurin signaling
- Inhibitors of DNA synthesis & chromatin remodeling
- Inhibitors of Hsp90

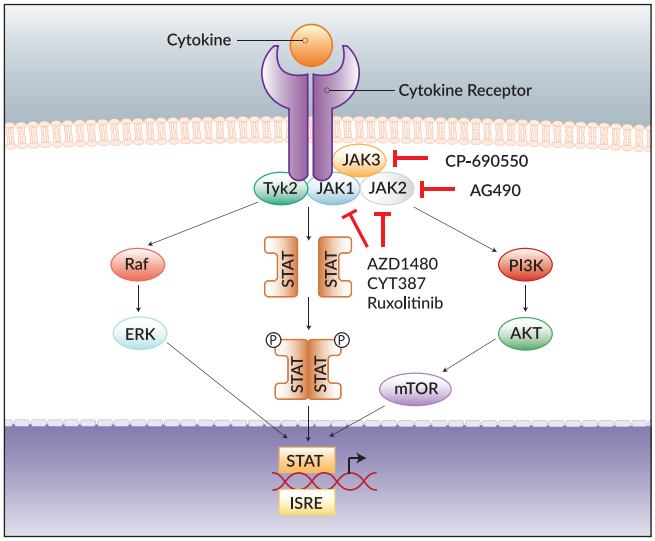
The inhibitors provided by InvivoGen are high quality products:

- Purity tested by HPLC,
- Inhibitory activity validated, when a reporter cell assay is available,
- Absence of TLR2 and TLR4 contaminant activities confirmed using HEK-Blue™ TLR cells.

PRODUCT	DESCRIPTION	TARGET	WORKING CONCENTRATION	QTY	CATALOG CODE	INFO
17-AAG	Geldanamycin (GA) analogue	Hsp90	1 nM - 10 µM	5 mg 25 mg	ant-agl-5 ant-agl-25	p. 123
17-DMAG	GA analogue	Hsp90	1 nM - 10 µM	5 mg 25 mg	ant-dgl-5 ant-dgl-25	p. 123
2-Aminopurine	PKR inhibitor	PRR signaling	1 - 10 mM	250 mg	tlrl-apr	p. 117
3-Methyladenine	PI3K inhibitor	mTOR / Calcineurin	5 mM	50 mg	tlrl-3ma	p.120
5-Aza-2'-deoxycytidine (Decitabine)	DNA methyltransferase inhibitor	DNA / Chromatin	0.1 - 10 µM	10 mg 50 mg	met-adc-1 met-adc-5	p. 122
5-Fluorocytosine	Thymidine synthesis inhibitor	DNA / Chromatin	200 - 500 µg/ml	250 mg	sud-5fc	p. 122
5-Fluorouracil	Thymidine synthesis inhibitor	DNA / Chromatin	1 - 300 µg/ml	250 mg	sud-5fu	p. 122
A-769662	AMPK activator / mTOR inhibitor	mTOR / Calcineurin	100 - 300 µM	10 mg	inh-a769	p. 120
Ac-YVAD-cmk	Caspase-1 inhibitor	PRR signaling	0.1 - 30 µg/ml	5 mg	inh-yvad	p. 117
AG490	JAK family tyrosine kinase inhibitor	JAK / STAT	20-50 µM	10 mg	tlrl-ag4	p. 114
Amlexanox	TBK1/IKKε inhibitor	PRR signaling	1 - 300 µg/ml	50 mg	inh-amx	p. 117
AZD1480	JAK2 inhibitor	JAK / STAT	0.5 -2.5 µM	5 mg	inh-ad14	p. 114
AZD6244	MEK1/2 Inhibitor	NF-κB / MAPK	0.2 -150 µM	50 mg	inh-ad62	p. 115
Bafilomycin A1	V-ATPase inhibitor	PRR signaling	0.1 - 1 µM	10 mg	tlrl-baf	p. 117
Bay11-7082	IkB-α inhibitor	NF-κB / MAPK	1 - 10 µM	10 mg	tlrl-b82	p. 115
BEZ235	PI3K/mTOR inhibitor	mTOR / Calcineurin	1-500 nM	50 mg	inh-bez2	p. 120
BX795	TBK1/IKKε and PDK1 inhibitor	PRR signaling	10 nM - 1 µM	5 mg	tlrl-bx7	p. 117
Celastrol	NF-κB inhibitor	NF-κB / MAPK	2.5 - 10 µM	1 mg	ant-cls	p. 115
Chloroquine	Endosomal acidification inhibitor	PRR signaling	10 µM	250 mg	tlrl-chq	p. 117
CLI-095	TLR4 signaling inhibitor	PRR signaling	50 nM - 1 µM	1 mg	tlrl-cli95	p. 117
CP-690550	JAK3 inhibitor	JAK / STAT	50 nM - 1 µM	5 mg	tlrl-cp69	p. 114
Cyclosporin A	Calcineurin inhibitor	mTOR / Calcineurin	50 nM - 1.5 µM	100 mg	tlrl-cyca	p. 120
CYT387	JAK1/JAK2 & TBK1/IKK-ε inhibitor	JAK / STAT	0.1 - 30 µg/ml	10 mg	inh-cy87	p. 114
Dexamethasone	NF-κB and MAPK inhibitor	NF-κB / MAPK	100 nM	100 mg	tlrl-dex	p. 115
Everolimus (RAD001)	mTOR inhibitor	mTOR / Calcineurin	1 - 300 nM	5 mg	tlrl-eve	p. 120
FITC-Geldanamycin	FITC-labeled Hsp90 inhibitor	Hsp90	1 nM - 10 µM	1 mg 5 mg	ant-fgl-1 ant-fgl-5	p. 123
FK506 (Tacrolimus)	Calcineurin inhibitor	mTOR / Calcineurin	1- 100 nM	10 mg	tlrl-fk5	p. 120
Ganciclovir	Thymidine synthesis inhibitor	DNA / Chromatin	1 - 100 µg/ml	250 mg	sud-gcv	p. 122
Gefitinib (Iressa)	RIP2 tyrosine kinase inhibitor	PRR signaling	10 µM	10 mg	tlrl-gef	p. 117
Geldanamycin (GA)	Hsp90 inhibitor	Hsp90	1 nM - 10 µM	5 mg 25 mg	ant-gl-5 ant-gl-25	p. 123
Glybenclamide (glyburide)	ATP-dependent K+ channel inhibitor	PRR signaling	25 µg/ml	1 g	tlrl-gly	p. 118
H-89	PKA inhibitor	PRR signaling	5 - 50 µM	5 mg	tlrl-h89	p. 118
Isoliquiritigenin	NLRP3 inflammasome inhibitor	PRR signaling	1 - 50 µg/ml	10 mg	inh-ilg	p. 118

PRODUCT	DESCRIPTION	TARGET	WORKING CONCENTRATION	QTY	CATALOG CODE	INFO
Leptomycin B	Nuclear export inhibitor	DNA / Chromatin	50 - 200 nM	5 µg	tlrl-lep	p. 122
LL-37	Antimicrobial peptide	PRR signaling	1 - 50 µg/ml	1 mg	tlrl-l37	p. 118
LY294002	PI3K inhibitor	mTOR / Calcineurin	50 - 100 µM	5 mg	tlrl-ly29	p. 120
Metformin	AMPK activator / mTOR inhibitor	mTOR / Calcineurin	2 - 10 mM	1 g	tlrl-metf	p. 120
MG-132	26S proteasome inhibitor	NF-κB / MAPK	10 µM	5 mg	tlrl-mg132	p. 115
OSU-03012 (AR-12)	PDK1 inhibitor	mTOR / Calcineurin	1 - 5 µM	10 mg	inh-os03	p. 121
OxPAPC	TLR2 and TLR4 inhibitor	PRR signaling	30 µg/ml	1 mg	tlrl-oxp1	p. 118
Parthenolide	Caspase-1 and inflammasome inhibitor	PRR signaling	0.5 - 50 µg/ml	50 mg	inh-ptd	p. 118
PD0325901	MEK inhibitor	NF-κB / MAPK	0.5 µM	2 mg	inh-pd32	p. 115
PD98059	MAP kinase kinase inhibitor	NF-κB / MAPK	50 - 100 µM	10 mg	tlrl-pd98	p. 115
Pepinh-MYD	MyD88 inhibitory peptide	PRR signaling	5 - 100 µM	2 mg	tlrl-pimyd	p. 118
Pepinh-TRIF	TRIF inhibitory peptide	PRR signaling	5 - 100 µM	2 mg	tlrl-pitrif	p. 118
Perifosine	Akt inhibitor	mTOR / Calcineurin	5 - 50 µM	5 mg	tlrl-peri	p. 121
PI-103	PI3K/mTOR inhibitor	mTOR / Calcineurin	0.1 - 10 µM	5 mg	inh-pi10	p. 121
Piceatannol	Syk inhibitor	PRR signaling	1 - 25 µM	5 mg	tlrl-pct	p. 118
PLX4032	B-Raf inhibitor	NF-κB / MAPK	0.1 - 30 µM	20 mg	inh-pl40	p. 115
PLX4720	B-Raf inhibitor	NF-κB / MAPK	1 - 30 µM	10 mg	inh-px47	p. 116
Polymyxin B	LPS-induced TLR4 activation inhibitor	PRR signaling	10 µg/ml	100 mg	tlrl-pmb	p. 118
R406	Syk inhibitor	PRR signaling	10 nM - 100 µM	2 mg	inh-r406	p. 118
Rapamycin (Sirolimus)	mTOR inhibitor	mTOR / Calcineurin	10-100 nM	5 mg	tlrl-rap	p. 121
Resveratrol	NF-κB and AP-1 inhibitor	NF-κB / MAPK	10 - 100 µM	100 mg	tlrl-resv	p. 116
Ruxolitinib	Pan JAK inhibitor	JAK / STAT	100 - 1000 nM	5 mg	tlrl-rux	p. 114
SAHA	Pan HDAC inhibitor	DNA / Chromatin	0.1 - 10 µM	25 mg	inh-saha	p. 122
SB202190	p38 MAP kinase inhibitor	NF-κB / MAPK	1 - 20 µM	5 mg	tlrl-sb90	p. 116
SB203580	p38 MAP kinase inhibitor	NF-κB / MAPK	1 - 20 µM	5 mg	tlrl-sb20	p. 116
SB431542	TGF-β receptor inhibitor	NF-κB / MAPK	2 µM	5 mg	inh-sb43	p. 116
SP600125	JNK inhibitor	NF-κB / MAPK	10 - 500 µM	10 mg	tlrl-sp60	p. 116
Temsirolimus	mTOR inhibitor	mTOR / Calcineurin	1 nM - 1 µM	10 mg	inh-tems	p. 121
Trichostatin A	Histone deacetylase inhibitor	DNA / Chromatin	150 - 300 nM	1 mg 5 mg	met-tsa-1 met-tsa-5	p. 122
Triptolide	NF-κB activation inhibitor	NF-κB / MAPK	10 - 100 nM	1 mg	ant-tpl	p. 116
U0126	MEK1-MEK2 inhibitor	NF-κB / MAPK	10 - 50 µM	5 mg	tlrl-u0126	p. 116
VX-765	Caspase-1 inhibitor	PRR signaling	10 - 100 µM	10 mg 50 mg	inh-vx765-1 inh-vx765-5	p. 118
Wortmannin	PI3K inhibitor	mTOR / Calcineurin	0.1 - 2.5 µM	5 mg	tlrl-wtm	p. 121
YM201636	PYKfyve inhibitor	PRR signaling	0.5 - 5 µM	5 mg	inh-ym20	p. 118
Z-VAD-FMK	Pan-caspase Inhibitor	PRR signaling	10 µg/ml (20 µM)	1 mg	tlrl-vad	p. 118

INHIBITORS OF JAK/STAT ACTIVATION



AG490 - JAK2 Inhibitor

AG490 is a specific and potent inhibitor of the Janus kinase 2 protein (JAK2)[1]. JAK2 regulates the phosphorylation of JNK, primarily through PI3K. It has been established that JAK2 plays an important role in TLR-mediated biological responses, blocking TLR4-mediated responses to LPS[2] and TLR5-mediated responses to flagellin[3].

AZD1480 - JAK1 & JAK2 Inhibitor

AZD1480 is an ATP-competitive inhibitor of JAK2, and to a lesser extent of JAK1. It exerts its biological effects by preventing the phosphorylation and activation of STAT3 (signal transducer and activator of transcription 3) both *in vitro* and *in vivo*[4] By suppressing STAT3 activation, AZD1480 blocks downstream targets of STAT3, such as the cell cycle regulator Cyclin D1 and the anti-apoptotic genes, Bcl-2 and Survivin. As a result, AZD1480 inhibits cell proliferation and induces cell apoptosis[4, 5].

CP-690550 - JAK3 Inhibitor

CP-690550 (Tofacitinib), an immunosuppressant, specifically inhibits JAK3, which has a pivotal role in cytokine signal transduction that governs lymphocyte survival, proliferation, differentiation, and apoptosis[6] In experimental models, treatment with CP-690550 decreases IL-6 production, a critical cytokine that drives inflammation[7]. Recently, it has been shown that CP-690550 also inhibits TNF-induced chemokine expression[8].

CYT387 - JAK1 & JAK2 Inhibitor

CYT387 (Momelotinib) is a potent ATP-competitive inhibitor of Janus kinases JAK1 and JAK2[9], thereby interrupting the signaling via the JAK-STAT pathway. CYT387 is significantly less active against other kinases, including JAK3. In addition, CYT387 is a potent inhibitor of the noncanonical IκB kinases IKK-ε and TANK-binding kinase 1 (TBK1)[10]. As a result, CYT387 prevents both NF-κB and STAT activation. Consequently, this multi-target inhibitor disrupts the expression of pro-tumorigenic cytokines, induces apoptosis and suppresses proliferation of many cells types, in particular cells harboring the JAK2V617F mutation which is associated with blood cancers[9, 11].

Ruxolitinib - Pan JAK Inhibitor

Ruxolitinib (INCB018424) is a small-molecule ATP mimetic that potently inhibits JAK1 and JAK2 in the blood[12]. Ruxolitinib is clinically used for the treatment of myelofibrosis, a bone marrow disorder[13], and is being investigated for the treatment of certain cancers and autoimmune diseases, such as psoriasis.

1. Levitzki A., 1990. Tyrphostins - potential antiproliferative agents and novel molecular tools. Biochem Pharmacol. 40:913-8. **2. Kimura A *et al.*, 2005.** Suppressor of cytokine signaling-1 selectively inhibits LPS-induced IL-6 production by regulating JAK–STAT. PNAS 102:17089-94. **3. Ha H. *et al.*, 2008.** Stimulation by TLR5 Modulates Osteoclast Differentiation through STAT1/IFN-β1. J. Immuno. 180:1382-9. **4. Yan S. *et al.*, 2013.** Inhibition of STAT3 with orally active JAK inhibitor, AZD1480, decreases tumor growth in neuroblastoma and pediatric sarcomas in vitro and in vivo. Oncotarget. 4(3):433.445. **5. Derenzini E. *et al.*, 2011.** The JAK inhibitor AZD1480 regulates proliferation and immunity in Hodgkin lymphoma. Blood Cancer J. 1(12):e46. **6. Wu W. & Sun X., 2012.** Janus kinase 3: the controller and the controlled. Acta Biochim Biophys Sin. 44(3):187-96. **7. Milici A. *et al.*, 2008.** Cartilage preservation by inhibition of Janus kinase 3 in two rodent models of rheumatoid arthritis. Arthritis Res Ther. 10: R14. **8. Sanna Rosengren S. *et al.*, 2012.** The JAK inhibitor CP-690,550 (tofacitinib) inhibits TNF-induced chemokine expression in fibroblast-like synoviocytes: autocrine role of type I interferon. Ann Rheum Dis 71:440-7. **9. Pardanani A. *et al.*, 2009.** CYT387, a selective JAK1/JAK2 inhibitor: In vitro assessment of kinase selectivity and preclinical studies using cell lines and primary cells from polycythemia vera patients. Leukemia 23:1441–5. **10. Zhu Z. *et al.*, 2014.** Inhibition of KRAS-driven tumorigenicity by interruption of an autocrine cytokine circuit. Cancer Discov. 4:452-65. **11. Tyner JW. *et al.*, 2010.** CYT387, a novel JAK2 inhibitor, induces hematologic responses and normalizes inflammatory cytokines in murine myeloproliferative neoplasms. Blood. 15:5232-40. **12. Quintás-Cardama A., 2010.** Preclinical characterization of the selective JAK1/2 inhibitor INCB018424: therapeutic implications for the treatment of myeloproliferative neoplasms. Blood 115(15):3109-17. **13. Mascarenhas J. & Hoffman R., 2012.** Ruxolitinib: The first FDA approved therapy for the treatment of myelofibrosis. Clin Cancer Res. 18:3008-14.

RECENT CITATIONS

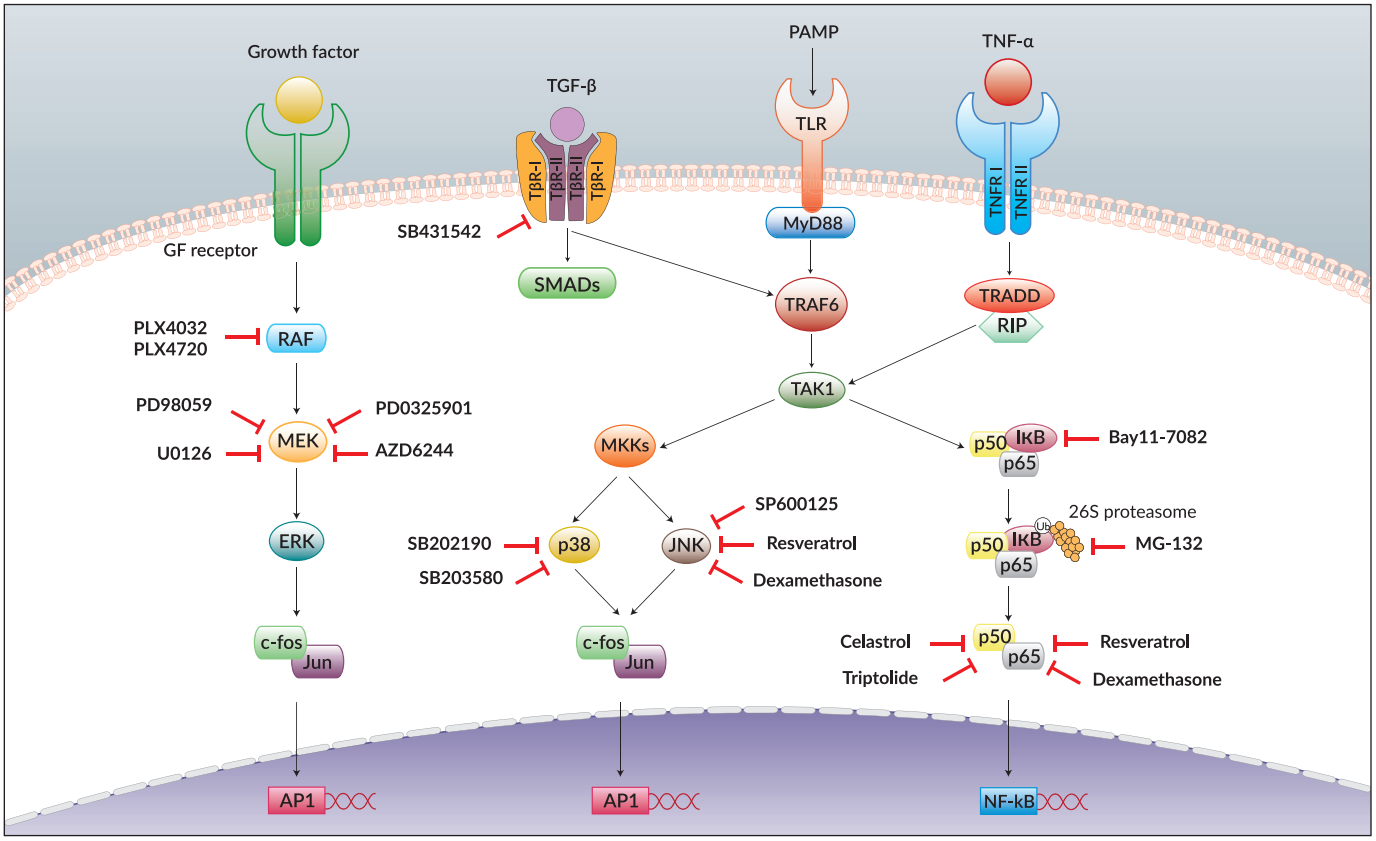
AG490 - Wang D. *et al.*, 2016. Combined administration of propranolol + AG490 offers better effects on portal hypertensive rats with cirrhosis. J Gastroenterol Hepatol. 31(5):1037-44.

Ruxolitinib - Cronin JG. *et al.*, 2016. Signal transducer and activator of transcription-3 licenses Toll-like receptor 4-dependent interleukin (IL)-6 and IL-8 production via IL-6 receptor-positive feedback in endometrial cells. Mucosal Immunol. [Ahead of print].

Chiappinelli KB. *et al.*, 2015. Inhibiting DNA Methylation Causes an Interferon Response in Cancer via dsRNA Including Endogenous Retroviruses.. Cell. 162(5):974-86.

Ruxolitinib & CP-690550 - Lamarthée B. *et al.*, 2016. Donor interleukin-22 and host type I interferon signaling pathway participate in intestinal graft-versus-host disease via STAT1 activation and CXCL10. Mucosal Immunol. 9(2):309-21.

INHIBITORS OF NF-κB & MAPK ACTIVATION



AZD6244 - MEK1/2 Inhibitor

AZD6244 (Selumetinib) is a specific inhibitor of MEK1/2, a key enzyme that regulates multiple cellular functions, including proliferation, differentiation and survival. AZD6244 inhibits cell proliferation in multiple cancer cell lines[1]. In experimental animal models, AZD6244 effectively inhibits tumor growth by blocking cell proliferation and angiogenesis. These anti-tumor and anti-angiogenic effects can be enhanced by combining AZD6244 with the mTOR inhibitor rapamycin[2] or with the PI3K/mTOR inhibitor BEZ235[1].

Bay 11-7082 - IkB-α Inhibitor

Bay 11-7082 is an irreversible inhibitor of TNF-α-induced IkB-α phosphorylation resulting in the inactivation of NF-κB[3]. TNF-α-dependent effects of NF-κB are important for TLR expression and cytokine production[4]. Recently, Bay-11-7082 was identified as a potent inhibitor of the NLRP3 inflammasome independent of its inhibitory effect on NF-κB. It is believed to act by suppressing the ATPase activity of NLRP3[5].

Celastrol - NF-κB Inhibitor

Celastrol is a triterpenoid compound isolated from the medicinal plant *Tripterygium wilfordii* known for its anti-inflammatory properties. Its mode of action and spectrum of cellular targets are still poorly understood. Celastrol was recently shown to act as an effective inhibitor of the transcription factor NF-κB resulting in the attenuation of nitric oxide and proinflammatory cytokine production[6, 7].

Dexamethasone - NF-κB & MAPK Inhibitor

Dexamethasone is a synthetic glucocorticoid (GC) compound with potent anti-inflammatory activities. It activates the GC receptor which binds to specific DNA sites located in the promoter of GC-regulated genes and also down-regulates the activity of transcription factors[8]. Dexamethasone has been shown to repress a large set of pro-inflammatory genes by blocking NF-κB and MAPK activation during TLR engagement[9].

MG-132 - 26S Proteasome Inhibitor

MG-132 is a peptide aldehyde (Z-Leu-Leu-Leu-al) that selectively blocks the proteolytic activity of the 26S proteasome. This potent inhibitor is used as a tool for disrupting the proteasome-regulated degradation of intracellular proteins, such as IκB. Inhibition of IκB proteasomal degradation by MG-132 leads to the suppression of NF-κB activation[10].

PD98059 - MAP Kinase Kinase Inhibitor

PD98059 is a potent and selective inhibitor of MAP kinase kinase (also known as MAPK/ERK kinase or MEK kinase)[11]. It mediates its inhibitory properties by binding to the ERK-specific MAP kinase MEK, therefore preventing phosphorylation of ERK1/2 (p44/p42 MAPK) by MEK1/2.

PD0325901 - MEK Inhibitor

PD0325901 is a selective inhibitor of mitogen-activated protein kinase kinase (MEK), with potential antineoplastic activity[12]. MEK is a key component of the RAS/RAF/MEK/ERK signaling pathway that is frequently activated in human tumors. This pathway plays also an important role in the self-renewing state of embryonic stem cells.

PLX4032 - B-Raf Inhibitor

PLX4032 (Vemurafenib) is a reversible, ATP-competitive inhibitor of B-Raf, a serine/threonine protein kinase with regulatory functions in MAPK signaling. PLX4032 also inhibits Raf-1 and several non-RAF kinases[13]. Interestingly, PLX4032 specifically blocks the MAPK pathway in cells expressing B-Raf-V600E, a B-Raf mutant associated with different types of cancer[13]. The V600E mutation in B-Raf leads to constitutive activation of MAPK signaling and increased cell proliferation. Inhibition of B-Raf-V600E with PLX4032 induces cell cycle arrest and apoptosis[14].

PLX4720 - B-Raf Inhibitor

PLX4720 is a potent and selective inhibitor of B-Raf-V600E, a B-Raf mutant associated with different types of cancer[15]. In fact, PLX4720 has a 10-fold greater selectivity for B-Raf-V600E than the wild-type B-Raf. Inhibition of B-Raf-V600E with PLX4720 blocks the activation of MAPK signaling, and thereby induces cell cycle arrest and apoptosis[16].

Resveratrol - NF-κB & MAPK Inhibitor

Resveratrol (3,4',5-trihydroxy-trans-stilbene), a polyphenol found in plants, possesses anti-inflammatory and chemopreventive properties. It inhibits the expression of proinflammatory markers, including inducible nitric oxide synthase and cyclooxygenase-2 in macrophages and cancer cells[17], blocks TRIF-dependent signaling pathway of TLR3 and TLR4[18] and modulates bacterial phagocytosis of macrophages[19]. Resveratrol may exert these diverse effects by suppressing NF-κB and/or AP-1 activation in a cell or tissue-specific manner[20].

SB202190 - p38/RK MAP Kinase Inhibitor

SB202190, a close relative of SB203580, is widely used to assess the physiological roles of p38a and p38b MAPKs. Recent studies have identified other protein kinases, including GAK, CK1 and RIP2, that are potently inhibited by SB202190 (as well as SB203580)[21]. Further, SB202190 was shown to induce autophagic vacuoles through cross-inhibition of the PI3K/mTOR pathway[22].

SB203580 - p38/RK MAP Kinase Inhibitor

SB203580 is a pyridinyl imidazole inhibitor widely used to elucidate the roles of p38 mitogen-activated protein (MAP) kinase[23]. SB203580 inhibits also the phosphorylation and activation of protein kinase B (PKB, also known as Akt)[24].

SB431542 - TGF-β Receptor Inhibitor

SB431542 is a potent and selective inhibitor of activin receptor-like kinase (ALK)5, also known as the TGF-β type I receptor[25]. It blocks TGF-β-induced transcription, gene expression, apoptosis, and growth suppression[26].

SP600125 - JNK Inhibitor

SP600125 is a potent, cell-permeable, selective and reversible inhibitor of c-Jun N-terminal kinase (JNK)[27]. It inhibits in a dose-dependent manner the phosphorylation of JNK. JNK is a member of the mitogen-activated protein kinase (MAPK) family and plays an essential role in TLR-mediated inflammatory responses.

Triptolide - NF-κB Inhibitor

Triptolide, a diterpenoid isolated from the Chinese herb *Tripterygium wilfordii* hook F, has been used for centuries in traditional Chinese medicine to treat immune-related disorders and has attracted extensive research interest due to its multiple biological activities. Recently, the cellular targets of triptolide, such as NF-κB, MKP-1, HSP, 5-Lox, RNA polymerase and histone methyl-transferases have been demonstrated[28].

U0126 - MEK1 and MEK2 Inhibitor

U0126 is a selective inhibitor of the MAP kinase kinases, MEK1 and MEK2. It acts by inhibiting the kinase activity of MEK1/2 thus preventing the activation of MAP kinases p42 and p44 which are encoded by the *erk2* and *erk1* genes, respectively[29].

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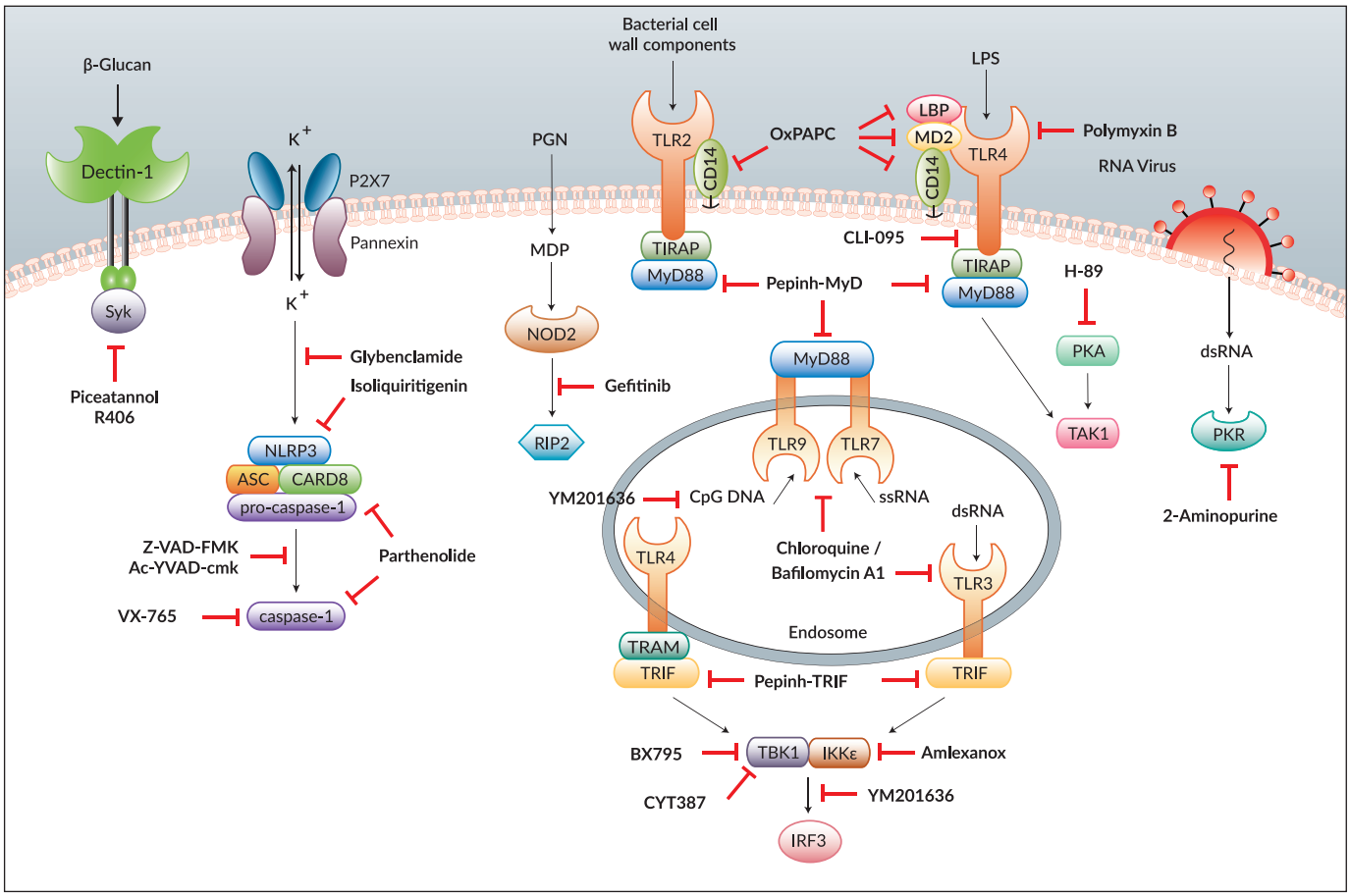
INHIBITORS OF PRR SIGNALING



2-Aminopurine - PKR Inhibitor

2-aminopurine (2-AP) is a potent inhibitor of double-stranded RNA (dsRNA)-activated protein kinase (PKR), a critical mediator of apoptosis. PKR is phosphorylated and activated by dsRNA and poly(I:C)[1] and contributes to the induction of type I interferons, such as IFN-β, which can further increase its expression[2]. PKR plays also a role in TLR-induced antiviral activities as an intermediary in TLR3, TLR4 and TLR9 signaling[3].

Ac-YVAD-cmk - Caspase-1 Inhibitor

Ac-YVAD-cmk is a cell-permeable, selective and irreversible inhibitor of caspase-1, with some activity against caspase-4 and caspase-5[4]. Ac-YVAD-cmk is a chloromethyl ketone tetrapeptide based on the target sequence in proIL-1β (YVHD)[5]. It blocks inflammasome activation, and hence displays anti-inflammatory, anti-apoptotic and anti-pyroptotic effects[6, 7].

Amlexanox - TBK/IKKε Inhibitor

Amlexanox is a specific inhibitor of the noncanonical IκB kinases IKKε and TANK-binding kinase 1 (TBK1), in a manner competitive with ATP[8]. At the concentrations that block IKKε and TBK1, it has no effect on the canonical IκB kinases IKKα and IKKβ, or a large panel of other kinases. Amlexanox is approved for the treatment of asthma, allergic rhinitis and aphthous ulcers, due to its anti-inflammatory and anti-allergic properties.

Bafilomycin A1 - Proton Pump Inhibitor

Bafilomycin A1 is a specific inhibitor of the lysosomal proton pump, thus it indirectly inhibits lysosomal enzymes which have acidic pH optima. Bafilomycin treatment has been shown to inhibit autophagy by preventing the fusion of autophagosomes with both endosomes and lysosomes[9, 10]. Furthermore, by blocking endo-lysosomal compartment acidification, bafilomycin interferes with intracellular TLR signaling[11].

BX795 - TBK/IKKε Inhibitor

BX795, an aminopyrimidine compound, was developed as an inhibitor of 3-phosphoinositide-dependent kinase 1 (PDK1)[12]. It was recently shown to be a potent inhibitor of the IKK-related kinases, TANK-binding kinase 1 (TBK1) and IKKε, and hence of IRF3 activation and IFN-β production[13]. BX795 inhibits the catalytic activity of TBK1/IKKε by blocking their phosphorylation.

Chloroquine - Inhibitor of Endosomal Acidification

Chloroquine is a lysomotropic agent that inhibits autophagic flux by disturbing lysosome pH and function[14]. It accumulates inside the endosomes and lysosomes leading to the inhibition of lysosomal enzymes that require an acidic pH, and prevents fusion of endosomes and lysosomes. Chloroquine is commonly used to study the role of endosomal acidification in cellular processes, such as the signaling of intracellular TLRs[15, 16].

CLI-095 - TLR4 Signaling Inhibitor

CLI095 (TAK-242), is a novel cyclohexene derivative that specifically suppresses TLR4 signaling, inhibiting the production of NO and pro-inflammatory cytokines[17]. It acts by blocking the signaling mediated by the intracellular domain of TLR4, but not the extracellular domain. It potently suppresses both ligand-dependent and -independent signaling of TLR4[18].

Gefitinib - RIP2 Tyrosine Kinase Inhibitor

Gefitinib (Iressa) is a selective inhibitor of epidermal growth factor. It inhibits NOD2-induced cytokine release and NF-κB activation by inhibiting RIP2 (receptor-interacting protein 2) tyrosine phosphorylation which is critical for activation of NOD2 downsream signaling pathways[19]. Gefitinib is used in the treatment of advanced non-small cell lung cancer[20].

Glybenclamide (glyburide) - Potassium Channel Inhibitor
Glybenclamide, also known as glyburide, blocks the maturation of caspase-1 and pro-IL-1β by inhibiting K+ efflux[21]. Glybenclamide was shown to potently block the activation of the NLRP3 inflammasome induced by PAMPs, DAMPs and crystalline substances[22]. Recent data suggest that glybenclamide works downstream of the $P2X_7$ receptor but upstream of NLRP3.

H-89 - PKA Inhibitor
H-89 is a selective, potent cell permeable inhibitor of cAMP-dependent protein kinase (PKA)[23]. It can be used to determine the role of PKA in TLR and other PRR mediated signaling. PKA has be shown to participate in the TLR-mediated TREM-1 expression on macrophages following LPS stimulation[24].

Isoliquiritigenin - NLRP3 Inflammasome Inhibitor
Isoliquiritigenin (ILG), a simple chalcone-type flavonoid isolated from licorice root (Glycyrrhiza uralensis), exhibits anti-oxidant, anti-inflammatory, and anti-tumor activities[25]. ILG was recently reported to block LPS-induced TLR4/MD2 complex signaling and NF-κB activation[26], and to inhibit NLRP3-activated ASC oligomerization[27]. Interestingly, NLRP3-dependent IL-1β production has been inhibited with low concentrations of Isoliquiritigenin (1 to 10 μM). Thus, ILG can block the NLRP3 inflammasome at both the priming step and the activation step.

LL-37 - Antimicrobial Peptide
LL-37, also known as hCAP18, is the C-terminal part of the only human cathelicidin identified to date called human cationic antimicrobial protein (hCAP). LL-37 exhibits a variety of immunomodulatory functions such as bactericidal action, chemotaxis, activation of chemokine secretion and antisepsis effect[28]. The synthetic LL-37 peptide has been shown to suppress the inflammatory response induced by LPS and other TLR ligands[29].

OxPAPC - TLR2 and TLR4 Inhibitor
OxPAPC (1-palmitoyl-2-arachidonyl-sn-glycero-3-phosphorylcholine), is an oxidized phospholipid that has been shown to inhibit the signaling induced by bacterial lipopeptide and lipopolysaccharide (LPS). It acts by competing with CD14, LBP and MD2, the accessory proteins that interact with bacterial lipids, thus blocking the signaling of TLR2 and TLR4[30, 31].

Parthenolide - Caspase-1 and inflammasome inhibitor
Parthenolide, a sesquiterpene lactone derived from feverfew, is a known inhibitor of NF-κB activation. It is also a direct inhibitor of caspase-1 and consequently of multiple inflammasomes, including the NLRP3 and NLRP1 inflammasomes[32]. Parthenolide has been found to block caspase-1 activation by alkylation of the p20 subunit. Further, parthenolide directly inhibits the NLRP3 inflammasome by interfering with NLRP3 ATPase activity.

Pepinh-MYD - MyD88 Inhibitory Peptide
Pepinh-MYD is a 26 aa peptide that blocks MyD88 signaling by inhibiting its homodimerization through binding. Pepinh-MYD contains a sequence from the MyD88 TIR homodimerization domain (RDVLPGT)[33] preceeded by a protein transduction sequence (RQIKIWFQNRRMKWKK) derived from antennapedia which enables the peptide to translocate through the cell membrane[34]. Pepinh-MYD is provided with a control peptide.

Pepinh-TRIF - TRIF Inhibitory Peptide
Pepinh-TRIF is a 30 aa peptide that blocks TRIF signaling by interfering with TLR-TRIF interaction. Pepinh-TRIF contains the 14 aa that correspond to the sequence of the BB loop of TRIF (FCEEFQVPGRGELH)[35] linked to the cell-penetrating segment of the antennapedia homeodomain (RQIKIWFQNRRMKWKK)[34]. Pepinh-TRIF is provided with a control peptide.

Piceatannol - Syk Inhibitor
Piceatannol (3, 4', 3', 5-trans-trihydroxystilbene) is a resveratrol analogue with antioxidant, anticancer and anti-inflammatory activities. Piceatannol has been shown to inhibit NF-κB and JAK-1, two key players in the immune response. Piceatannol also inhibits Syk which plays a crucial role in the signaling pathway of C-type lectin receptors[36].

Polymyxin B - Inhibitor of LPS-Induced TLR4 Activation
Polymyxin B (PMB) is a cyclic cationic polypeptide antibiotic produced by the soil bacterium *Paenibacillus polymixa*. PMB blocks the biological effects of Gram negative LPS (endotoxin) through binding to lipid A, the toxic component of LPS, which is negatively charged[37,38]. The neutralizing effect of PMB on LPS is dose-dependent and specific for LPS[39]. PMB is widely used to eliminate the effects of endotoxin contamination, both *in vitro* and *in vivo*.

R406 - Syk Inhibitor
R406 (the active metabolite of Fostamatinib) is a specific, ATP-competitive inhibitor of spleen tyrosine kinase (Syk), which plays a key role in the signaling of activating Fc receptors and the B-cell receptor. R406 was shown to potently inhibit IgE- and IgG-mediated activation of Fc receptor signaling and to reduce inflammation in animal models of arthritis[40]. Inhibition of Syk with R406 selectively abrogates inflammasome activation by *Candida albicans* but not by inflammasome activators such as the bacterial toxin nigericin[41].

VX-765 - Caspase-1 Inhibitor
VX-765 is an orally absorbed prodrug of VRT-043198, a potent and selective inhibitor of caspases belonging to the ICE/caspase-1 subfamily[42]. VX-765 is converted to VRT-043198 under the action of plasma and liver esterases. The active metabolite of VX-765 exhibits potent inhibition of caspase-1 and caspase-4 and at least 100-fold lower potency against other non-ICE subfamily caspases. VX-765 was shown to reduce the production of IL-1β and IL-18 both in vitro and in vivo in correlation with tissue-protective effects in animal models of inflammatory disease[42]. VX-765 was found in a phase II a trial to be safe and well tolerated. Recent data demonstrate that VX-765 prevents CD4 T-cell pyroptotic death in a dose-dependent manner in HIV-infected lymphoid tissues[43].

YM201636 - PYKfyve Inhibitor
YM201636 is a potent inhibitor of mammalian phosphatidylinositol phosphate kinase PIP5KIII (PIKfyve) [1]. Inhibition of PIKfyve with YM201636 disrupts endomembrane transport[1. YM201636 blocks TLR9-signaling by preventing endosomal translocation of CpG-containing oligodeoxynucleotides (CpG ODNs), thus preventing co-localization of agonist and receptor [4]. YM201636 also inhibits TBK-1/IRF3-mediated type I IFN production without affecting NF-κB dependent cytokine production [5].

Z-VAD-FMK - Caspase Inhibitor
Z-VAD-FMK is a cell-permeable pan-caspase inhibitor that irreversibly binds to the catalytic site of caspase proteases[44]. The peptide is O-methylated in the P1 position on aspartic acid, providing enhanced stability and increased cell permeability. Z-VAD-FMK is used in apoptosis studies and also in inflammasome studies. It is a potent inhibitor of caspase-1 activation in NLRP3-induced cells[45].

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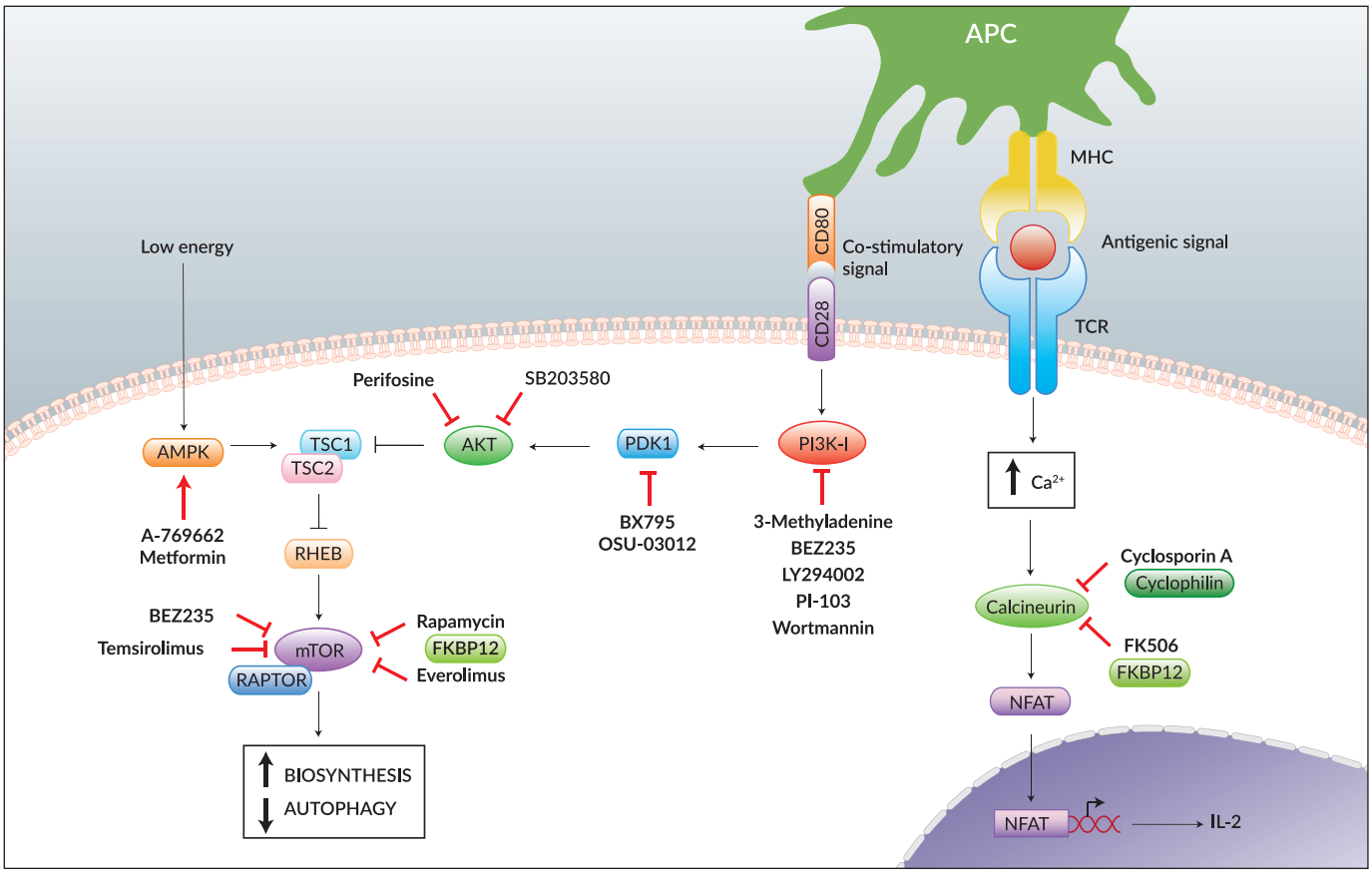
INHIBITORS OF mTOR & CALCINEURIN SIGNALING



3-Methyladenine - PI3K Inhibitor

3-Methyladenine (3-MA) is commonly used as a specific inhibitor of autophagic sequestration[1]. It blocks autophagy by the inhibition of phosphatidylinositol 3-kinase (PI3K) activity, an enzyme required for autophagy[2]. Further, it has been suggested that 3-MA inhibits autophagosome formation by inactivating p38[3].

A-769662 - AMPK Activator (mTOR Inhibitor)

A-769662 is a potent and reversible activator of AMPK (AMP-activated protein kinase), an energy sensing serine/threonine protein kinase important in cellular metabolism[4]. Through the activation of AMPK, A-769662 inhibits mammalian target of rapamycin (mTOR). Studies demonstrate that A-769662 inhibits cell proliferation of various cell types[5, 6].

BEZ235 - PI3K Inhibitor

BEZ235, an imidazoquinoline derivative, inhibits PI3K and mTOR kinase activity by binding to the ATP-binding cleft of these enzymes. By inhibiting mTOR activity, BEZ235 induces autophagy and promotes cells survival[7]. In contrast, the combination of BEZ235 with autophagy inhibitors, such as 3-methyladenine, inhibits cell growth and induces apoptosis[8, 9].

Cyclosporin A - Calcineurin inhibitor

Cyclosporin A (CsA) is an immunosuppressant drug widely used in organ transplantation to prevent rejection. CsA blocks calcineurin activation leading to the down-regulation of nuclear factor of activated T cells (NFAT), thus preventing the transcription of T cell effector cytokines[10]. The inhibitory activity of CsA on calcineurin depends on its ability to bind to the immunophilin named cyclophilin, a cis-trans peptidyl-prolyl isomerase protein[11].

Everolimus - mTOR inhibitor

Everolimus (RAD001), a derivative of rapamycin, is an inhibitor of mTOR. mTOR is a key downstream protein kinase of PI3K/AKT signaling pathway, and plays a central role in controlling cancer cell growth. Everolimus is currently used as an immunosuppressant to prevent the rejection of organ transplants and for the treatment of certain types of cancer[12]. It has been demonstrated that in cancer models Everolimus promotes autophagy through the inhibition of mTOR[13]

FK506 - Calcineurin inhibitor

FK506 (Tacrolimus) is commonly used as an immunosuppressant to prevent the rejection of organ transplants. Similarly to cyclosporin A (CsA), FK506 blocks the activation of calcineurin through the formation of complexes with immunophilins. FK506 binds to a different immunophilin than CsA, called FK506 binding protein (FKBP) 12[14].

LY294002 - PI3K Inhibitor

LY294002, a compound derived from the flavonoid quercetin, is a potent, cell permeable inhibitor of PI3K that acts on the ATP binding site of the enzyme[15]. LY294002 is often used to study the role of PI3K in apoptosis[10] and autophagy[16].

Metformin - AMPK activator

Metformin activates adenosine monophosphate-activated protein kinase (AMPK), an enzyme that controls cell survival, mediates cell activation and metabolism. Metformin is used as an antidiabetic drug and displays significant growth inhibitory effects in several cancer models[17]. Experimental data indicate that metformin blocks lymphoma cell growth through the inhibition of the mTOR pathway and the induction of autophagy[18].

OSU-03012 - PDK1 Inhibitor

OSU-03012, a derivative of the cyclooxygenase-2 (COX2) inhibitor celecoxib but lacking COX2 inhibitory activity, is a potent inhibitor of PDK1 (phosphoinositide-dependent kinase-1), a protein in the PI3K/Akt pathway that is involved in the growth and proliferation of cells[19]. OSU-03012 has been shown to induce cell death in various types of cancer cells through the inhibition of PDK1, although other mechanisms of action of this agent may be involved[20, 21].

Perifosine - Akt inhibitor

Perifosine is an alkylphospholipid exhibiting antitumor activity. It induces cell cycle arrest and apoptosis through the inhibition of the serine-threonine protein kinase Akt[22]. In addition, perifosine inhibits mTOR signaling through a different mechanism than classical mTOR inhibitors such as rapamycin[23].

PI-103 - PI3K/mTOR Inhibitor

PI-103 is a potent ATP-competitive dual inhibitor of PI3K and mTOR. By inhibiting mTOR activity, PI-103 induces autophagy and promotes cell survival[24, 25]. Conversely, combining PI-103 with inhibitors of autophagy, such as 3-methyladenine or bafilomycin A1, leads to apoptosis[24].

Rapamycin - mTOR Inhibitor

Rapamycin (Sirolimus) is an inhibitor of the serine-threonine protein kinase named "mammalian target of rapamycin" (mTOR) that regulates cell growth and metabolism. Rapamycin binds to the immunophilin FKBP12 and blocks the formation of the mTOR signaling complex by preventing the interaction of mTOR with RAPTOR leading to an increase in autophagy[26].

Temsirolimus - mTOR inhibitor

Temsirolimus is a derivative and prodrug of rapamycin (also known as sirolimus). Temsirolimus specifically inhibits the serine/threonine protein kinase mTOR[27, 28]. To inhibit mTOR signaling, Temsirolimus interacts with the cytosolic FK506-binding protein 12 (FKBP12) to form a complex which binds the mTOR Complex 1. Through its effects on mTOR, Temsirolimus can inhibit cell proliferation and induce apoptosis, in addition to the inhibiton of angiogenesis in cancer models[29, 30].

Wortmannin - PI3K Inhibitor

Wortmannin is a cell-permeable, fungal metabolite that acts as a potent, selective and irreversible inhibitor of PI3K[31]. Wortmannin has been used to determine the involvement of PI3K in many cellular processes, such as apoptosis[10], autophagy[2] and TLR signaling[32].

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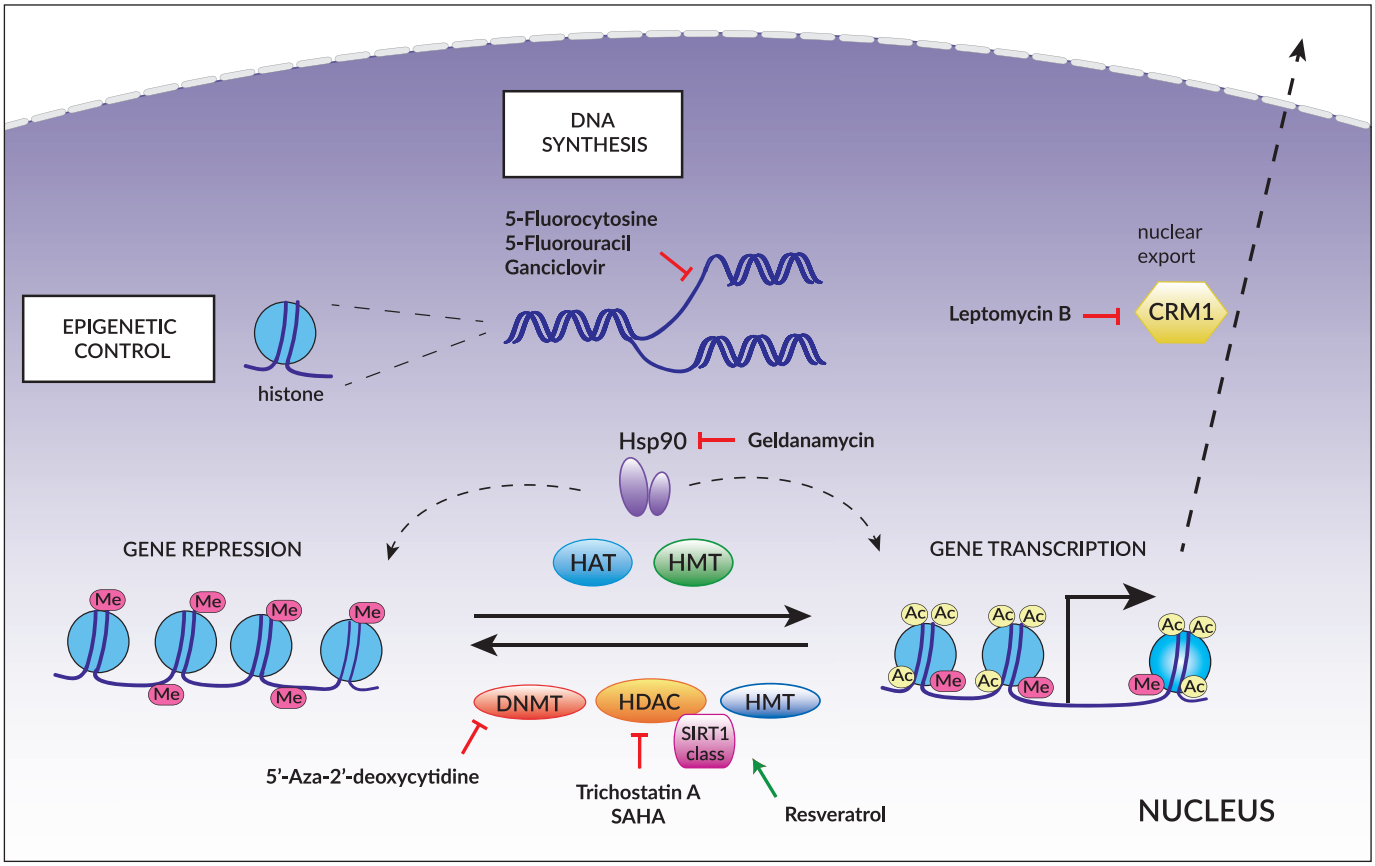
INHIBITORS OF DNA SYNTHESIS & CHROMATIN REMODELING



5-Aza-2'-deoxycytidine - DNA Methyltransferase Inhibitor
5-Aza-2'-deoxycytidine (5-AzadCyD, 5-Aza-CdR, decitabine) is a specific inhibitor of DNA methylation. 5-AzadCyD is incorporated into the DNA of dividing cells where it specifically inhibits DNA methylation by forming covalent complexes with the DNA methyltransferases (DNMTs). 5-AzadCyD is a potent antineoplastic agent against leukemia and tumors in animal models[1, 2].

5-Fluorocytosine - DNA Synthesis Inhibitor
5-Fluorocytosine (5-FC) is a fluorinated cytosine analogue, clinically approved as an antifungal agent. 5-FC is nontoxic to mammalian cells due to their lack of the enzyme cytosine deaminase (CD). CD converts 5-FC into 5-fluorouracil (5-FU), a highly cytotoxic compound routinely used in cancer chemotherapy. 5-FC is used in combination with the *E. coli* CD gene (*codA*) or *S. cerevisiae* CD gene (*fcy*) in suicide gene therapy protocols[3].

5-Fluorouracil - Thymidylate Synthase Inhibitor
5-Fluorouracil (5-FU), a fluorinated analogue of uracil, is approved for cancer chemotherapy as an antineoplastic, antimetabolic agent. 5-FU is converted to the active metabolite 5-fluoro-uridine monophosphate (5-FUMP) which incorporates into RNA, inhibiting RNA processing and cell growth. Another active metabolite, 5-fluoro-deoxyuridine monophosphate (5-FdUMP) inhibits thymidylate synthase, and leads to cell death by blocking DNA synthesis through deprivation of deoxythymidine triphosphate.

Ganciclovir - DNA Synthesis Inhibitor
Ganciclovir (GCV) is a synthetic analogue of 2'-deoxy-guanosine, clinically approved for the treatment of cytomegalovirus (CMV) infections. GCV is used as a prodrug to obtain a suicide effect in cells transfected with the herpes virus thymidine kinase gene (HSV-*tk*)[3]. HSV-TK phosphorylates GCV to GCV-monophosphate which is further converted to GCV-diphosphate and GCV-triphosphate by host kinases. GCV-triphosphate causes premature DNA chain termination and apoptosis. The HSV-tk gene and ganciclovir are used in molecular biology for negative selection[4].

Leptomycin B - Nuclear export inhibitor
Leptomycin B is an inhibitor of nuclear export used to study nucleo-cytoplasmic translocation. Leptomycin B can causes the accumulation of proteins that shuttle the cytosol and nucleus, such as IRAK-1 and NLRC5[5]. The cellular target of leptomycin B has been idenetified as CRM1 (exportin 1), an evolutionarily conserved receptor for the nuclear export signal of proteins.

SAHA - Pan-Histone deacetylase (HDAC) Inhibitor
SAHA (Vorinostat) is a pan-HDAC inhibitor. It binds to the active site of histone deacetylases and act as a chelator for Zinc ions also found in the active site of histone deacetylases. SAHA has been in clinical development as an anti-cancer drug, and has has antifibrotic and anti-inflammatory potential[6, 7]. The antitumor activity of SAHA has been demonstrated both *in vitro* and *in vivo* with little or no toxicity to normal cells. Under its trade name Vorinostat, SAHA has been approved by the FDA for the treatment of advanced cutaneous T-cell-lymphoma[8].

Trichostatin A - Histone deacetylase Inhibitor
Trichostatin A (TSA) is a potent and specific inhibitor of histone deacetylase (HDAC). TSA suppresses the activity of HDAC leading to an increase in histone acetylation. TSA has been shown to induce apoptosis in many cancer cells at submicromolar concentrations with very low toxicity toward normal cells[9]. Furthermore, TSA is used to improve the genomic reprogramming of embryos generated by somatic cell nuclear transfer[10].

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INHIBITORS OF HEAT SHOCK PROTEIN 90

Geldanamycin - HSP90 Inhibitor

Geldanamycin (GA) is a natural product produced by *Streptomyces hygroscopicus*. InvivoGen produces GA from a mutant strain of *S. hygroscopicus*, inactivated for the synthesis of nigericin, a common contaminant of GA. GA binds with high affinity into the ATP binding pocket of Hsp90. Binding of GA to Hsp90 causes the destabilization and degradation of its client proteins[1], thereby inhibiting the oncogenic activity of these proteins[2].

17-AAG - Less Toxic and More Stable GA Analog

17-Allylamino-17-demethoxygeldanamycin (17-AAG) is an analog chemically derived from GA. 17-AAG is a less toxic and more stable analog of geldanamycin (GA)[3]. Even though 17-AAG binding to Hsp90 is weaker than GA, 17-AAG displays similar antitumor effects as GA and a better toxicity profile. 17-AAG is currently in phase I clinical trial in several centers worldwide. Preliminary data obtained from these trials demonstrate that antitumor activity is achieved at concentrations below the maximum tolerated dose[4].

17-DMAG - Water-soluble GA Analog

17-(Dimethylaminoethylamino)-17-demethoxygeldanamycin (17-DMAG, NSC 707545) is the first water-soluble analog of 17-AAG. This Hsp90 inhibitor shows promises in preclinical models[5]. 17-DMAG has excellent bioavailability, is widely distributed to tissues, and is quantitatively metabolized much less than is 17-AAG[6].

The use of 17-DMAG is covered under US Patent 6,890,917 owned and licensed by the NIH to InvivoGen.

FITC-Geldanamycin - FITC-Labeled GA

FITC-geldanamycin is a fluorescent derivative of geldanamycin. The fluorescein-5-isothiocyanate dye was linked to geldanamycin at the C17 position[7]. FITC-geldanamycin binds tightly to Hsp90. This interaction can be competed by Hsp90 inhibitors such as 17-AAG. Therefore, FITC-geldanamycin can be used to screen for new Hsp90 inhibitors by measuring its binding to Hsp90 through a fluorescent polarization assay.

1. Whitesell L. *et al.*, 1994. Inhibition of heat shock protein HSP90-pp60v-src heteroprotein complex formation by benzoquinone ansamycins: essential role for stress proteins in oncogenic transformation. PNAS 91(18):8324-8. **2. Neckers L., 2002.** Hsp90 inhibitors as novel cancer chemotherapeutic agents. Trends Mol Med 8(4 Suppl):S55-61. **3. Schulte TW. & Neckers LM., 1998.** The benzoquinone ansamycin 17-allylamino-17-demethoxygeldanamycin binds to HSP90 and shares important biologic activities with geldanamycin. Cancer Chemother Pharmacol 42(4):273-9. **4. Agnew EB. *et al.* 2001.** Measurement of the novel antitumor agent 17-(allylamino)-17-demethoxygeldanamycin in human plasma by high-performance liquid chromatography. J Chromatogr B Biomed Sci Appl 755:237-43. **5. Workman P., 2003.** Overview: translating Hsp90 biology into Hsp90 drugs. Curr Cancer Drug Targets 3(5):297-300. **6. Egorin MJ, *et al.*, 2002.** Pharmacokinetics, tissue distribution, and metabolism of 17-(dimethylaminoethylamino)-17-demethoxygeldanamycin (NSC 707545) in CD2F1 mice and Fischer 344 rats. Cancer Chemother Pharmacol 49(1):7-19. **7. Llauger-bufi L. *et al.*, 2003.** Synthesis of novel fluorescent probes for the molecular chaperone Hsp90. Bioorg Med Chem Lett. 13(22):3975-8.

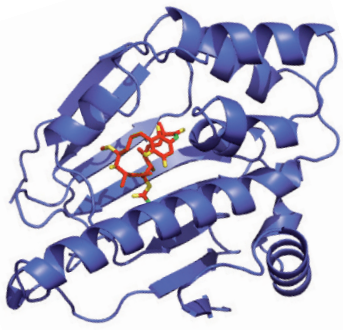
RECENT CITATIONS

Geldanamycin - Couceiro JR. *et al.*, 2015. Sequence-dependent internalization of aggregating peptides. J Biol Chem. 290(1):242-58.

17-AAG - Wang J. *et al.*, 2016. Suppression of KRas-mutant cancer through the combined inhibition of KRAS with PLK1 and ROCK. Nat Commun. 2016 7:11363.

17-DMAG - Wang YL. *et al.*, 2016. 17-DMAG, an HSP90 Inhibitor, Ameliorates Multiple Organ Dysfunction Syndrome via Induction of HSP70 in Endotoxemic Rats. PLoS One. 11(5):e0155583.

FITC-Geldanamycin - Riccardi Sirtori F. *et al.*, 2015. Establish an automated flow injection ESI-MS method for the screening of fragment based libraries: Application to Hsp90. Eur J Pharm Sci. 76:83-94.



Hsp90-geldanamycin complex (Stebbins et al., Cell 1997)

6 ANTIBODIES & RECOMBINANT PROTEINS

Antibodies

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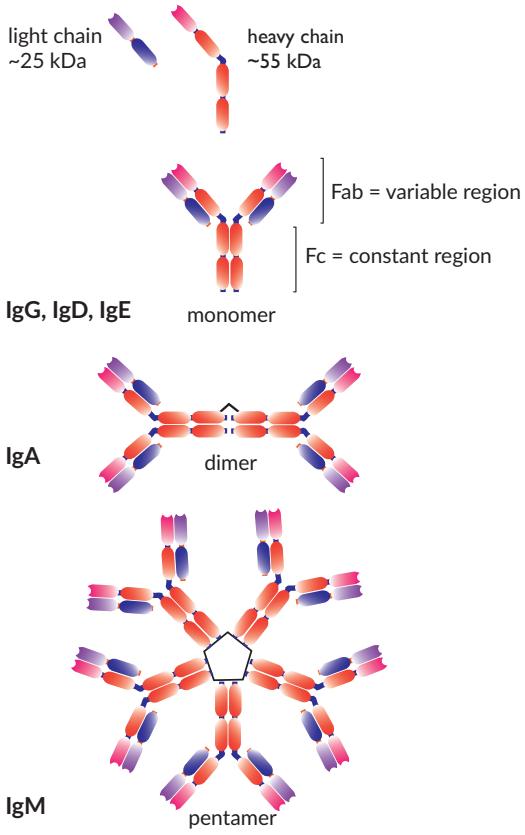
ANTIBODIES

Antibodies are immunoglobulin (Ig) molecules made up of 2 large heavy chains (~55 kDa each) and 2 small light chains (~25 kDa each). Heavy chains are bound to light chains by sulfhydryl linkages to form a Y shaped structure. The stem of the Y contains the constant region (Fc) and the two prongs of the Y contain the variable region (Fab). The Fab interacts with the antigen and therefore is unique to each antibody, while the Fc is common to all antibodies and interacts with the immune system. The Fc portion of the heavy chains defines the class of antibody, of which there are five in mammals: IgG, IgA, IgM, IgD and IgE. The classes differ in their biological properties, otherwise known as effector functions, and their functional localization to ensure an appropriate immune response for a given antigen.



Isotypes of Immunoglobulins

IgG is the most abundant circulating antibody, making up 80% of the total antibodies and 75% of that found in serum. IgG provides the majority of antibody-based immunity against pathogens. IgG can be split into 4 sub-isotypes, each with its own effector function.

IgA is a dimeric antibody present in mucosal secretions in the respiratory, gastrointestinal and urogenital tracts, in saliva, tears, sweat, milk as well as in serum. IgA protects mucosal surfaces by neutralizing bacterial toxins and inhibiting adhesion to epithelial cells. IgA can be split into 2 sub-isotypes.

IgM is the largest antibody, with five Y structures being joined by their Fc regions in a circular configuration. IgM is expressed on the surface of B cells and present in serum, making up about 10 % of antibodies in the blood. **IgD** is monovalent and found on the surface of B-lymphocytes and together with monomeric IgM, serves as antigen receptor for the activation of B cells. **IgE** is a monomeric antibody that accounts for only 0.002 % of the total serum antibodies. IgE is bound to tissue cells, especially mast cells and associated with allergic reactions.

Characteristics of Immunoglobulin Isotypes

Human Isotype	IgG	IgA	IgM	IgD	IgE
Sub-isotype	g1 / g2 / g3 / g4	a1 / a2			
Structure	monomer	monomer/ dimer	pentamer	monomer	monomer
Size (kDa)	150	160-350	950	170-180	190
% carbohydrates	2-3	7-11	10-12	9-14	12-13
Localization	serum	serum & secretions	serum & membrane	serum & membrane	serum
% in serum	75	20	10	0.2	0.002
Effector functions					
Virus neutralization	++/++/++/++	++	+	-	-
Activation of complement (CDC)	++/+/+++/-	+	+++	-	-
Cytotoxicity (ADCC)	++/±/++/±	-	-	-	+
Opsonization/ phagocytosis (ADCP)	+++/±/++/+	+	-	-	-

Isotype switching

During antibody switching, the Fc portion of the antibody heavy chain is changed from one isotype or class to another. Since the Fab region, and therefore antigen specificity, remains the same, the switch in isotype permits a change in the ability of the antibody to interact with different effector molecules of the immune system. Class switch recombination is a biological mechanism that occurs in activated B cells, triggered by cytokines.The isotype generated depends on which cytokines are present in the B cell environment. Class switching may reduce or potentiate effector functions, which include the complement-dependent cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cell phagocytosis (ADCP).

Immunoglobulin binding affinities

Isotype	Protein A	Protein G	Protein L	Peptide M
Human IgA1	+	-	++++	++++
Human IgA2	+	-	++++	++++
Human IgD	-	-	++++	n/a
Human IgE	+	-	++++	n/a
Human IgG1	++++	++++	++++	-
Human IgG2	++++	++++	++++	-
Human IgG3	-	++++	++++	-
Human IgG4	++++	++++	++++	-
Human IgM	+	-	++++	-
Mouse IgA	-	-	++++	-
Mouse IgD	-	-	++++	n/a
Mouse IgE	-	-	++++	n/a
Mouse IgG1	+	++++	++++	-
Mouse IgG2a	++++	++++	++++	-
Mouse IgG2b	+++	+++	++++	-
Mouse IgG3	++	+++	++++	-
Mouse IgM	-	-	++++	-
Rabbit IgG	++++	+++	+	-

Protein A, Protein G, Protein L and Peptide M are recombinant proteins or peptides of microbial origin that bind to mammalian immunoglobulins. Protein A binds the heavy chain of most human immunoglobulins and many other species IgGs, Protein G binds exclusively the heavy chain of IgGs while Peptide M binds only human IgAs. Protein L will bind to all immunoglobulins if they contain the appropriate kappa light chain.

ANTIBODY GENERATION

pFUSE-CHIg and pFUSE2-CLIg plasmids are designed to change a monoclonal antibody from one immunoglobulin subclass to another, such as IgG1 to IgG4 or IgG1 to IgA2, therefore enabling the generation of antibodies with the same antigen affinity but with different effector functions. They can also be used to produce entire IgG antibodies from fragment antigen-binding (Fab) or single-chain variable fragment (scFv) fragments that are either chimeric, humanized, or fully human depending on the nature of the variable region. Lastly, pFUSE-CHIg and pFUSE2-CLIg plasmids can be used to generate recombinant antibodies from different species: human, mouse, rabbit, rat or rhesus monkey.

Description

pFUSE-CHIg and pFUSE2-CLIg plasmids express the constant regions of heavy (CH) and the light (CL) chains, respectively. They contain a multiple cloning site (MCS) upstream of these constant regions to enable the cloning of the variable (VH and VL) regions of a given antibody, Fab or ScFv. Co-transfection of mammalian cell lines with the recombinant pFUSE-CHIg and pFUSE2-CLIg plasmids allows the generation of antibodies that can be purified from the supernatant using the appropriate affinity chromatography (page 125).

pFUSE-CHIg - Heavy chain constant region

pFUSE-CHIg plasmids express the three constant domains CH1, CH2, CH3 (and hinge region) of the heavy chain of the five primary classes of immunoglobulins, IgG, IgM, IgA, IgD and IgE. The CH regions of IgG1 and IgG4 have been engineered to modify their effector functions (page 21).

pFUSEss-CHIg plasmids derive from the corresponding pFUSE-CHIg plasmids by addition of the IL-2 signal sequence (IL2ss) upstream of the MCS. It allows the secretion of the recombinant heavy chains generated using Fab or scFv fragments selected from phage display libraries that lack a signal sequence.

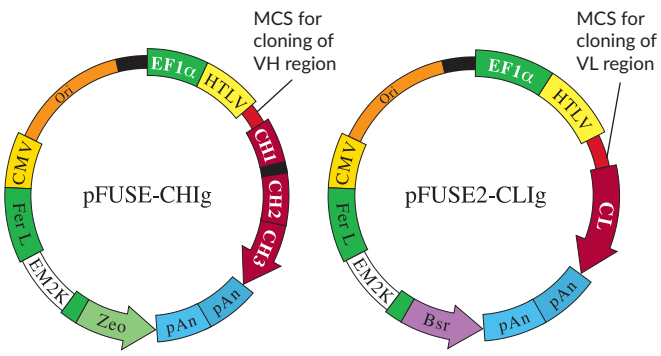
pFUSE-CHIg and pFUSEss-CHIg plasmids are selectable in *E. coli* and mammalian cells with Zeocin™.

pFUSE2-CLIg - Light chain constant region

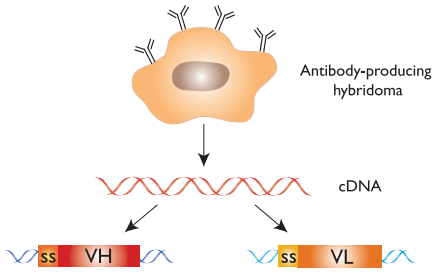
pFUSE2-CLIg plasmids express the constant domain CL of the two main types of light chains, kappa and lambda, from different species.

pFUSE2ss-CLIg: Similarly to pFUSEss-CHIg plasmids, pFUSE2ss-CLIg plasmids contain the IL-2ss upstream of the MCS for proper secretion of light chains missing a signal sequence.

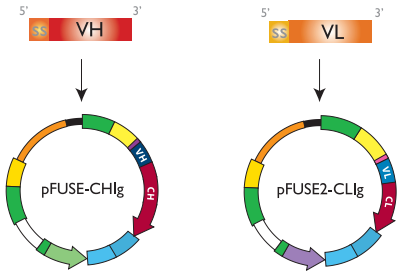
pFUSE2-CLIg and pFUSE2ss-CLIg plasmids are selectable in *E. coli* and mammalian cells with blasticidin.



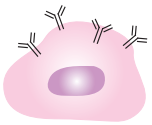
Antibody generation using pFUSE-CHIg/pFUSE2-CLIg



1- Obtaining VH and VL sequences: To obtain the cDNA sequence of the VH and VL regions from an antibody producing hybridoma, total RNA or mRNA is extracted and reverse transcribed to cDNA. PCR is performed with 5' degenerate primers to anneal to the unknown VH and VL regions and the 3' primers designed to anneal to the 'known' CH and CL regions. Alternatively 5' RACE can be used. The resulting amplicons are sequenced.

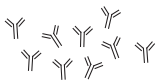


2- Cloning into pFUSE-CHIg and pFUSE2-CLIg: Once the VH and VL sequences are known, inserts that will be cloned into the pFUSE-CHIg and pFUSE2-CLIg plasmids, respectively, can be generated. When generating the insert for VH, a Nhe I site must be introduced at the 3' end to maintain the integrity of the constant region. Similarly, when generating the insert for VL, a Bsi WI (human VL) or Bst API (mouse VL) site must be introduced at the 3' end. There is a choice of restriction sites at the 5' end.



Mammalian cell line co-transfected with recombinant pFUSE-CHIg and pFUSE2-CLIg plasmids

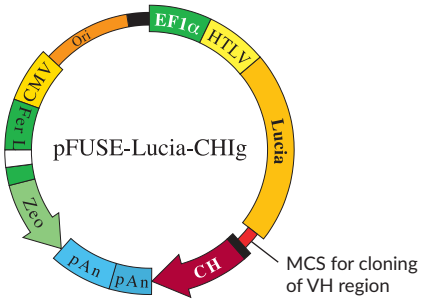
3- Antibody production: Antibody production depends greatly on the ratio of heavy chain (HC) to light chain (LC) expression. Typically, a pFUSE-CHIg (HC):pFUSE2-CLIg (LC) ratio of 2:3 is used to cotransfect mammalian cells, such as HEK293 or CHO cells. Production of antibodies in cell supernatants can be analyzed using different methods including ELISA, flow cytometry or bioactivity assays.



4- Antibody purification: Many antibody purification methods are available, among them isotype-specific affinity chromatography using Protein A, Protein G, Protein L or Peptide M.

pFUSE-Lucia-CHIg

Lucia luciferase is a secreted luciferase reporter protein with high protein carrier ability. pFUSE-Lucia-CHIg plasmids contain the Lucia luciferase gene upstream of the MCS and the CH region to serve as a tag to facilitate the detection and quantification of recombinant antibodies. As heavy chains cannot be properly secreted in the absence of light chains, detection of Lucia luciferase in the cell supernatants reflects the presence of full-length antibodies.
pFUSE-Lucia-CHIg plasmids are selectable in *E. coli* and mammalian cells with Zeocin™.



PRODUCT	ISOTYPE	QUANTITY	CAT. CODE (No IL2ss)	CAT. CODE (With IL2ss)
pFUSE-CHIg - Native Heavy Chain				
pFUSE-CHIg-hA1	Human IgA1	20 µg	pfuse-hcha1	pfusess-hcha1
pFUSE-CHIg-hA2m1	Human IgA2 (allele m1)	20 µg	pfuse-hcha2m1	pfusess-hcha2m1
pFUSE-CHIg-hD	Human IgD (allele 2)	20 µg	pfuse-hchd	pfusess-hchd
pFUSE-CHIg-hE	Human IgE	20 µg	pfuse-hche	pfusess-hche
pFUSE-CHIg-hG1	Human IgG1	20 µg	pfuse-hchg1	pfusess-hchg1
pFUSE-CHIg-hG2	Human IgG2	20 µg	pfuse-hchg2	pfusess-hchg2
pFUSE-CHIg-hG3	Human IgG3 (allelle 1)	20 µg	pfuse-hchg301	pfusess-hchg301
pFUSE-CHIg-hG4	Human IgG4	20 µg	pfuse-hchg4	pfusess-hchg4
pFUSE-CHIg-hM	Human IgM (allele 3)	20 µg	pfuse-hchm	pfusess-hchm
pFUSE-CHIg-mA	Mouse IgA	20 µg	pfuse-mcha	pfusess-mcha
pFUSE-CHIg-mD	Mouse IgD	20 µg	pfuse-mchd	pfusess-mchd
pFUSE-CHIg-mE	Mouse IgE (allele 1)	20 µg	pfuse-mche	pfusess-mche
pFUSE-CHIg-mG1	Mouse IgG1	20 µg	pfuse-mchg1	pfusess-mchg1
pFUSE-CHIg-mG2a	Mouse IgG2a	20 µg	pfuse-mchg2a	pfusess-mchg2a
pFUSE-CHIg-mG2b	Mouse IgG2b	20 µg	pfuse-mchg2b	pfusess-mchg2b
pFUSE-CHIg-mG3	Mouse IgG3	20 µg	pfuse-mchg3	pfusess-mchg3
pFUSE-CHIg-mM	Mouse IgM (allele 1)	20 µg	pfuse-mchm	pfusess-mchm
pFUSE-CHIg-rG	Rabbit IgG	20 µg	pfuse-rchg	pfusess-rchg
pFUSE-CHIg-ratG1	Rat IgG1	20 µg	pfuse-rtchg1	pfusess-rtchg1
pFUSE-CHIg-ratG2a	Rat IgG2a	20 µg	pfuse-rtchg2a	pfusess-rtchg2a
pFUSE-CHIg-ratG2b	Rat IgG2b	20 µg	pfuse-rtchg2b	pfusess-rtchg2b
pFUSE-CHIg-ratG2c	Rat IgG2c	20 µg	pfuse-rtchg2c	pfusess-rtchg2c
pFUSE-CHIg-rhG1	Rhesus monkey IgG1	20 µg	pfuse-rhchg1	pfusess-rhchg1
pFUSE-CHIg-rhG2	Rhesus monkey IgG2	20 µg	pfuse-rhchg2	pfusess-rhchg2
pFUSE-CHIg-rhG3	Rhesus monkey IgG3	20 µg	pfuse-rhchg3	pfusess-rhchg3
pFUSE-CHIg-rhG4	Rhesus monkey IgG4	20 µg	pfuse-rhchg4	pfusess-rhchg4
pFUSE-CHIg - Engineered Heavy Region (see decription page 21)				
pFUSE-CHIg-hG1e1	Human IgG1 - Increased half-life	20 µg	pfuse-hchg1e1	pfusess-hchg1e1
pFUSE-CHIg-hG1e2	Human IgG1 - Increased half-life	20 µg	pfuse-hchg1e2	pfusess-hchg1e2
pFUSE-CHIg-hG1e3	Human IgG1 - Reduced ADCC and CDC	20 µg	pfuse-hchg1e3	pfusess-hchg1e3
pFUSE-CHIg-hG1e4	Human IgG1 - Increased ADCC and CDC	20 µg	pfuse-hchg1e4	pfusess-hchg1e4
pFUSE-CHIg-hG1e5	Human IgG1 - Increased ADCC	20 µg	pfuse-hchg1e5	pfusess-hchg1e5
pFUSE-CHIg-hG1e6	Human IgG1 - Unchanged Half-life	20 µg	pfuse-hchg1e6	pfusess-hchg1e6
pFUSE-CHIg-hG1e7	Human IgG1 - Increased CDC	20 µg	pfuse-hchg1e7	pfusess-hchg1e7
pFUSE-CHIg-hG1e9	Human IgG1 - Increased Phagocytosis	20 µg	pfuse-hchg1e9	pfusess-hchg1e9
pFUSE-CHIg-hG4e1	Human IgG4 - Reduced Fab-arm exchange	20 µg	pfuse-hchg4e1	pfusess-hchg4e1

PRODUCT	ISOTYPE	QUANTITY	CAT. CODE (No IL2ss)	CAT. CODE (With IL2ss)
pFUSE2-CLIg - Light Chain				
pFUSE2-CLIg-hk	Human kappa	20 µg	pfuse2-hclk	pfuse2ss-hclk
pFUSE2-CLIg-hl2	Human lambda 2	20 µg	pfuse2-hcll2	pfuse2ss-hcll2
pFUSE2-CLIg-mk	Mouse kappa	20 µg	pfuse2-mclk	pfuse2ss-mclk
pFUSE2-CLIg-ml1	Mouse lambda 1	20 µg	pfuse2-mcll1	pfuse2ss-mcll1
pFUSE2-CLIg-ml2	Mouse lambda 2	20 µg	pfuse2-mcll2	pfuse2ss-mcll2
pFUSE2-CLIg-ratk	Rat kappa	20 µg	pfuse2-rtclk	pfuse2ss-rtclk
pFUSE2-CLIg-ratl1	Rat lambda 1	20 µg	pfuse2-rtcll1	pfuse2ss-rtcll1
pFUSE2-CLIg-rhk	Rhesus monkey kappa	20 µg	pfuse2-rhclk	pfuse2ss-rhclk
pFUSE2-CLIg-rk1	Rabbit kappa 1	20 µg	pfuse2-rclk1	pfuse2ss-rclk1
pFUSE2-CLIg-rk2	Rabbit kappa 2	20 µg	pfuse2-rclk2	pfuse2ss-rclk2
pFUSE-Lucia-CHIg				
pFUSE-Lucia-CHIg-hG1	Human IgG1	20 µg	pfuselc-hchg1	-
pFUSE-Lucia-CHIg-hG2	Human IgG2	20 µg	pfuselc-hchg2	-
pFUSE-Lucia-CHIg-hG3	Human IgG3	20 µg	pfuselc-hchg3	-
pFUSE-Lucia-CHIg-hG4	Human IgG4	20 µg	pfuselc-hchg4	-
pFUSE-Lucia-CHIg-mG1	Mouse IgG1	20 µg	pfuselc-mchg1	-
pFUSE-Lucia-CHIg-mG2a	Mouse IgG2a	20 µg	pfuselc-mchg2a	-
pFUSE-Lucia-CHIg-mG2b	Mouse IgG2b	20 µg	pfuselc-mchg2b	-
pFUSE-Lucia-CHIg-mG3	Mouse IgG3	20 µg	pfuselc-mchg3	-

Contents

pFUSE-CHIg and pFUSE2-CLIg plasmids are provided as 20 µg of lyophilized DNA. Products are shipped at room temperature and should be stored at -20°C.

RECENT CITATIONS

pFUSE-CHIg-hG1 - Ternant D. *et al.*, 2016. IgG1 Allotypes Influence the Pharmacokinetics of Therapeutic Monoclonal Antibodies through FcRn Binding. Oncotarget. 6(33):34617-28.

Romani C. *et al.*, 2015. Evaluation of a novel human IgG1 anti-claudin3 antibody that specifically recognizes its aberrantly localized antigen in ovarian cancer cells and that is suitable for selective drug delivery. J Immunol. 196(2):607-13.

pFUSE-CHIg-hG1 & pFUSE2-CLIg-hk - Faitschuk E. *et al.*, 2016. A dual chain chimeric antigen receptor (CAR) in the native antibody format for targeting immune cells towards cancer cells without the need of an scFv. Gene Ther. [Ahead of print].

pFUSE-CHIg-hG1, pFUSE-CHIg-hG4 & pFUSE2-CLIg-hk Yamashita M. *et al.*, 2015. Discovery of anti-claudin-1 antibodies as candidate therapeutics against hepatitis C virus. J Pharmacol Exp Ther. 353(1):112-8.

pFUSE-CHIg-hG1e3 - Miyazaki-Komine K. *et al.*, 2016. High avidity chimeric monoclonal antibodies against the extracellular domains of human aquaporin-4 competing with the neuromyelitis optica autoantibody, NMO-IgG. Br J Pharmacol. 173(1):103-14.

pFUSE-CHIg-hE - Sun Y *et al.*, 2015. A Human Platelet Receptor Protein Microarray Identifies the High Affinity Immunoglobulin E Receptor Subunit α (FcεR1α) as an Activating Platelet Endothelium Aggregation Receptor 1 (PEAR1) Ligand. Mol Cell Proteomics. 14(5):1265-74.

ANTIBODY PURIFICATION

Protein G and Protein A are currently used to purify IgG antibodies, but these supports are not appropriate for IgA antibody purification. InvivoGen provides four different methods, Peptide M, SSL7, Protein L and Protein G, that allow fast and efficient purification of IgG and IgA antibodies from biological samples. The correct choice of purification method depends upon the class of the antibody, the species in which it was raised and the intended use of the antibody. Antibodies from tissue culture supernatant or serum can be purified using one or more of the following antibody binding proteins offered by InvivoGen. These proteins are provided coupled to agarose beads.

Protein L / Agarose - κ light chain specific

Protein L is an immunoglobulin-binding protein expressed by the anaerobic bacterial species *Peptostreptococcus magnus*[1]. Protein L binds specifically to the variable domain of Ig κ light chain, as a consequence Protein L has the capacity to purifiy κ light containing IgA antibodies[2]. It binds strongly to human κ light chain subclasses I, III and IV, and also to most κ light chains of other species such as rat and mouse. As it recognizes κ light chains, protein L can bind to all classes of Ig, in contrast to Protein A and Protein G which interact with the Fc region and bind exclusively to IgG heavy chains. Protein L does not bind bovine immunoglobulins which are present in the fetal bovine serum (FBS) and thus provides a convenient way to purify κ light chain-containing monoclonal antibodies from culture supernatant.

Binding capacity >5 mg of human IgA/IgG per ml of gel

Protein G / Agarose - IgG purification

Protein G is an immunoglobulin-binding protein expressed in group C and G Streptococcal bacteria. It is a 65-kDa (G148 protein G) and a 58 kDa (C40 protein G) cell surface protein that has found application in purifying antibodies through its binding to the Fc region[3]. Protein G binds to most mammalian IgGs through the Fc region, but some binding also occurs through the Fab region. Native protein G also binds albumin, however, because serum albumin is a major contaminant of antibody sources, the albumin binding site has been removed from recombinant forms of Protein G[4] Protein G / Agarose from InvivoGen uses the recombinant form of protein G lacking the albumin-binding region.

Binding capacity 20 mg of human IgG per ml of gel

Peptide M / Agarose - IgA1 and IgA2 purification

Peptide M is a 50 aa synthetic peptide derived from a streptococcal M protein containing an additional C-terminal cysteine residue. Peptide M binds monomeric and dimeric human IgA of both subclasses (IgA1 and IgA2) with high specificity and affinity. It also binds bovine IgA but not murine IgA. Peptide M binding occurs at a site in IgA-Fc conserved in human IgA1 and IgA2 and bovine IgA but not in mouse IgA. Peptide M can be used for single-step affinity purification of IgA and for specific detection of antigen-bound IgA[5].

Binding capacity: 6 mg human IgA per ml of gel

Contents

Each product is provided as 50% v/v gel slurry in PBS 20% v/v ethanol. It is shipped at room temperature. Store at 4°C. Do not freeze.

Isotype	Protein G	Protein L	Peptide M
Human κ light chain	-	++++	-
Human λ light chain	-	-	-
Human IgA1	-	++++	++++
Human IgA2	-	++++	++++
Human IgG	++++	++++	-
Human IgM	-	++++	-
Human IgE		++++	n/a
Human IgD	-	++++	n/a
Mouse IgA	-	++++	-
Rat IgA	-	++++	n/a
Bovine IgA	-	-	+

1. Björck L., 1988. Protein L. A novel bacterial cell wall protein with affinity for Ig L chains. J Immunol. 1988 Feb 15;140(4): 1194-7. **2. Nilson BH. *et al.*, 1993.** Purification of antibodies using protein L-binding framework structures in the light chain variable domain. J Immunol Methods. 164(1):33-40. **3. Sjobring U. *et al.*, 1991.** Streptococcal Protein G. Gene structure and protein binding properties. J Biol Chem. 140:1194-1197. **4. Goward CR. et al., 1990.** Expression and purification of truncated recombinant streptococccal Protein G. Biochem J. 267:171-177. **5. Sandin C. *et al.*, 2002.** Isolation and detection of human IgA using a streptococcal IgA-binding peptide. J Immunol. 169(3):1357-64.

PRODUCT	QUANTITY	CAT. CODE
Peptide M / Agarose	2 ml 5 ml	gel-pdm-2 gel-pdm-5
Protein G / Agarose	2 ml 5 ml	gel-agg-2 gel-agg-5
Protein L / Agarose	2 ml 10 ml	gel-protl-2 gel-protl-10

CLINICALLY RELEVANT ANTIBODIES

Monoclonal antibodies (mAbs) have become a major tool in cancer therapy. They function through various mechanisms with the ultimate effect of priming either the innate or adaptive arm of the immune system to target tumor cells for destruction. The efficacy of antibodies is governed by their bifunctional nature: the variable region confers antigen specificity and the constant region triggers antibody-mediated effector functions by engaging a variety of Fc receptors. These effector functions include complement-dependent cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP). One approach employed to enhance the efficacy of therapeutic antibodies is to modify the immunoglobulin (Ig) constant region. InvivoGen provides a series of clinically relevant antibodies, such as anti-hCD20 (rituximab), anti-HER2 (trastuzumab) and anti-hPD1 (nivolumab), available in their original format or with different Ig isotypes, either native or engineered, thus constituting an antibody isotype family (3 different isotypes) or collection (up to 14 different isotypes). These antibody isotype families or collections will help you study the effector functions between different isotypes and determine which isotype is the most suitable for your application.

Effector functions	Native human isotypes								Engineered human isotypes			Native mouse isotypes		
	IgG1	IgG2	IgG3	IgG4	IgM	IgA1	IgA2	IgE	IgG1NQ	IgG1fut	IgG4S228P	IgG1	IgG2a	IgA
ADCC	++	+/-	++	+/-	+	+	+	+	-	++++	+/-	+/-	++	+
ADCP	+++	+/-	++	+	-	+	+	-	-	+++	+	+	+++	+
CDC	++	+	+++	-	+++	-	-	-	+/-	+	-	-	++	-

InvivoGen's clinically relevant antibodies are generated by recombinant DNA technology, produced in CHO cells and purified by affinity chromatography. They are validated by flow cytometry, are sterile and contain no sodium azide. All of these antibodies feature the human (or murine) kappa light chain. Larger quantities are available upon request.

Antibody Isotype Families

Description

Antibody isotype families consist of clinically relevant monoclonal antibodies comprising the same variable region and the constant region of three different human isotypes. The Ig isotypes chosen are **human IgG1, IgG4 (S228P) and IgA.** These isotypes exhibit distinct properties and therefore, differ in their suitability for a given application.

Anti-hCTLA4 isotype family

The anti-hCTLA4 isotype family features the variable region of ipilimumab. Ipilimumab is a fully human IgG1 monoclonal antibody that targets CTLA-4 (also known as CD152), a negative regulator of T cell activation. By binding CTLA-4, ipilimumab inhibits negative signals that physiologically downregulate T cell activation and exerts its therapeutic activity by upregulating the antitumor activity of T lymphocytes.

Anti-hEGFR isotype family

The anti-hEGFR isotype family features the variable region of cetuximab. Cetuximab is a chimeric human/mouse IgG1 monoclonal antibody that targets EGFR, a cell surface receptor overexpressed in many types of cancer. Binding of cetuximab to EGFR blocks ligand/receptor binding, and induces receptor internalization and subsequent degradation.

Anti-HER2 isotype family

The anti-HER2 isotype family features the variable region of trastuzumab.Trastuzumab is a humanized IgG1 monoclonal antibody that targets the HER2 receptor (HER2/neu, ERBB2) found on the cell membrane of epithelial cells. HER2 is over-expressed in certain types of cancers, particularly in breast and ovarian cancers. Binding of trastuzumab to HER2 results in cell death through different mechanisms including ADCC and ADCP.

PRODUCT	ISOTYPE	QTY	CAT. CODE
Anti-hCTLA4 isotype family			
Anti-hCTLA4-hIgG1	Human IgG1	100 µg	hctla4-mab1
Anti-hCTLA4-hIgG4 (S228P)	Human IgG4 (S228P)	100 µg	hctla4-mab14
Anti-hCTLA4-hIgGA2	Human IgA2	100 µg	hctla4-mab7
Anti-hEGFR isotype family			
Anti-hEGFR-hIgG1	Human IgG1	100 µg	hegfr-mab1
Anti-hEGFR-hIgG4 (S228P)	Human IgG4 (S228P)	100 µg	hegfr-mab14
Anti-hEGFR-hIgGA2	Human IgA2	100 µg	hegfr-mab7
Anti-HER2 isotype family			
Anti-HER2-Tra-hIgG1	Human IgG1	100 µg	her2tra-mab1
Anti-HER2-Tra-hIgG4 (S228P)	Human IgG4 (S228P)	100 µg	her2tra-mab14
Anti-HER2-Tra-hIgA2	Human IgA2	100 µg	her2tra-mab7
Anti-hPD1 isotype family			
Anti-hPD1-Ni-hIgG1	Human IgG1	100 µg	hpd1ni-mab1
Anti-hPD1-Ni-hIgG4 (S228P)	Human IgG4 (S228P)	100 µg	hpd1ni-mab14
Anti-hPD1-Ni-hIgA2	Human IgA2	100 µg	hpd1ni-mab7
Anti-hPD-L1 isotype family			
Anti-hPD-L1-hIgG1	Human IgG1	100 µg	hpdl1-mab1
Anti-hPD-L1-hIgG1 (N298A)	Human IgG1 (N298A)	100 µg	hpdl1-mab12
Anti-hPD-L1-mIgG1	Mouse IgG1	100 µg	hpdl1-mab9
Anti-hPD-L1-mIgG1 InvivoFit	Mouse IgG1	5 mg	hpdl1-mab9-5

Anti-hPD1 isotype family

The anti-hPD1 isotype family features the variable region of nivolumab. Nivolumab is a fully human IgG4 (S228P) monoclonal antibody that targets the programmed cell death 1 (PD-1) receptor found on activated T cells, B cells, and myeloid cells. Nivolumab binds and blocks the activation of the PD-1 receptor resulting in the activation of T cells and cell-mediated immune responses. This antibody contains an engineered hinge region mutation (S228P) designed to prevent exchange of IgG4 molecules.

Anti-hPD-L1 isotype family

The anti-hPD-L1 isotype family features the variable region of atezolizumab, a therapeutic mAb that targets human programmed cell death ligand 1 (PD-L1) and thus blocks the interaction with its receptor PD-1. Atezolizumab is a fully humanized IgG1 mAb that contains an Asp to Ala change introduced at position 298 (N298A) eliminating its ability to bind to human Fcγ receptors. Atezolizumab targets both human and mouse PD-L1. For studies in mice, InvivoGen has developed anti-hPD-L1-mIgG1, which contains the Fc domain of mouse IgG1 to prevent anti-drug-antibodies (ADAs). This mAb is available in a preclinical grade (InvivoFit™): it is guaranteed sterile and endotoxin-free (<1 EU/mg) and is provided in 5-mg units.

Anti-hVEGF isotype family

The anti-hVEGF isotype family features the variable region of bevacizumab. Bevacizumab is a humanized IgG1 monoclonal antibody that targets VEGF, a signaling protein that promotes the growth of new blood vessels. Bevacizumab neutralizes VEGF and blocks its interaction with the VEGF receptor thus inhibiting downstream pathways that regulate cell growth and angiogenesis.

PRODUCT	ISOTYPE	QTY	CAT. CODE
Anti-hVEGF isotype family			
Anti-hVEGF-hIgG1	Human IgG1	100 µg	hegfr-mab1
Anti-hVEGF-hIgG4 (S228P)	Human IgG4 (S228P)	100 µg	hegfr-mab14
Anti-hVEGF-hIgGA2	Human IgA2	100 µg	hegfr-mab7

Antibody Isotype Collections

Description

Antibody isotype families consist of clinically relevant monoclonal antibodies comprising the same variable region and the constant region of up to fourteen different human or mouse isotypes:
- **human isotypes:** IgG1, IgG2, IgG3, IgG4, IgM, IgA1, IgA2, IgE
- **mouse isotypes:** IgG1, IgG2a, IgA

In addition to these native isotypes, three engineered human isotypes are available: **IgG1NQ** (aglycosylated mAb), **IgG1fut** (afucosylated mAb) and **IgG4 (S228P)** (reduced Fab arm exchange).

Anti-hCD20 isotype collection

The anti-hCD20 isotype collection features the variable region of rituximab. Rituximab is a chimeric human/mouse IgG1 monoclonal antibody that targets the CD20 antigen found on the surface of malignant and normal B lymphocytes. Binding of rituximab to CD20 results in cell destruction through different mechanisms including direct signaling of apoptosis, complement activation and cell-mediated cytotoxicity.

Anti-hTNF-α isotype collection

The anti-hTNF-α isotype collection features the variable region of adalimumab. Adalimumab is a fully human monoclonal antibody against the pro-inflammatory cytokine TNF-α. Adalimumab binds to TNF-α and blocks its interaction with TNF receptors thereby downregulating the inflammatory reactions associated with autoimmune diseases.

Contents

Clinically relevant antibodies are provided azide-free and lyophilized. Products are shipped at room temperature and should be stored at -20°C.

PRODUCT	ISOTYPE	QTY	CAT. CODE
Anti-hCD20 isotype collection			
Anti-hCD20-hIgG1	Human IgG1	100 µg	hcd20-mab1
Anti-hCD20-hIgG1NQ	Human IgG1	100 µg	hcd20-mab12
Anti-hCD20-hIgG1fut	Human IgG1	100 µg	hcd20-mab13
Anti-hCD20-hIgG2	Human IgG2	100 µg	hcd20-mab2
Anti-hCD20-hIgG3	Human IgG3	100 µg	hcd20-mab3
Anti-hCD20-hIgG4	Human IgG4	100 µg	hcd20-mab4
Anti-hCD20-hIgG4 (S228P)	Human IgG4 (S228P)	100 µg	hcd20-mab14
Anti-hCD20-hIgM	Human IgM	100 µg	hcd20-mab5
Anti-hCD20-hIgA1	Human IgA1	100 µg	hcd20-mab6
Anti-hCD20-hIgA2	Human IgA2	100 µg	hcd20-mab7
Anti-hCD20-hIgE	Human IgE	100 µg	hcd20-mab8
Anti-hCD20-mIgG1	Mouse IgG1	100 µg	hcd20-mab9
Anti-hCD20-mIgG2a	Mouse IgG2a	100 µg	hcd20-mab10
Anti-hCD20-mIgA	Mouse IgA	100 µg	hcd20-mab11
Anti-hTNF-α isotype collection			
Anti-hTNF-α-hIgG1	Human IgG1	100 µg	htnfa-mab1
Anti-hTNF-α-hIgG2	Human IgG2	100 µg	htnfa-mab2
Anti-hTNF-α-hIgG3	Human IgG3	100 µg	htnfa-mab3
Anti-hTNF-α-hIgG4	Human IgG4	100 µg	htnfa-mab4
Anti-hTNF-α-hIgM	Human IgM	100 µg	htnfa-mab5
Anti-hTNF-α-hIgA1	Human IgA1	100 µg	htnfa-mab6
Anti-hTNF-α-hIgA2	Human IgA2	100 µg	htnfa-mab7
Anti-hTNF-α-hIgE	Human IgE	100 µg	htnfa-mab8
Anti-hTNF-α-mIgG1	Mouse IgG1	100 µg	htnfa-mab9
Anti-hTNF-α-mIgG2a	Mouse IgG2a	100 µg	htnfa-mab10
Anti-hTNF-α-mIgA	Mouse IgA	100 µg	htnfa-mab11

RELATED PRODUCTS

PRODUCT	INFO
Anti-β-Gal-hIgA2	Visit website
Anti-β-Gal-hIgG1	Visit website
Anti-β-Gal-hIgG2	Visit website
Anti-β-Gal-hIgG3	Visit website
Anti-β-Gal-hIgG4	Visit website
Anti-β-Gal-hIgG4 (S228P)	Visit website

ANTIBODY COLLECTION

Primary Antibodies

InvivoGen offers a selection of monoclonal and polyclonal antibodies that target proteins involved in the innate immune response, such as pattern recognition receptors and cytokines. These antibodies have been generated by immunization of mice or rats with DNA or recombinant proteins or peptides. These antibodies can be used for different applications. They have been tested in our laboratories for neutralization and/or flow cytometry.

TARGET	ANTIBODY	SPECIFICITY	DESCRIPTION	APPLICATIONS*	QTY	CAT. CODE
CD14	Anti-hCD14-IgA	Human CD14	Monoclonal human IgA2	Neutralization, FC	100 µg	maba-hcd14
CD20	Anti-hCD20-hIgG1	Human CD20	Monoclonal human IgG1	FC	100 µg	hcd20-mab1
CD40L	Anti-hCD40L-hIgG	Human CD40L	Monoclonal mouse IgG2b	Neutralization	3x100 µg	mabg-h40l-3
DC-SIGN	MAb-hDC-SIGN	Human DC-SIGN	Monoclonal mouse IgG1	Neutralization, FC	100 µg	mab-hdcsg
Dectin-1	Anti-hDectin-1-IgG	Human Dectin-1	Monoclonal mouse IgG1	Neutralization, FC	100 µg	mabg-hdect
	Anti-hDectin-1-Biotin	Human Dectin-1	Monoclonal mouse IgG1	Neutralization, FC	100 µg	bmab-hdect
	Anti-mDectin-1-IgG	Mouse Dectin-1	Monoclonal rat IgG2a	Neutralization, FC	100 µg	mabg-mdect
	Anti-mDectin-1-Biotin	Mouse Dectin-1	Monoclonal rat IgG2a	Neutralization, FC	100 µg	bmab-mdect
	Anti-mDectin-1a-IgG	Mouse Dectin-1a	Monoclonal rat IgG2b	Neutralization, FC	100 µg	mabg-mdecta
	Anti-mDectin-1a-Biotin	Mouse Dectin-1a	Monoclonal rat IgG2b	Neutralization, FC	100 µg	bmab-mdecta
Dectin-2	Anti-hDectin-2-IgG	Human Dectin-2	Monoclonal mouse IgG2a	Neutralization, FC	100 µg	mabg-hdect2
	Anti-hDectin-2-Biotin	Human Dectin-2	Monoclonal mouse IgG2a	Neutralization, FC	100 µg	bmab-hdect2
Dectin-3	Anti-hDectin-3-IgG	Human Dectin-3	Monoclonal mouse IgG2a	FC	100 µg	mabg-hdect3
	Anti-hDectin-3-Biotin	Human Dectin-3	Monoclonal mouse IgG2a	FC	100 µg	bmab-hdect3
EGFR	Anti-hEGFR-hIgG1	Human EGFR	Monoclonal human IgG1	FC	100 µg	hegfr-mab1
Flagellin FliC	Anti-Flagellin FliC	*S. typhimurium* flagellin	Monoclonal mouse IgG1	WB	100 µg	mabg-flic
HER2	Anti-HER2-Tra-hIgG1	HER2 (ERBB2)	Monoclonal human IgG1	FC	100 µg	her2tra-mab1
IFN-α	Anti-hIFN-α-IgA	Human Interferon α	Monoclonal human IgA2	Neutralization, FC	3x100 µg	maba-hifna-3
IFN-α7	Anti-hIFN-α7-IgG	Human Interferon α 7	Monoclonal mouse IgG1	Neutralization	100 µg	mabg-hifna7
IFN-α8	Anti-hIFN-α8-IgG	Human Interferon α 8	Monoclonal mouse IgG1	Neutralization	100 µg	mabg-hifna8
IFN-β	Anti-hIFN-β-IgG	Human Interferon β	Monoclonal mouse IgG2a	Neutralization	3x100 µg	mabg-hifnb-3
IFN-γ	Anti-hIFN-γ-IgA	Human Interferon γ	Monoclonal human IgA2	Neutralization	3x100 µg	maba-hifng-3
IL-1α	Anti-hIL-1α-IgG	Human Interleukin 1α	Monoclonal mouse IgG1	Neutralization	3x100 µg	mabg-hil1a-3
	Anti-mIL-1α-IgG	Mouse Interleukin 1α	Monoclonal mouse IgG1	Neutralization	100 µg	mabg-mil1a
IL-1β	Anti-hIL-1β-IgG	Human Interleukin 1β	Monoclonal mouse IgG1	Neutralization	3x100 µg	mabg-hil1b-3
	Anti-mIL-1β-IgG	Mouse Interleukin 1β	Monoclonal mouse IgG1	Neutralization	100 µg	mabg-mil1b
IL-4	Anti-hIL-4-IgA	Human Interleukin 4	Monoclonal human IgA2	Neutralization	3x100 µg	maba-hil4-3
IL-6	Anti-hIL-6-IgG	Human Interleukin 6	Monoclonal mouse IgG1	Neutralization	3x100 µg	mabg-hil6-3
	Anti-mIL-6-IgG	Mouse Interleukin 6	Monoclonal rat IgG2a	Neutralization	3x100 µg	mabg-mil6-3
IL-13	Anti-hIL-13-IgG	Human Interleukin 13	Monoclonal mouse IgG2b	Neutralization	3x100 µg	mabg-hil13-3
	Anti-mIL-13-IgG	Mouse Interleukin 13	Monoclonal mouse IgG1	Neutralization	100 µg	mabg-mil13
IL-17a	Anti-hIL-17a-IgG	Human Interleukin 17a	Monoclonal mouse IgG1	Neutralization	3x100 µg	mabg-hil17a-3

TARGET	ANTIBODY	SPECIFICITY	DESCRIPTION	APPLICATIONS*	QTY	CAT. CODE
IL-18	Anti-hIL-18-IgA	Human Interleukin 18	Monoclonal human IgA2	Neutralization	3x100 µg	maba-hil18-3
IL-28	Anti-hIL-28a-IgG	Human Interleukin 28a	Monoclonal mouse IgG1	Neutralization	3x100 µg	mabg-hil28a-3
	Anti-hIL-28b-IgG	Human Interleukin 28b	Monoclonal mouse IgG1	Neutralization	3x100 µg	mabg-hil28b-3
	Anti-mIL-28b-IgG	Mouse Interleukin 28b	Monoclonal mouse IgG1	Neutralization	100 µg	mabg-mil28b
IL-29	Anti-hIL-29-IgG	Human Interleukin 28 & 29	Monoclonal mouse IgG2a	Neutralization	3x100 µg	mabg-hil29-3
IL-33	Anti-hIL-33-IgG	Human Interleukin 33	Monoclonal mouse IgG1	Neutralization	3x100 µg	mabg-hil33-3
MD-2	MAb-hMD2	Human MD-2	Monoclonal mouse IgG2b	Neutralization, FC	100 µg	mab-hmd2
MR	MAb-hMR	Human Mannose Receptor	Monoclonal mouse IgG1	Neutralization, FC	100 µg	mab-hmr
Mincle	Anti-hMincle-IgG	Human Mincle	Monoclonal mouse IgG2b	Neutralization	100 µg	mabg-hmcl
	Anti-hMincle-Biotin	Human Mincle	Monoclonal mouse IgG2b	Neutralization	100 µg	bmab-hmcl
	Anti-mMincle-IgG	Mouse Mincle	Monoclonal rat IgG2b	Neutralization, FC	100 µg	mabg-mmcl
	Anti-mMincle-Biotin	Mouse Mincle	Monoclonal rat IgG2b	Neutralization, FC	100 µg	bmab-mmcl
PD-1	Anti-hPD1-Ni-hIgG4S228P	Human PD-1	Monoclonal human IgG4 (S228P)	FC	100 µg	hpd1ni-mab14
TGF-β	Anti-hTGFβ-IgA	Human TGF-β1	Monoclonal human IgA2	Neutralization	3x100 µg	maba-htgfb-3
TLR1	Anti-hTLR1-IgG	Human TLR1	Monoclonal mouse IgG1	Neutralization	100 µg	mabg-htlr1
	PAb-hTLR1	Human TLR1	Polyclonal rat IgG	Neutralization	200 µg	pab-hstlr1
TLR2	Anti-hTLR2-IgA	Human TLR2	Monoclonal human IgA2	Neutralization, FC	100 µg	maba2-htlr2
	MAb-hTLR2	Human TLR2	Monoclonal mouse IgG2a (clone TL2.1)	FC, IHC, IP, WB	100 µg	mab-htlr2
	PAb-hTLR2	Human TLR2	Polyclonal rat IgG	Neutralization	200 µg	pab-hstlr2
	Anti-mTLR2-IgG	Mouse TLR2	Monoclonal mouse IgG2a (clone C9A12)	Neutralization	100 µg	mabg-mtlr2
	MAb-mTLR2	Human/mouse TLR2	Monoclonal mouse IgG1 (clone T2.5)	Neutralization·FC, IHC	100 µg	mab-mtlr2
TLR3	Anti-hTLR3-IgA	Human TLR3	Monoclonal human IgA2	FC	100 µg	maba-htlr3
	MAb-hTLR3	Human TLR3	Monoclonal mouse IgG1 (clone TLR3.7)	FC, WB	100 µg	mab-htlr3
TLR4	Anti-hTLR4-IgG	Human TLR4	Monoclonal mouse IgG1	Neutralization	100 µg	mabg-htlr4
	MAb-hTLR4	Human/monkey TLR4	Monoclonal mouse IgG2a (clone HTA125)	FC, IP, IHC	100 µg	mab-htlr4
	MAb2-hTLR4	Human TLR4	Monoclonal mouse IgG1	Neutralization, FC	100 µg	mab2-htlr4
	PAb-hTLR4	Human TLR4	Polyclonal rat IgG	Neutralization	200 µg	pab-hstlr4
TLR4/MD2	MAb-hTLR4/MD2	Human TLR4/MD2	Monoclonal mouse IgG1	Neutralization, FC	100 µg	mab-htlr4md2
	MAb-mTLR4/MD2	Mouse TLR4/MD2	Monoclonal rat IgG2a (clone MTS510)	Neutralization, FC, IP, IHC	100 µg	mab-mtlr4md2
TLR5	Anti-hTLR5-IgA	Human TLR5	Monoclonal human IgA2	Neutralization, FC	100 µg	maba2-htlr5
	Anti-mTLR5-IgG	Mouse TLR5	Monoclonal rat IgG2a (clone Q23D11)	Neutralization	100 µg	mabg-mtlr5
	PAb-hTLR5	Human TLR5	Polyclonal rat IgG	Neutralization	200 µg	pab-hstlr5
TLR6	Anti-hTLR6-IgG	Human TLR6	Monoclonal mouse IgG1 (clone C5C8)	Neutralization	100 µg	mabg-htlr6
	PAb-hTLR6	Human TLR6	Polyclonal rat IgG	Neutralization	200 µg	pab-hstlr6
TLR9	MAb-mTLR9	Human/mouse TLR9	Monoclonal mouse IgG2a (clone 5G5)	FC, IHC, IP, WB	100 µg	mab-mtlr9
TNF-α	Anti-hTNF-α-hIgG1	Human TNF-alpha	Monoclonal human IgG1	Neutralization	100 µg	htnfa-mab1

Isotype Controls

InvivoGen provides several isotype control antibodies that target E. coli β-galactosidase (β-Gal) and offers a control serum for polyclonal rat IgG. These negative control antibodies are designed for use with the corresponding primary antibodies of the same isotype. Choosing the appropriate negative control is critical for confirming the specificity of primary antibody binding and for ruling out non-specific Fc receptor binding.

ANTIBODY	DESCRIPTION	APPLICATIONS	QUANTITY	CAT. CODE
Human IgA2 Control	Monoclonal human IgA2, (*E. coli* β-Gal)	Isotype control	100 µg	maba2-ctrl
Mouse IgG1 Control	Monoclonal mouse IgG1, (*E. coli* β-Gal)	Isotype control	100 µg	mabg1-ctrlm
Mouse IgG2a Control	Monoclonal mouse IgG2a, (*E. coli* β-Gal)	Isotype control	100 µg	mabg2a-ctrlm
Mouse IgG2b Control	Monoclonal mouse IgG2b, (*E. coli* β-Gal)	Isotype control	100 µg	mabg2b-ctrlm
Rat IgG1 Control	Monoclonal rat IgG1, (*E. coli* β-Gal)	Isotype control	100 µg	mabg1-ctlrt
Rat IgG2a Control	Monoclonal rat IgG2a, (*E. coli* β-Gal)	Isotype control	100 µg	mabg2a-ctlrt
Rat IgG2b Control	Monoclonal rat IgG2b, (*E. coli* β-Gal)	Isotype control	100 µg	mabg2b-ctlrt
PAb Control	Polyclonal rat IgG	Control	200 µg	pab-sctr

STREPTAVIDIN CONJUGATE - Streptavidin-Lucia

The common techniques of ELISA and Western Blot analysis widely use the Streptavidin-Biotin system for signal amplification. InvivoGen introduces Streptavidin-Lucia, a new bioluminescent streptavidin-conjugate featuring the Lucia luciferase. Streptavidin-Lucia has been optimized for ELISA and can be adapted for use in a variety of assays requiring the detection of biotin and biotinylated proteins.

- **Highly sensitive -** Detect low levels of target with low background
- **Accurate -** Tight linear correlation over several logs
- **Convenient -** Reveal using one-step QUANTI-Luc™ detection reagent
- **Rapid -** Measure 96 samples in a microplate in less than a minute

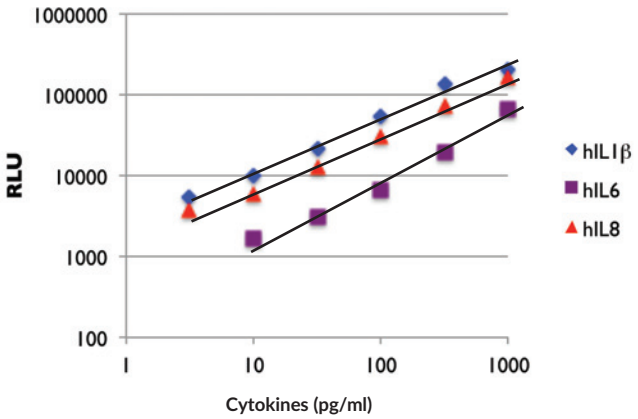
Description

Streptavidin-Lucia consists of a streptavidin peptide fused to the Lucia luciferase, a small secreted coelenterazine-utilizing luciferase with strong and stable bioluminescence properties. Streptavidin-Lucia uses the advantages of two systems, streptavidin for strength of binding to biotin labeled proteins, together with an optimized luciferase with a strong and stable bioluminescence for high sensitive detection with low background. Thus, this product is used for the detection of streptavidin-biotin interactions with high sensitivity and accuracy.

The unique long-lasting bioluminescence property of Lucia luciferase allows for ease in reading measurements. After addition of the QUANTI-Luc™ assay reagent to each well of a 96-well plate, results can be obtained in less than a minute using a luminometer without injectors.

Contents

Streptavidin-Lucia is provided lyophilized in 2 vials, allowing to prepare 100x 96-well plates. Once reconstituted, each vial contains 500 ml.



Typical data obtained using Strepavidin-Lucia for the detection of cytokines with commercially available ELISA. Graph shows detection of hIL1-b, hIL-8 and hIL-6. Streptavidin-Lucia was detected using QUANTI-Luc™. Linear regression indicates detection range over at least 3 logs.

PRODUCT	QUANTITY	CAT. CODE
Streptavidin-Lucia	2 x 500 ml	rep-strlc

INTERFERON ELISA KITS - LumiKine™

LumiKine™ is InvivoGen's new immunoassay for rapid, sensitive and specific detection of type I interferons. By replacing the absorbance-based enzymatic detection reaction of a standard ELISA with its lightning-fast Lucia luciferase/QUANTI-Luc™ bioluminescence detection system, InvivoGen has created an assay that can save you precious time in the laboratory. Unlike many ELISA kits, which are based on E. coli-produced cytokines, LumiKine™ has been designed using cytokines produced in mammalian cells, for accurate detection of species-specific mammalian cytokines. LumiKine™ kits are currently available for human and murine interferon (IFN)-α and IFN-β. They allow you to detect IFN-α/β in a broad concentration range that makes quantification and calibration curves a snap.

- **Rapid -** Optimized to reduce assay time - With LumiKine Xpress kits, save at least 1½ hours compared to standard ELISAs
- **Specific -** Antibodies raised against natural cytokines by DNA immunization
- **Precise -** Wide concentration range
- **Economical -** Each kit contains enough reagents to run five ELISA plates

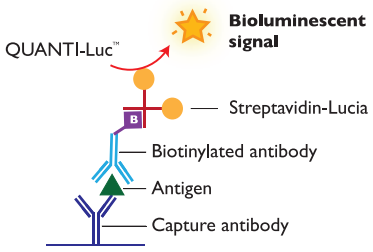
LumiKine™ IFN-α/β Kits

Four step bioluminescent ELISA

Description

LumiKine™ IFN-α/β kits are bioluminescent ELISA kits designed to quantify the levels of of human or mouse IFN-α or IFN-β in cell culture supernatant, serum and plasma samples using the classical "sandwich" ELISA method.
LumiKine™ IFN-α/β kits include a capture antibody, a biotinylated secondary antibody and a streptavidin-Lucia conjugate for detection (see previous page). This conjugate consists of a streptavidin peptide fused to Lucia luciferase, a small secreted coelenterazine-utilizing luciferase.
After addition of QUANTI-Luc™, a solution that contains the Lucia luciferase substrate, a bioluminescent signal is instantaneously emitted. This signal is directly proportional to the concentration of the cytokine produced and quantifiable by a luminometer. The streptavidin-Lucia conjugate can be replaced by the streptavidin conjugate of your choice.

Principle



Contents

- Capture antibody (50 µg)
- Biotinylated antibody (5 µg)
- Recombinant cytokine (100 ng)
- QUANTI-Luc™ (2 pouches)
- Strepatividin-Lucia (1 vial)
- White immunoplates (5 plates)

PRODUCT	QUANTITY	CAT. CODE
LumiKine™ hIFN-α (human)	5 plates	lumi-hifna
LumiKine™ mIFN-α (mouse)	5 plates	lumi-mifna
LumiKine™ hIFN-β (human)	5 plates	lumi-hifnb
LumiKine™ mIFN-β (mouse)	5 plates	lumi-mifnb

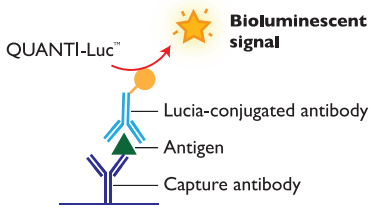
LumiKine™ Xpress IFN-α/β Kits

Three step bioluminescent ELISA - Even faster!

Description

LumiKine™ Xpress IFN-α/β kits are bioluminescent ELISA kits designed to quantify the levels of human or mouse IFN-α or IFN-β in cell culture supernatant, serum and plasma samples, more rapidly than classical "sandwich" ELISA kits (at least 1½ hours).
In the LumiKine™ Xpress IFN-α/β kits, the biotinylated secondary antibody and the streptavidin-Lucia conjugate are replaced by a Lucia-conjugated secondary antibody, eliminating an incubation step for faster detection. Lucia luciferase is a small secreted luciferase with strong and stable bioluminescence properties.
Levels of IFN-α or IFN-β are determined by measuring the luminescence instantaneously produced by Lucia luciferase after hydrolysis of its substrate provided in QUANTI-Luc™. The bioluminescent signal is assessed by luminometry.

Principle



Content

- Capture antibody (50 µg)
- Lucia-conjugated antibody (5 µg)
- Recombinant cytokine (100 ng)
- QUANTI-Luc™ (2 pouches)
- White immunoplates (5 plates)

PRODUCT	QUANTITY	CAT. CODE
LumiKine™ Xpress hIFN-α (human)	5 plates	luex-hifna
LumiKine™ Xpress mIFN-α (mouse)	5 plates	luex-mifna
LumiKine™ Xpress hIFN-β (human)	5 plates	luex-hifnb
LumiKine™ Xpress mIFN-β (mouse)	5 plates	luex-mifnb

RECOMBINANT PROTEINS

RECOMBINANT CYTOKINES

InvivoGen provides a selection of human cytokines produced by recombinant DNA technology in E. coli or mammalian cells. To ensure consistent and reliable results, our recombinant cytokines are of the highest quality; their endotoxin level is extremely low (<0.1 EU/µg) and their biological activity high. Each batch is validated using our cytokine reporter cells. InvivoGen's recombinant cytokines are provided with human serum albumin as a carrier protein.

Recombinant human CD40L

CD40 ligand, CD40L (also known as CD154, TRAP or gp39), is a type II transmembrane glycoprotein belonging to the TNF family. CD40L is predominantly expressed on activated $CD4^+$ T lymphocytes. It binds to CD40 present on antigen-presenting cells to regulate both humoral and cellular immune responses.
Recombinant human CD40L is a 149 amino acid, non-glycosylated protein with a molecular mass of 16 kDa.
Source: *E. coli*
Uniprot ID: P29965
Purity: >95%, by SDS-PAGE
Bioactivity: Validated using HEK-Blue CD40L cells

Recombinant human IFN-γ

Interferon gamma (IFN-γ), a Type II interferon, is secreted from CD4+ T-helper 1 (Th1) cells and activated natural killer (NK) cells. It plays a role in activating lymphocytes to enhance anti-microbial and anti-tumor effects. In addition, IFN-γ plays a role in regulating the proliferation, differentiation, and response of lymphocyte subsets.
Recombinant human IFN-γ is a single, non-glycosylated, polypeptide chain containing 144 amino acids and having a molecular mass of 17 kDa.
Source: *E. coli*
Uniprot ID: P01579
Purity: >95%, by SDS-PAGE
Bioactivity: Validated using HEK-Blue IFN-γ cells

Recombinant human IL-1β

Interleukin 1 beta (IL-1β) is a secreted pro-inflammatory cytokine that participates in the generation of systemic and local responses to infection and injury. Human IL-1β is produced as a precursor (269 amino acids) that is cleaved intracellularly by the cysteine protease Caspase-1 to generate the active cytokine.
Recombinant human IL-1β is a non-glycosylated polypeptide chain containing 153 amino acids with a molecular mass of 17 kDa.
Source: *E. coli*
Accession #: NP_000576
Purity: >95%, by SDS-PAGE
Bioactivity: Validated using HEK-Blue IL-1β cells

Recombinant human IL-4

Interleukin-4 (IL-4) is a cytokine that plays an important role in regulating inflammation and immune responses. It induces the differentiation of naïve helper T cells to Th2 cells. This cytokine binds the IL-4 receptor that also binds another cytokine interleukin 13 (IL-13), which may explain the overlapping functions of IL-4 and IL-13.
Recombinant human IL-4 is a 119 amino acid non-glycosylated, polypeptide chain with a molecular weight of 15 kDa.
Source: *E. coli*
Uniprot ID: P05112
Purity: >95%, by SDS-PAGE
Bioactivity: Validated using HEK-Blue IL-4/IL-13 cells

Recombinant human IL-6 (*E. coli*)

Interleukin-6 (IL-6) is a multifunctional cytokine that plays an important role in the regulation of the immune response, hematopoiesis, and inflammation. IL-6 is secreted by many cell types, including endothelial cells, fibroblasts, osteoblasts and T cells. It exerts its action by binding to the IL-6 receptor (IL-6R).
Recombinant human IL-6 is a 185 amino acid non-glycosylated, polypeptide chain with a molecular weight of 21 kDa.
Source: *E. coli*
Uniprot ID: P05231
Purity: >95%, by SDS-PAGE
Bioactivity: Validated using HEK-Blue IL6 cells

Recombinant human IL-12 (CHO)

Interleukin-12 (IL-12), also known as IL-12p70, cytotoxic lymphocyte maturation factor (CLMF) and natural killer cell stimulatory factor (NKSF), is primarily produced by dendritic cells and macrophages in response to bacterial pathogens. It promotes Th1 responses and is a powerful inducer of interferon gamma (IFN-γ). IL-12 is a heterodimeric 70 kDa cytokine composed of two subunits, p35 and p40.
Recombinant human IL-12 is produced in Chinese hamster ovary (CHO) cells transfected with a fusion gene encoding the p35 and p40 subunits linked by a decapeptide containing two elastin motifs. These transfected cells produce and secrete biologically active IL-12. The recombinant protein is purified by size exclusion chromatography.
Source: CHO
Uniprot ID: P29459
Purity: >90%, by SDS-PAGE
Bioactivity: Validated using HEK-Blue IL-12 cells

Recombinant human IL-13

Interleukin-13 (IL-13) is a Th2-type cytokine secreted from $CD4^+$ T cells, mast cells, basophils and eosinophils. IL-13 is a key mediator in the pathogenesis of allergic inflammation. The functions of IL-13 overlap considerably with those of the closely related cytokine IL-4.
Recombinant human IL-13 is a single, non-glycosylated, 112 amino acid protein with a molecular mass of 12 kDa.
Source: *E. coli*
Accession #: NM_002188
Purity: >95%, by SDS-PAGE
Bioactivity: Validated using HEK-Blue IL-4/IL-13 cells

Recombinant human IL-17A

Interleukin 17A (IL-17A) is a pro-inflammatory cytokine that belongs to a family of six cytokines; IL-17A, IL-17B, IL-17C, IL-17D, IL-E (also known as IL-25) and IL-17F. Although this cytokine exerts a protective role against many pathogens, the overproduction of IL-17A is associated with autoimmune diseases.
Recombinant human IL-17A is produced in CHO cells transfected with the full-length sequence for the human IL-17A gene. These transfected cells produce and secrete biologically active IL-17A. Recombinant human IL-17A is purified by affinity chromatography.
Source: CHO
Uniprot ID: Q16552
Purity: >95%, by SDS-PAGE
Bioactivity: Validated using HEK-Blue IL-17 cells

Recombinant human IL-18

Interleukin-18 (IL-18) is a key pro-inflammatory cytokine and an important mediator of Th1 immune response[3]. IL-18 is produced by

macrophages and other cells, as a proprotein which is proteolytically processed to its active form by caspase-1.
Recombinant human IL-18 is a 157 amino acid protein with a molecular mass of 18 kDa.
Source: *E. coli*
Uniprot ID: Q14116
Purity: >95%, by SDS-PAGE
Bioactivity: Validated using HEK-Blue IL-18 cells

Recombinant human IL-33

Interleukin 33 (IL-33) is a pro-inflammatory protein that induces helper T cells, mast cells, eosinophils and basophils to produce type 2 cytokines[3]. Similar to IL-1β and IL-18, IL-33 is produced as a proprotein which is proteolytically processed to its active form.
Recombinant human IL-33 is a 159 amino acid protein with a molecular mass of 18 kDa.
Source: *E. coli*
Uniprot ID: O95760
Purity: >95%, by SDS-PAGE
Bioactivity: Validated using HEK-Blue IL-33 cells

Recombinant human TNF-α

Tumor necrosis factor-alpha (TNF-α) is a cytokine that plays a role in a variety of biological processes including cell proliferation, differentiation and apoptosis. It functions by activating transcription factors such as NF-κB and AP-1. TNF-α consists of three identical polypeptide chains of 157 amino acids combined to form a homotrimer with subunit mass of 17 kDa.
Recombinant human TNF-α produced in CHO cells transfected with the full-length sequence for the human TNF-α gene. These transfected cells produce and secrete biologically active TNF-α. The recombinant protein is purified by affinity chromatography.
Source: CHO
Uniprot ID: P01375
Purity: >97%, by SDS-PAGE
Bioactivity: Validated using HEK-Blue TNF-α cells

Contents

Recombinant cytokines are provided as sterile, endotoxin-free (<0.1 EU/µg) lyophilized powder with human serum albumin. Products are shipped at room temperature. Store at -20˚C.

PRODUCT	QUANTITY	CAT. CODE
Recombinant human CD40L	10 µg	rcyec-hcd40l
Recombinant human IFN-γ	20 µg	rcyec-hifng
Recombinant human IL-1β	10 µg	rcyec-hil1b
Recombinant human IL-4	10 µg	rcyec-hil4
Recombinant human IL-6	10 µg	rcyec-hil6
Recombinant human IL-12	10 µg	rcyc-hil12
Recombinant human IL-13	10 µg	rcyec-hil13
Recombinant human IL-17a	10 µg	rcyc-hil17a
Recombinant human IL-18	10 µg	rcyec-hil18
Recombinant human IL-33	10 µg	rcyec-hil33
Recombinant human TNF-α	20 µg	rcyc-htnfa

RELATED PRODUCTS

PRODUCT	INFO
HEK-Blue™ CD40L	See page 57
HEK-Blue™ IFN-γ	See page 49
HEK-Blue™ IL-1β	See page 50
HEK-Blue™ IL-4/IL-13	See page 54
HEK-Blue™ IL-6	See page 55
HEK-Blue™ IL-12	See page 55
HEK-Blue™ IL-17	See page 53
HEK-Blue™ IL-18	See page 52
HEK-Blue™ IL-33	See page 52
HEK-Blue™ TNF-α	See page 56

RECOMBINANT REPORTER PROTEINS

Recombinant Lucia luciferase protein

Recombinant Lucia Luciferase is a coelenterazine-utilizing luciferase that can be directly measured in the cell culture medium using bioluminescent assays. The recombinant protein is a monomeric protein expressed in CHO cells. It is composed of 192 amino acids and has an estimated molecular weight of 21 kDa.
Recombinant Lucia luciferase is provided in FBS-containing culture medium. It can be used as a positive control for luciferase reporter assays.

Recombinant SEAP protein

Recombinant SEAP (secreted embryonic alkaline phosphatase) protein is a truncated form of human placental alkaline phosphatase that comprises 520 amino acids. It is expressed in CHO cells and shows a 75 kDa band on SDS page.
Recombinant SEAP protein is formulated in 91 mM glycine, 91 mM TRIS and 5% w/v saccharose. It can be used as a positive control for SEAP reporter assays.

Contents

Recombinant reporter proteins are provided lyophilized. Products are shipped at room temperature. Store at -20˚C.

PRODUCT	QUANTITY	CAT. CODE
Recombinant Lucia luciferase protein	1 µg	rec-lucia
Recombinant human IFN-γ	20 µg	rcyec-hifng

SOLUBLE RECEPTOR:FC FUSION PROTEINS

InvivoGen provides soluble forms of the pattern recognition receptors, Dectin-1 and TLR5, that consist of the extracellular domain (ECD) of each receptor fused to an IgG1 Fc domain, engineered to reduce ADCC and enhance half-life. Dectin-1, which is a type-II transmembrane receptor, is fused to the C-terminus of the Fc domain while TLR5, which is a type-1 transmembrane receptor is fused to the N-terminus of the Fc domain. The Fc fusion proteins are expressed in CHO cells and purified by protein G affinity chromatography. The soluble Dectin-1 and TLR5 receptors can be used for receptor binding assays or neutralization studies.

Soluble Dectin-1 Receptors

InvivoGen provides soluble forms of the human and mouse Dectin-1 receptors, Fc-hDectin-1a and Fc-mDectin-1a, respectively. Human and murine Dectin-1 share 60% sequence identity, which may lead to differences in protein folding and β-glucans recognition of the Fc-Dectin-1 fusion proteins.

Fc-hDectin-1a

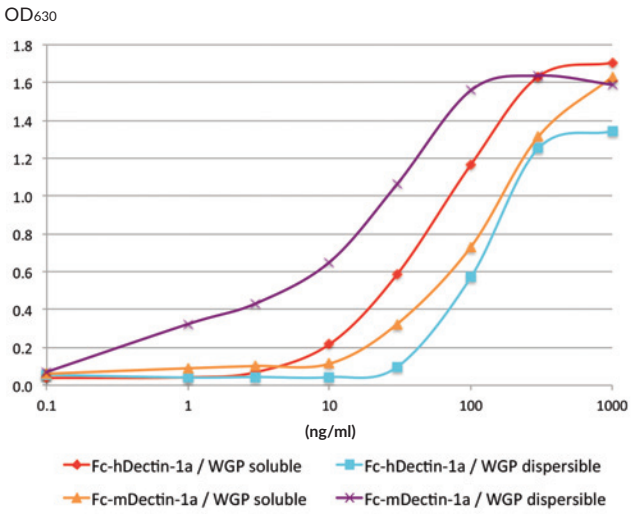
Fc-hDectin-1a is a soluble human Dectin-1 receptor constructed by fusing the C-terminal extracellular domain of human Dectin-1a (aa 67-247) to the C-terminus of an engineered human IgG1 Fc domain with a 10 amino acid linker. Fc-hDectin-1a has an apparent molecular weight of ~55 kDa on SDS-PAGE.

Fc-mDectin-1a

Fc-mDectin-1a is a soluble murine Dectin-1 receptor constructed by fusing the C-terminal extracellular domain of mouse Dectin-1a (aa 67-244) to the C-terminus of an engineered human IgG1 Fc domain with a 10 amino acid linker. Fc-mDectin-1a has an apparent molecular weight of ~55 kDa on SDS-PAGE.

Contents

Soluble Dectin-1 receptors are provided lyophilized. They are shipped at room temperature and should be stored at -20°C.



β-Glucan receptor binding assay - 96-well plates were coated with 0.5 µg of various β-glucans and incubated with increasing concentrations of Fc-hDectin-1a or Fc-mDectin-1a. After 2 hours, an anti-IgG secondary antibody conjugated to alkaline phosphatase was added. β-Glucan/Dectin-1 binding was assessed using the QUANTI-Blue™ assay.

Soluble TLR5 Receptor

Toll-like receptor 5 (TLR5) is a type-1 transmembrane receptor comprising an N-terminal extracellular leucine rich repeat domain and a C-terminal intracellular TIR signaling domain.

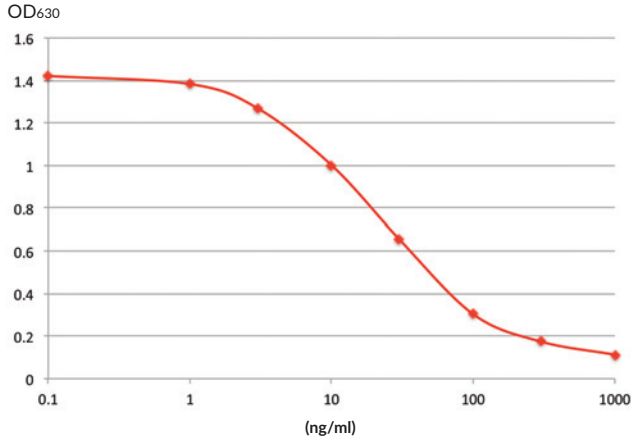
hTLR5-Fc - Soluble ectodomain of TLR5

The soluble TLR5 receptor, hTLR5-Fc, was generated by fusing the N-terminal extracellular domain of human TLR5 (aa 21-639) to the N- terminus of an engineered Fc region of human IgG1 with a 2 amino acid linker. The hTLR5-hFc fusion has an apparent molecular weight of 110 kDa on SDS-PAGE.

Contents

Soluble TLR5 receptor is provided lyophilized. Product is shipped at room temperature and should be stored at -20°C.

PRODUCT	QUANTITY	CAT. CODE
Fc-hDectin-1a	50 µg	fc-hdec1a
Fc-mDectin-1a	50 µg	fc-mdec1a
hTLR5-Fc	50 µg	fc-htlr5



Neutralization activity of hTLR5-Fc - Increasing concentrations of hTLR5-Fc were pre-incubated with HEK-Blue™ hTLR5 cells, which express the human TLR5 gene and an NF-κB-inducible SEAP gene, prior to the addition of 1 ng/ml FLA-ST (*S. typhimurium* flagellin). After 24h incubation, TLR5-induced NF-κB activation was assessed by measuring the levels of SEAP using the QUANTI-Blue™ assay.

7 VACCINATION

Vaccine Adjuvants

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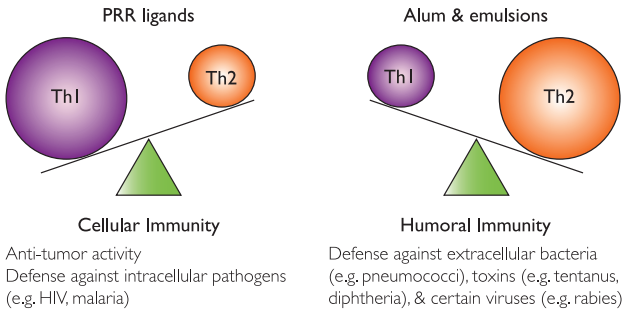
VACCINE ADJUVANTS

Adjuvants are essential for enhancing and directing the adaptative immune response to vaccine antigens. This response is mediated by two main types of lymphocytes, B and T cells. Upon activation by cytokines, B cells differentiate into memory B cells (long-lived antigen-specific B cells) or plasma cells (effector B cells that secrete large quantities of antibodies). Most antigens activate B cells using activated T helper (Th) cells, primarily Th1 and Th2 cells. Th1 cells secrete IFN-γ, which activates macrophages and induces the production of opsonizing antibodies by B cells. The Th1 response leads mainly to cell-mediated immunity (cellular response), which protects against intracellular pathogens (invasive bacteria, protozoa and viruses). The Th1 response activates cytotoxic T lymphocytes (CTL), a sub-group of T cells, which induce death of cells infected with viruses and other intracellular pathogens. Natural killer (NK) cells are also activated by the Th1 response. They play a major role in the induction of apoptosis in tumors and in cells infected by viruses. Th2 cells secrete cytokines, including IL-4, which induces B cells to make neutralizing antibodies. Th2 cells generally induce a humoral (antibody) response, which is critical in the defense against extracellular pathogens (helminths, extracellular microbes and toxins).



The magnitude and type of Th response to a vaccine can be greatly modulated through the use of adjuvants. For almost 80 years, aluminium salts (referred to as 'alum') have been the only adjuvant in use in human vaccines. Only in the last two decades, have novel adjuvants (MF59, AS04) been introduced into the formulation of new licensed vaccines. As our understanding of the mechanisms of 'immunogenicity' and 'adjuvancy' increases, new adjuvants and adjuvant formulations are being developed.

Mechanisms of adjuvants

Adjuvants may exert their effects through different mechanisms. Some adjuvants, such as alum and emulsions (e.g. MF59), function as delivery systems by generating depots that trap antigens at the injection site, providing slow release in order to continue the stimulation of the immune system. These adjuvants enhance antigen persistence at the injection site and increase recruitment and activation of antigen presenting cells (APCs). Particulate adjuvants (e.g. alum) have the capability to bind antigens to form multi-molecular aggregates which will encourage uptake by APCs[1]. Some adjuvants are also capable of directing antigen presentation by the major histocompatibility complexes (MHC)[1]. Other adjuvants, essentially ligands for pattern recognition receptors (PRR), act by inducing innate immunity, predominantly targeting APCs and consequently influencing the adaptive immune response. Members of nearly all of the PRR families are potential targets for adjuvants. These include Toll-like receptors (TLRs), NOD-like receptors (NLRs), RIG-I-like receptors (RLRs) and C-type lectin receptors (CLRs). They signal through pathways that involve distinct adaptor molecules, leading to the activation of different transcription factors. These transcription factors (NF-κB, IRF3) induce the production of cytokines and chemokines that play a key role in the priming, expansion and polarization of immune cells.

Alum & emulsions

Alum is the most commonly used adjuvant in human vaccination. It is found in numerous vaccines, including diphtheria-tetanus-pertussis, human papillomavirus and hepatitis vaccines[2]. Alum provokes a strong Th2 response, but is rather ineffective against pathogens that require Th1–cell-mediated immunity. Alum induces an immune response by a depot effect and activation of APCs. Recently, the NLRP3 inflammasome has been linked to the immunostimulatory properties of alum[3], although its role in adjuvant-induced antibody responses remains controversial.
Emulsions (either oil-in-water or water-in-oil), such as Freund's Incomplete Adjuvant (IFA) and MF59, can trigger depot generation and induction of MHC responses. IFA induces a predominantly Th2-biased response with some Th1 cellular response. MF59 is a potent stimulator of cellular (Th1) as well as humoral (Th2) immune responses[4]. However, the precise mode of action of emulsion-based adjuvants is still unclear.

PRR Ligands

The current challenge is to develop adjuvants which induce a strong Th1 bias important for vaccines against hepatitis, flu, malaria and HIV. New adjuvants are being developed that are natural ligands or synthetic agonists for PRRs, either alone or with various formulations. PRR activation stimulates the production of pro-inflammatory cytokines/chemokines and type I IFNs that increase the host's ability to eliminate the pathogen. Thus, the incorporation of pathogen-associated molecular patterns (PAMPs) in vaccine formulations can improve and accelerate the induction of vaccine-specific responses. A number of these agonists are now in clinical or late preclinical stages of development for hepatitis and human papillomavirus vaccines[5, 6].

1. Leroux-Roels G., 2010. Unmet needs in modern vaccinology adjuvants to improve the immune response. Vaccine. 28S(3):C25-3. **2. Marrack P. *et al.*, 2009.** Towards an understanding of the adjuvant action of aluminium. Nat Rev Immunol. 9(4):287-93. **3. Li H. *et al.*, 2008.** Cutting edge: Inflammasome activation by alum and alum's adjuvant effect are mediated by NLRP3. J Immunol. 181(1):17-21. **4. Ott G. *et al.*, 1995.** MF59. Design and evaluation of a safe and potent adjuvant for human vaccines. Pharm Biotechnol 6: 277-96. **5. Steinhagen F. *et al.*, 2010.** TLR-based immune adjuvants. Vaccine. 29(17):3341-55. **6. Mbow ML. *et al.*, 2010.** New adjuvants for human vaccines. Curr Opin Immunol. 22(3):411-6.

Adjuvants licensed for use in human vaccines

Adjuvant Name	Adjuvant Class	Components	Indications (Vaccines)
Alum	Mineral salts	Aluminium phosphate or aluminium hydroxide	Multiple
AS03	Oil-in-water emulsion	Squalene, Tween 80, α-tocopherol	Pandemic influenza (Pandemrix)
AS04	MPL® adsorbed to alum	Alum and 3-O-desacyl-4′-monophosphoryl lipid A	Human papillomavirus (Cervarix), hepatitis B (Fendrix)
MF59	Oil-in-water emulsion	Squalene, polysorbate 80, sorbitan trioleate	Seasonal influenza (Fluad), pandemic influenza (Aflunov, Focetria)
Influenza virosomes	Liposomes	Lipids, hemagglutinin	Seasonal influenza (Inflexal), hepatitis A (Epaxal)

InvivoGen provides different classes of vaccine adjuvants that are either already approved for use in human vaccination, such as alum, or under investigation, including TLR agonists such as Gardiquimod™ and CpG oligonucleotides. InvivoGen adjuvants are VacciGrade™, a specific grade for preclinical studies. They are prepared under strict aseptic conditions. They are guaranteed sterile and thoroughly tested for the presence of endotoxins.

PRODUCT	DESCRIPTION	Th RESPONSE	RATIO/WORKING CONCENTRATION	QTY	CATALOG CODE
Alum and Emulsions					
AddaVax™	Squalene-based-oil-in-water emulsion	Th2	1:1 (AddaVax™:antigen)	10 ml	vac-adx-10
Alhydrogel® adjuvant 2%	Aluminium hydroxide gel	Th2	1:9 - 1:1 (Alhydrogel®:antigen)	250 ml	vac-alu-250
CFA	Complete Freund's adjuvant Oil emulsion	Th1	1:1 (CFA:antigen)	10 ml 6 x 10 ml	vac-cfa-10 vac-cfa-60
IFA	Incomplete Freund's adjuvant Oil emulsion	Th2	1:1 (IFA:antigen)	10 ml 6 x 10 ml	vac-ifa-10 vac-ifa-60
Quil-A® adjuvant	Saponin vaccine adjuvant	Th1/Th2	1:1 (Quil-A®:antigen)	1 g	vac-quil
PRR Ligands					
2'3'-cGAMP VacciGrade™	2'5'-3'5' Cyclic GMP-AMP - STING agonist	Th1	5 - 50 µg/mouse	1 mg	vac-nacga23
3'3'-cGAMP VacciGrade™	3'5'-3'5' Cyclic GMP-AMP - STING agonist	Th1	5 - 50 µg/mouse	1mg	vac-nacga
c-di-AMP VacciGrade™	Cyclic diadenylate monophosphate - STING agonist	Th1/Th2	5 - 50 µg/mouse	1 mg	vac-nacda
c-di-GMP VacciGrade™	Cyclic diguanylate monophosphate - STING agonist	Th1/Th2	5 - 50 µg/mouse	1 mg	vac-nacdg
Chitosan VacciGrade™	Deacetylated derivative of chitin - NLRP3 inducer	Th1/Th2	100 µg/mouse	100 mg	vac-cht
CL401 VacciGrade™	Pam2C-conjugated hydroxyadenine compound TLR2 & TLR7 agonist	Th1/Th2	20 - 50 µg/mouse	1 mg	vac-c401
CL413 VacciGrade™	Pam2CK4-conjugated hydroxyadenine compound TLR2 & TLR7 agonist	Th1/Th2	20 - 50 µg/mouse	1 mg	vac-c413
CL429 VacciGrade™	Pam2C-conjugated murabutide TLR2 & NOD2 agonist	Th1/Th2	20 - 50 µg/mouse	5 mg	vac-c429
Flagellin FliC VacciGrade™	Recombinant flagellin from *S. typhimurium* -TLR5 agonist	Th1/Th2	1 - 10 µg/mouse	50 µg	vac-fla
Gardiquimod™ VacciGrade™	Imidazoquinoline compound - TLR7 agonist	Th1	10 - 100 µg/mouse	5 mg	vac-gdq
Imiquimod VacciGrade™	Imidazoquinoline compound - TLR7 agonist	Th1	10 - 100 µg/mouse	5 mg	vac-imq
LPS-EB VacciGrade™	*E. coli 0111:B4* Lipopolysaccharide - TLR4 agonist	Th1	0.1 - 25 µg/mouse	5 x 10^6 EU	vac-3pelps
MPLA-SM VacciGrade™	Detoxified monophosphoryl Lipid A - TLR4 agonist	Th1	2 - 20 µg/mouse	1 mg	vac-mpla
MPLAs VacciGrade™	Synthetic monophosphoryl Lipid A - TLR4 agonist	Th1	2 - 20 µg/mouse	1 mg	vac-mpls
N-glycolyl-MDP VacciGrade™	N-glycolyted muramyldipeptide - NOD2 agonist	Th1	5 - 30 µg/mouse	5 mg	vac-gmdp
ODN 1585 VacciGrade™	CpG ODN, type A (mouse) - TLR9 agonist	Th1	20 - 50 µg/mouse	1 mg	vac-1585-1
ODN 1826 VacciGrade™	CpG ODN, type B (mouse) - TLR9 agonist	Th1	20 - 50 µg/mouse	1 mg	vac-1826-1
ODN 2006 VacciGrade™	CpG ODN, type B (human) - TLR9 agonist	Th1	20 - 50 µg/mouse	1 mg	vac-2006-1
ODN 2395 VacciGrade™	CpG ODN, type C (human / mouse) - TLR9 agonist	Th1	20 - 50 µg/mouse	1 mg	vac-2395-1
Pam3CSK4 VacciGrade™	Synthetic triacylated lipoprotein - TLR1/2 agonist	Th1	2 - 20 µg/mouse	1 mg	vac-pms
Poly(I:C) (HMW) VacciGrade™	Polyinosine-polycytidylic acid - TLR3 agonist	Th1	10 - 100 µg/mouse	10 mg	vac-pic
R848 VacciGrade™	Imidazoquinoline compound - TLR7/8 agonist	Th1	10 - 100 µg/mouse	5 mg	vac-r848
TDB VacciGrade™	Synthetic analog of the cord factor - Mincle agonist	Th1	1 - 100 µg/ml	2 mg	vac-tdb

Contents

Alhydrogel® adjuvant 2% is provided as a ready-to-use wet gel suspension. AddaVax™, CFA and IFA are provided as ready-to-use sterile solutions. Quil-A® adjuvant and VacciGrade™ PRR Ligands are provided lyophilized. Products are shipped at room temperature.

Alhydrogel® adjuvant 2% should be kept at room temperature; AddaVax™, CFA and IFA should be stored at 4 °C. DO NOT FREEZE. Quil-A® adjuvant and VacciGrade™ PRR Ligands should be stored at 4 °C or -20 °C according to the product label. Alhydrogel® and Quil A® are trademarks of Brenntag Nordic A/S (Brenntag Biosector A/S).

ALUM & EMULSIONS

AddaVax™

AddaVax™ is a squalene-based oil-in-water nano-emulsion with a formulation similar to MF59® that has been licensed in Europe for adjuvanted flu vaccines[1]. Squalene is an oil that is more readily metabolized than the paraffin oil used in Freund's adjuvants[1]. Squalene-based oil-in-water nano-emulsions promote a significant increase in antibody titers with reportedly more balanced Th1/Th2 responses than those obtained with alum[2]. This class of adjuvants is believed to act through recruitment and activation of APCs and stimulation of cytokine and chemokine production by macrophages and granulocytes[1].

Alhydrogel® Adjuvant 2%

Alhydrogel® adjuvant 2%, referred to as alum, is an aluminium hydroxide wet gel suspension. Alum improves attraction and uptake of antigen by APCs[2]. More recently, it has been suggested that the antigens absorbed on the aluminum salts are presented in a particulate form, making them more efficiently internalized by APCs. Moreover, alum activates the NLRP3 inflammasome complex implicated in the induction of several pro-inflammatory cytokines, including IL-1β and IL-18[3]. Alum increases Th2 antibodies but does not promote significant Th1 cellular response.

CFA - Complete Freund's Adjuvant

Complete Freund's adjuvant (CFA) consists of heat-killed *Mycobacterium tuberculosis* in an oil emulsion. CFA contains ligands for Mincle, TLR2, TLR4 and TLR9[2]. Injection of antigen in CFA induces a Th1-dominated response, while injection in Incomplete Freund's Adjuvant (IFA), which lacks mycobacterial components, induces a Th2-dominated response[4].

IFA - Incomplete Freund's Adjuvant

IFA (Incomplete Freund's adjuvant), an oil emulsion, is one of the most commonly used adjuvants in research. It is prepared from non-metabolizable oils (paraffin oil and mannide monooleate). IFA does not contain killed *Mycobacterium tuberculosis* found in Complete Freund's Adjuvant and is thus less inflammatory. IFA induces a predominantly Th2 biased response through the formation of a depot at the injection site and the stimulation of antibody producing plasma cells[4]. It has been suggested that NOD2 modulates the adjuvant effects of IFA[5].

Quil-A® Adjuvant

Quil-A® adjuvant is a saponin adjuvant produced under GMP by Brenntag Biosector, and purified through a proprietary process that ensures consistency and immunostimulatory potential. Quil-A® adjuvant is used in a wide variety of veterinary vaccines, as well as in immunological research on human and veterinary applications[6, 7]. Quil-A® adjuvant contains the water-extractable fraction of saponins from the South American tree, *Quillaja saponaria* Molina. Saponins induce a strong adjuvant response to T-dependent as well as T-independent antigens[4]. Saponins also induce strong cytotoxic CD8+ lymphocyte responses and potentiate the response to mucosal antigens[4, 7].

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PRR LIGANDS

cGAMPs VacciGrade™

Cyclic guanosine monophosphate–adenosine monophosphate (cGAMP) is a hybrid cyclic dinucleotide (CDN). cGAMP isomers include the mammalian molecule 2'3'-cGAMP and the microbial molecule 3'3'-cGAMP. CDNs represent a recently discovered class of adjuvants that have been shown to increase vaccine potency[1]. CDNs activate innate immunity by directly binding the endoplasmic reticulum-resident receptor STING (stimulator of interferon genes), activating a signaling pathway that induces the expression of IFN-β and also NF-κB-dependent inflammatory cytokines[3]. Recently, 2′3′-cGAMP was reported to function as an effective adjuvant that boosts the production of antigen-specific antibodies and T cell responses in mice[2].

c-di-AMP & c-di-GMP VacciGrade™

Cyclic diguanylate monophosphate (c-di-GMP) and cyclic diadenylate monophosphate (c-di-AMP) are intracellular signaling molecules produced by bacteria. Both CDNs can induce a strong immune response by triggering the production of cytokines, such as type I interferons[3]. Due to their immunostimulatory properties, these molecules have been investigated as vaccine adjuvants. Mucosal delivery of c-di-AMP[4] and c-di-GMP[5] has been reported to elicit a balanced Th1/Th2 profile and Th17 response, which is crucial against intracellular pathogens. These adjuvants act through recruitment of monocytes and granulocytes, and maturation of dendritic cells[4, 6].

Chitosan VacciGrade™

Chitosan is the deacetylated derivative of chitin, a polysaccharide found in the exoskeletons of crustaceans and in the cell walls of fungi. Chitosan is used in drug delivery and as a vaccine adjuvant[7, 8]. Due to its bioadhesive properties, chitosan is efficiently taken up by phagocytotic cells, leading the strong induction of systemic and mucosal immune responses. Although the activity of chitosan is not fully understood, there are some clues to its mode of action. Chitosan stimulates the immune system by activating the NLRP3 inflammasome, leading to potent IL-1β production[9]. When tested in experimental models of vaccination, chitosan elicits a balanced Th1/Th2 response[10].

CL401 & CL413 VacciGrade™

CL401 and CL413 are chimeric molecules that co-activate the surface receptor TLR2 and the endosomal receptor TLR7. They are composed of 8-hydroxyadenine (TLR7 ligand) conjugated to Pam2C (TLR2 ligand) or to the terminal acid group of Pam2CSK4 (TLR2 ligand), respectively. Both chimeric compounds display strong adjuvant activity. In mice, administration of OVA with CL401 or CL413 leads to a significant increase of anti-OVA IgG titers in sera. The predominant IgG subclass is IgG1, a Th2-associated isotype. The IgG2a subclass, a Th1-associated isotype, is also significantly increased, resulting in a decreased IgG1/IgG2a ratio.

CL429 VacciGrade™

CL429 is a chimeric compound that stimulates both TLR2 and NOD2. This compound is composed of murabutide (NOD2 ligand) covalently linked to Pam2C (TLR2 ligand) via a spacer[11]. CL429 possesses a very potent adjuvant activity, with no apparent toxicity. It enables strong induction of both systemic and mucosal immunity against a vaccine antigen. This activity seems to be mediated by an optimal DC maturation process, which induces strong B and T cell stimulation as well as autophagy.

Flagellin FliC VacciGrade™

Flagellin FliC is a recombinant flagellin protein encoded by the *fliC* gene from *Salmonella typhimurium*. Bacterial flagellin, a TLR5 ligand, is a potent T-cell antigen and has potential as a vaccine adjuvant. Unlike other TLR agonists, flagellin tends to produce mixed Th1 and Th2 responses rather than strongly Th1 responses[12]. It has been demonstrated that flagellin can act as a potent adjuvant in flu vaccines[13, 14]. Furthermore, flagellin can also signal through the NLRC4 inflammasome[15], although it is not known whether this pathway contributes to the adjuvant activity of flagellin.

Gardiquimod™, Imiquimod & R848 VacciGrade™

The imidazoquinoline compounds Gardiquimod, Imiquimod and R848, are guanosine derivatives and agonists for TLR7 and TLR8. These TLR7/8 agonists, originally developed as type I IFN inducers, are effective adjuvants by activating dendritic cells (DCs) and B cells to induce cytokines optimal for Th1 cell immunity, and antibody production[16]. More specifically, R848 (Resiquimod) activates NF-κB and MAP kinase pathways in B cells, thereby promoting the production of antibodies[17]. R848 is a good inducer of IFN-related innate immunity pathways[18].

LPS-EB VacciGrade™

LPS-EB is a lipopolysaccharide (LPS) extracted from *E. coli 0111:B4* strain. LPS is a natural adjuvant synthesized by gram-negative bacteria and is a potent activator of the immune system. LPS stimulates immune responses through TLR4. Similar to other TLR-based adjuvants, LPS drives Th1 immunity, although in certain circumstances low-dose inhaled LPS can promote Th2 responses. Although LPS is a potent adjuvant, its pyrogenic activity has prevented clinical use of LPS in vaccines. LPS-EB VacciGrade™ is an ultrapure preparation that only activates the TLR4 pathway.

MPLA-SM & MPLAs VacciGrade™

The TLR4 agonist, MPLA (monophosphoryl lipid A)-SM is a detoxified derivative of lipid A from *S. minnesota* lipopolysaccharide (LPS or endotoxin). MPLA is considerably less toxic than LPS but maintains the immunostimulatory activity[19]. Preclinical studies indicate that MPLA induces a strong Th1 response[20]. MPLAs is a chemically synthesized TLR4 agonist combining 6 acyl chains with a single phosphorylation site. Its structure is reminiscent of the hexaacyl lipid found in monophosphoryl lipid A (MPL®), a component of AS04, which, when adsorbed onto alum, constitutes the licensed adjuvant AS04. Synthetic MPLA combined with an oil-in-water emulsion has been shown to elicit high titers of vaccine-specific antibodies and high levels of Th1 cytokine responses in mice[21, 22].

N-glycolyl-MDP VacciGrade™

MDP (muramyl dipeptide), is the minimal bioactive peptidoglycan (PGN) motif common to all bacteria and the essential structure required for PGN adjuvant activity in vaccines. MDP has been shown to be recognized by NOD2, but not TLR2, TLR2/1 or TLR2/6 associations[23]. The cell wall of mycobacteria is known to be extremely immunogenic. This potent activity is attributed to their MDP, which is N-glycolylated in contrast to the MDP of most bacteria which is N-acetylated. N-glycolyl-MDP has been reported to display a stronger NOD2-mediated activity than N-acetyl-MDP and thus to be a more potent vaccine adjuvant than N-acetyl-MDP[24]. Furthermore, MDP leads to activation of the NLRP3 inflammasome[25].

CpG ODN VacciGrade™

Synthetic oligodeoxynucleotides containing unmethylated CpG motifs (CpG ODNs) have been extensively studied as adjuvants. Three types of CpG ODNs exist: types A, B and C. All CpG ODN types are recognized by TLR9, which is expressed exclusively in human B cells and plasmacytoid dendritic cells, thereby inducing Th1-dominated immune responses. Pre-clinical studies and human clinical trials have demonstrated that CpG ODNs can significantly improve vaccine-specific antibody responses[20]. Clinical data indicate that the type B ODN 2006 (aka ODN 7909) is highly effective for enhancing antigen-specific antibody responses against a variety of antigens[26]. Numerous preclinical studies report the efficacy of ODN 1826 (another type B CpG ODN), used alone or in combination with other adjuvants to enhance the protective immunity of vaccination with diverse antigens[27, 28]. ODN 1585, a type A CpG ODN, was recently shown to provide protective immunity against HPV16-associated-tumors in mice when combined with a peptide vaccine[29]. *In vivo* studies have demonstrated that type C ODNs which combine the effects of types A and B ODNs, such as ODN 2395, are very potent Th1 adjuvants[30].

Pam3CSK4 VacciGrade™

Pam3CSK4, a synthetic lipopeptide and a TLR1/2 ligand, is an efficient adjuvant for the influenza vaccine. In a recent preclinical study, Pam3CSK4 was reported to increase antibody responses to flu antigens, unlike other TLR ligands[18]. It was shown to exert a strong local response, enhance IgG2a and IgG1 titers and upregulate proinflammatory and Th1 cytokine genes. Pam3CSK4 was also used as adjuvant to improve the efficacy of a DNA-based vaccine against *Leishmania*[31]. It increased antigen specific CD8 cells in immunized mice and induced higher levels of IFN-γ.

Poly(I:C) VacciGrade™

Synthetic double-stranded RNA, namely poly(I:C), can activate immune responses through two distinct PRRs[13]. Endosomal poly(I:C) activates TLR3 while cytosolic poly(I:C) activates RIG-I/MDA-5. Triggering the TLR3 pathway induces IL-12 and type I IFNs production, and improves MHC class II expression and cross-presentation of antigen[17]. Stimulation of MDA-5 enhances the production of type I IFNs that play a critical role in enhancing T and B cell immunity. Poly(I:C) promotes Th1-biased immunity through its induction of IL-12 and type I IFN[16]. Promising results have been obtained using poly(I:C) as an adjuvant in flu vaccine delivered intranasally[9].

TDB VacciGrade™

Trehalose-6,6-dibehenate (TDB) is a non-toxic synthetic analog of the mycobacterial cell wall component trehalose 6,6' dimycolate (TDM, also known as cord factor). TDB was recently shown to rely on the C-type lectin Mincle and the signaling molecules Syk and Card9 to trigger innate immunity[32]. Incorporation of TDB combined with the synthetic amphiphilic cationic lipid compound dimethyldioctadecylammonium (DDA) into liposomes, known as CAF01, has been shown to strongly enhance cellular and humoral responses against a protein antigen[33]. The adjuvanticity of the cationic DDA:TDB liposomes and sustained protection against disease challenge has been demonstrated with a tuberculosis vaccine candidate[34, 35] and has good potential for application in a range of other diseases[36].

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OVA ANTIGENS

Ovalbumin (OVA) is a key reference protein for immunization and biochemical studies (Western blots, ELISA). It is the major protein constituent of chicken egg whites. Ovalbumin is a glycoprotein that is sufficiently large and complex to be mildly immunogenic. Consequently, it is widely used as an antigen for immunization experiments. Commercially available ovalbumin is often contaminated with endotoxins that influence in vivo *results. InvivoGen provides two grades of ovalbumin, a standard grade and an LPS-free grade (EndoFit™), as well as two standard OVA peptides and an OVA-expressing CpG-free plasmid.*

OVALBUMIN

Ovalbumin

Ovalbumin (OVA) is widely used as a model antigen for the characterization of antigen uptake, processing and presentation. OVA is a glycoprotein that comprises approximately 54% of the total proteins of egg white. This abundant protein has been widely studied by immunologists who have defined the immunogenic epitopes of the OVA antigen for many strains of mice. Ovalbumin is purified using chromatography (≥98% purity).
Application: detection (Western blot, ELISA)

EndoFit™ Ovalbumin - LPS-free, sterile OVA

Commercially available ovalbumin is often contaminated with endotoxins that influence *in vivo* results. EndoFit™ Ovalbumin is a high purity (≥ 98%), LPS-free OVA (endotoxin level < 1 EU/mg). Furthermore, EndoFit™ Ovalbumin is guaranteed sterile.
Application: *in vivo* use

OVA PEPTIDES

OVA 257-264

OVA 257-264 is a class I (Kb)-restricted peptide epitope of ovalbumin, presented by the class I major histocompatibility complex molecule, H-2Kb[1]. The OVA 257-264 peptide has been shown to induce a strong CD8+ cytolytic T cell response[2]. OVA 257-264 can be used to trigger T cell activation.
Sequence: SIINFEKL (MW 963.2)
Application: detection (ELISPOT)

OVA 323-339

OVA 323-339 encompasses an allergenic and antigenic epitope of the ovalbumin protein[3]. OVA 323-339 peptide binds to I-A(d) MHC class II protein[4]. OVA 323-339 has been used extensively to study the nature of class II MHC-peptide binding and T-cell activation[5, 6]. This peptide is often used for studies of immunological allergic responses.
Sequence: ISQAVHAAHAEINEAGR (MW 1773.9)
Application: detection (ELISPOT)

OVA PLASMID

pCpGfree-OVA

pCpGfree-OVA is a plasmid completely devoid of CpG dinucleotides. It expresses a synthetic OVA gene, a CpG-free allele of the OVA gene constructed by chemical synthesis. It is designed for DNA immunization experiments in animal models. This plasmid possesses dual functions: it can be used as a DNA vaccine carrier for antigen presentation, and as an immunostimulatory adjuvant[7].

Contents

Products are provided lyophilized and shipped at room temperature. Store at 4 °C or -20 °C according to the product label.

PRODUCT	QUANTITY	CAT. CODE
EndoFit™ Ovalbumin	10 mg	vac-efova
Ovalbumin	1 g	vac-stova
OVA 257-264	1 mg	vac-sin
OVA 323-339	1 mg	vac-isq
pCpGfree-OVA	20 µg	pcpgf-ova

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PRODUCT INFORMATION

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If the purchaser is not willing to accept the limitations of this limited use statement, InvivoGen is willing to accept return of the product with a full refund. For information on purchasing a license to this product for purposes other than research, contact InvivoGen at business@invivogen.com.

Returns

All product returns must have prior authorization and approval. Contact our customer service or technical service department for a return authorization number. Return authorization numbers are valid for 30 days from issuance. Items that are authorized for return must arrive at InvivoGen Corporation in resalable condition to be eligible for a product credit. A restocking charge of 20% will be charged on returns that are through no error or fault of InvivoGen Corporation and shipping charges will not be credited. Products may not be returned for credit after 20 days of receipt of material.

Trademarks of InvivoGen

A549-Dual™ - B16-Blue™ - EndoFit™ - Fast-Media® - Fungin™ - HCT116-Dual™ - HEK-Blue™ - HygroGold™ - InvivoFit™ - InvivoGen® - J774-Dual™ - Jurkat-Dual™ - LipoGen™ - LyoVec™ - Normocin™ - Normocure™ - Plasmocin™ - Plasmocure™ - PlasmoTest™ - Primocin™ - QUANTI-Blue™ - QUANTI-Luc™ - RAW-Blue™ - RAW-Lucia™ - THP1-Blue™ - THP1-Dual™ - THP1-Lucia™ - VacciGrade™ - Zeocin™

PRODUCT INFORMATION

PRODUCT (QUANTITY)	CAT. CODE	PAGE
17-AAG (5 mg)	ant-agl-5	123
17-AAG (25 mg)	ant-agl-25	123
17-DMAG (5 mg)	ant-dgl-5	123
17-DMAG (25 mg)	ant-dgl-25	123
2-Aminopurine (250 mg)	tlrl-apr	117
2'2'-cGAMP (500 µg)	tlrl-nacga22	102
2'2'-cGAMP (1 mg)	tlrl-nacga22-1	102
2'3'-c-di-AMP (500 µg)	tlrl-nacda23	103
2'3'-c-di-AM(PS)2 (Rp,Rp) (100 µg)	tlrl-nacda2r-01	103
2'3'-c-di-AM(PS)2 (Rp,Rp) (500 µg)	tlrl-nacda2r	103
2'3'-c-di-GMP (500 µg)	tlrl-nacdg23	103
2'3'-cGAMP (500 µg)	tlrl-nacga23	102
2'3'-cGAMP (1 mg)	tlrl-nacga23-1	102
2'3'-cGAMP (5 mg)	tlrl-nacga23-5	102
2'3'-cGAMP Control (1 mg)	tlrl-nagpap	102
2'3'-cGAMP VacciGrade™ (1 mg)	vac-cga23	142
2'3'-cGAM(PS)2 (Rp/Sp) (250 µg)	tlrl-nacga2srs	102
293/hMD2-CD14 (3-7 x 10^6 cells)	293-hmd2cd14	website
293/hNOD1 (3-7 x 10^6 cells)	293-hnod1	website
293/hNOD2 (3-7 x 10^6 cells)	293-hnod2	website
293/hTLR1-HA (3-7 x 10^6 cells)	293-htlr1ha	website
293/hTLR2 (3-7 x 10^6 cells)	293-htlr2	website
293/hTLR2-CD14 (3-7 x 10^6 cells)	293-htlr2cd14	website
293/hTLR2-HA (3-7 x 10^6 cells)	293-htlr2ha	website
293/hTLR2/6 (3-7 x 10^6 cells)	293-htlr2/6	website
293/hTLR3 (3-7 x 10^6 cells)	293-htlr3	website
293/hTLR3-HA (3-7 x 10^6 cells)	293-htlr3ha	website
293/hTLR4A (3-7 x 10^6 cells)	293-htlr4a	website
293/hTLR4A-MD2-CD14 (3-7 x 10^6 cells)	293-htlr4md2cd14	website
293/hTLR4-HA (3-7 x 10^6 cells)	293-htlr4ha	website
293/hTLR5 (3-7 x 10^6 cells)	293-htlr5	website
293/hTLR5-CD14 (3-7 x 10^6 cells)	293-htlr5cd14	website
293/hTLR5-HA (3-7 x 10^6 cells)	293-htlr5ha	website
293/hTLR6-HA (3-7 x 10^6 cells)	293-htlr6ha	website
293/hTLR10-HA (3-7 x 10^6 cells)	293-htlr10ha	website
293/LacZ (3-7 x 10^6 cells)	293-lacz	website
293/mNOD1 (3-7 x 10^6 cells)	293-mnod1	website
293/mNOD2 (3-7 x 10^6 cells)	293-mnod2	website
293/mTLR1 (3-7 x 10^6 cells)	293-mtlr1	website
293/mTLR1/2 (3-7 x 10^6 cells)	293-mtlr1/2	website
293/mTLR2 (3-7 x 10^6 cells)	293-mtlr2	website
293/mTLR2/6 (3-7 x 10^6 cells)	293-mtlr2/6	website
293/mTLR3 (3-7 x 10^6 cells)	293-mtlr3	website
293/mTLR4 (3-7 x 10^6 cells)	293-mtlr4	website
293/mTLR4-MD2-CD14 (3-7 x 10^6 cells)	293-mtlr4md2cd14	website
293/mTLR5 (3-7 x 10^6 cells)	293-mtlr5	website
293/mTLR6 (3-7 x 10^6 cells)	293-mtlr6	website
293/mTLR9 (3-7 x 10^6 cells)	293-mtlr9	website
293/null (3-7 x 10^6 cells)	293-null	website
293XL/hTLR7 (3-7 x 10^6 cells)	293xl-htlr7	website
293XL/hTLR7-HA (3-7 x 10^6 cells)	293xl-htlr7ha	website
293XL/hTLR8 (3-7 x 10^6 cells)	293xl-htlr8	website

PRODUCT (QUANTITY)	CAT. CODE	PAGE
293XL/hTLR8-HA (3-7 x 10^6 cells)	293xl-htlr8ha	website
293XL/hTLR9 (3-7 x 10^6 cells)	293xl-htlr9	website
293XL/hTLR9-HA (5-7 x 10^6 cells)	293xl-htlr9ha	website
293XL/mTLR7 (5-7 x 10^6 cells)	293xl-mtlr7	website
293XL/null (5-7 x 10^6 cells)	293xl-null	website
3'3'-cGAMP (500 µg)	tlrl-nacga	103
3'3'-cGAMP (1 mg)	tlrl-nacga-1	103
3'3'-cGAMP Control (1 mg)	tlrl-napgpa	103
3'3'-cGAMP Fluorinated (100 µg)	tlrl-nacgaf	103
3'3'-cGAMP VacciGrade™ (1 mg)	vac-nacga	142
3-Methyladenine (50 mg)	tlrl-3ma	120
5-Aza-2'-deoxycytidine (10 mg)	met-adc-1	122
5-Aza-2'-deoxycytidine (50 mg)	met-adc-5	122
5-Fluorocytosine (250 mg)	sud-5fc	122
5-Fluorouracil (250 mg)	sud-5fu	122
5'ppp-dsRNA (25 µg)	tlrl-3prna	99
5'ppp-dsRNA (100 µg)	tlrl-3prna-100	99
5'ppp-dsRNA / LyoVec™ (25 µg)	tlrl-3prnalv	99
5'ppp-dsRNA / LyoVec™ (100 µg)	tlrl-3prnalv-100	99
5'ppp-dsRNA Control (25 µg)	tlrl-3prnac	99
5'ppp-dsRNA Control (100 µg)	tlrl-3prnac-100	99
5'ppp-dsRNA Control / LyoVec™ (25 µg)	tlrl-3prnaclv	99
5'ppp-dsRNA Control / LyoVec™ (100 µg)	tlrl-3prnaclv-100	99
A549-Dual ™ (3-7 x 10^6 cells)	a549d-nfis	64
A-769662 (10 mg)	inh-a769	120
Ac-YVAD-cmk (5 mg)	inh-yvad	117
AddaVax™ (10 ml)	vac-adx-10	142
AG490 (10 mg)	tlrl-ag4	114
Alhydrogel® 2% (250 ml)	vac-alu-250	142
Alum Crystals (1 g)	tlrl-alk	97
Amlexanox (50 mg)	inh-amx	117
Anti-Flagellin FliC (100 µg)	mabg-flic	132
Anti-hCD14-IgA (100 µg)	maba-hcd14	132
Anti-hCD20-hIgA1 (100 µg)	hcd20-mab6	131
Anti-hCD20-hIgA2 (100 µg)	hcd20-mab7	131
Anti-hCD20-hIgE (100 µg)	hcd20-mab8	131
Anti-hCD20-hIgG1 (100 µg)	hcd20-mab1	131
Anti-hCD20-hIgG1fut (100 µg)	hcd20-mab13	131
Anti-hCD20-hIgG1NQ (100 µg)	hcd20-mab12	131
Anti-hCD20-hIgG2 (100 µg)	hcd20-mab2	131
Anti-hCD20-hIgG3 (100 µg)	hcd20-mab3	131
Anti-hCD20-hIgG4 (100 µg)	hcd20-mab4	131
Anti-hCD20-hIgG4 (S228P) (100 µg)	hcd20-mab14	131
Anti-hCD20-hIgM (100 µg)	hcd20-mab5	131
Anti-hCD20-mIgA (100 µg)	hcd20-mab11	131
Anti-hCD20-mIgG1 (100 µg)	hcd20-mab9	131
Anti-hCD20-mIgG2a (100 µg)	hcd20-mab10	131
Anti-hCD40L-IgG (3 x 100 µg)	mabg-h40l-3	132
Anti-hCTLA4-hIgA2 (100 µg)	hctla4-mab7	130
Anti-hCTLA4-hIgG1 (100 µg)	hctla4-mab1	130
Anti-hCTLA4-hIgG4 (S228P) (100 µg)	hctla4-mab14	130
Anti-hDectin-1-IgG (100 µg)	mabg-hdect	132

PRODUCT INFORMATION

PRODUCT (QUANTITY)	CAT. CODE	PAGE
Anti-hDectin-1-IgG-Biotin (100 µg)	bmab-hdect	132
Anti-hDectin-2-IgG (100 µg)	mabg-hdect2	132
Anti-hDectin-2-IgG-Biotin (100 µg)	bmab-hdect2	132
Anti-hDectin-3-IgG (100 µg)	mabg-hdect3	132
Anti-hDectin-3-IgG-Biotin (100 µg)	bmab-hdect3	132
Anti-hEGFR-hIgA2 (100 µg)	hegfr-mab7	130
Anti-hEGFR-hIgG1 (100 µg)	hegfr-mab1	130
Anti-hEGFR-hIgG4 (S228P) (100 µg)	hegfr-mab14	130
Anti-HER2-Tra-hIgA2 (100 µg)	her2tra-mab7	130
Anti-HER2-Tra-hIgG1 (100 µg)	her2tra-mab1	130
Anti-HER2-Tra-hIgG1 (S228P) (100 µg)	her2tra-mab14	130
Anti-hIFN-α-IgA (3 x 100 µg)	maba-hifna-3	132
Anti-hIFN-α7-IgG (100 µg)	mabg-hifna7	132
Anti-hIFN-α8-IgG (100 µg)	mabg-hifna8	132
Anti-hIFN-β-IgG (3 x 100 µg)	mabg-hifnb-3	132
Anti-hIFN-γ-IgA (3 x 100 µg)	maba-hifng-3	132
Anti-hIL-1α-IgG (3 x 100 µg)	mabg-hil1a-3	132
Anti-hIL-1β-IgG (3 x 100 µg)	mabg-hil1b-3	132
Anti-hIL-4-IgA (3 x 100 µg)	maba-hil4-3	132
Anti-hIL-6-IgG (3 x 100 µg)	mabg-hil6-3	132
Anti-hIL-13-IgG (3 x 100 µg)	mabg-hil13-3	132
Anti-hIL-17a-IgG (3 x 100 µg)	mabg-hil17a-3	132
Anti-hIL-18-IgA (3 x 100 µg)	maba-hil18-3	132
Anti-hIL-28a-IgG (3 x 100 µg)	mabg-hil28a-3	132
Anti-hIL-28b-IgG (3 x 100 µg)	mabg-hil28b-3	132
Anti-hIL-29-IgG (3 x 100 µg)	mabg-hil29-3	133
Anti-hIL-33-IgG (3 x 100 µg)	mabg-hil33-3	133
Anti-HLA Class I Control (100 µg)	hla-c1	website
Anti-HLA Class I Control Kit (75 tests)	kc-hla-c1	website
Anti-HLA Class I hIgG2 (100 µg)	hla-c1g2	website
Anti-HLA Class I hIgG4 (100 µg)	hla-c1g4	website
Anti-HLA Class II Control (100 µg)	hla-c2	website
Anti-HLA Class II Control Kit (75 tests)	kc-hla-c2	website
Anti-HLA Class II hIgG2 (100 µg)	hla-c2g2	website
Anti-HLA Class II hIgG4 (100 µg)	hla-c2g4	website
Anti-hMincle-Biotin (100 µg)	bmab-hmcl	132
Anti-hMincle-IgG (100 µg)	mabg-hmcl	132
Anti-hPD1-Ni-hIgA2 (100 µg)	hpd1ni-mab7	130
Anti-hPD1-Ni-hIgG1 (100 µg)	hpd1ni-mab1	130
Anti-hPD1-Ni-hIgG4 (S228P) (100 µg)	hpd1ni-mab14	130
Anti-hPD-L1-hIgG1 (100 µg)	hpdl1-mab1	website
Anti-hPD-L1-hIgG1 (N298A) (100 µg)	hpdl1-mab12	website
Anti-hPD-L1-mIgG1 (100 µg)	hpdl1-mab9	131
Anti-hPD-L1-mIgG1 InvivoFit™ (5 x 1 mg)	hpdl1-mab9-5	131
Anti-hTGFβ-IgA (3 x 100 µg)	maba-htgfb-3	133
Anti-hTLR1-IgG (100 µg)	mabg-htlr1	133
Anti-hTLR2-IgA (100 µg)	maba2-htlr2	133
Anti-hTLR3-IgA (100 µg)	maba-htlr3	133
Anti-hTLR4-IgG (100 µg)	mabg-htlr4	133
Anti-hTLR5-IgA (100 µg)	maba2-htlr5	133
Anti-hTLR6-IgG (100 µg)	mabg-htlr6	133
Anti-hTNF-α-hIgA1 (100 µg)	htnfa-mab6	131

PRODUCT (QUANTITY)	CAT. CODE	PAGE
Anti-hTNF-α-hIgA2 (100 µg)	htnfa-mab7	131
Anti-hTNF-α-hIgE (100 µg)	htnfa-mab8	131
Anti-hTNF-α-hIgG1 (100 µg)	htnfa-mab1	131
Anti-hTNF-α-hIgG2 (100 µg)	htnfa-mab2	131
Anti-hTNF-α-hIgG3 (100 µg)	htnfa-mab3	131
Anti-hTNF-α-hIgG4 (100 µg)	htnfa-mab4	131
Anti-hTNF-α-hIgM (100 µg)	htnfa-mab5	131
Anti-hTNF-α-mIgA (100 µg)	htnfa-mab11	131
Anti-hTNF-α-mIgG1 (100 µg)	htnfa-mab9	131
Anti-hTNF-α-mIgG2a (100 µg)	htnfa-mab10	131
Anti-hVEGF-hIgA2 (100 µg)	hvegf-mab7	130
Anti-hVEGF-hIgG1 (100 µg)	hvegf-mab1	130
Anti-hVEGF-hIgG4 (S228P) (100 µg)	hvegf-mab14	130
Anti-mDectin-1-IgG (100 µg)	mabg-mdect	132
Anti-mDectin-1-IgG-Biotin (100 µg)	bmab-mdect	132
Anti-mDectin-1a-IgG (100 µg)	mabg-mdecta	132
Anti-mDectin-1a-IgG-Biotin (100 µg)	bmab-mdecta	132
Anti-mIL-1α-IgG (100 µg)	mabg-mil1a	132
Anti-mIL-1α-IgG (5 x 100 µg)	mabg-mil1a-5	website
Anti-mIL-1β-IgG (100 µg)	mabg-mil1b	132
Anti-mIL-1β-IgG (5 x 100 µg)	mabg-mil1b-5	website
Anti-mIL-6-IgG (100 µg)	mabg-mil6	132
Anti-mIL-13-IgG (100 µg)	mabg-mil13	132
Anti-mIL-13-IgG (5 x 100 µg)	mabg-mil13-5	website
Anti-mIL-28b-IgG (100 µg)	mabg-mil28b	132
Anti-mIL-28b-IgG (5 x 100 µg)	mabg-mil28b-5	website
Anti-mMincle-Biotin (100 µg)	bmab-mmcl	132
Anti-mMincle-IgG (100 µg)	mabg-mmcl	132
Anti-mTLR2-IgG (100 µg)	mabg-mtlr2	133
Anti-mTLR5-IgG (100 µg)	mabg-mtlr5	133
Anti-β-Gal-hIgA2 (200 µg)	bgal-mab7	website
Anti-β-Gal-hIgG1 (200 µg)	bgal-mab1	website
Anti-β-Gal-hIgG2 (200 µg)	bgal-mab2	website
Anti-β-Gal-hIgG3 (200 µg)	bgal-mab3	website
Anti-β-Gal-hIgG4 (200 µg)	bgal-mab4	website
Anti-β-Gal-hIgG1 (S228P) (200 µg)	bgal-mab14	website
ATP (1 g)	tlrl-atp	97
AZD1480 (5 mg)	inh-ad14	114
AZD6244 (50 mg)	inh-ad62	115
B16-Blue™ IFN-α/β Cells (3-7 x 10^6 cells)	bb-ifnt1	49
B16-Blue™ ISG Cells (3-7 x 10^6 cells)	bb-ifnabg	58
B16-Blue™ ISG-KO-STING Cells (3-7 x 10^6 cells)	bb-kostg	67
Bafilomycin A1 (10 µg)	tlrl-baf1	117
Bay11-7082 (10 mg)	tlrl-b82	115
Beta-glucan peptide (50 mg)	tlrl-bgp	99
BEZ235 (50 mg)	inh-bez2	120
Blasticidin (100 mg)	ant-bl-1	13
Blasticidin (500 mg)	ant-bl-5	13
Blasticidin (500 mg, bottle)	ant-bl-5b	13
Blasticidin (1 g powder)	ant-bl-10p	13
BX795 (5 mg)	tlrl-bx7	117
C12-iE-DAP (1 mg)	tlrl-c12dap	96

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PRODUCT (QUANTITY)	CAT. CODE	PAGE
c-di-AMP (1 mg)	tlrl-nacda	103
c-di-AMP Control (1 mg)	tlrl-napapa	103
c-di-AMP Fluorinated (100 µg)	tlrl-nacdaf	103
c-di-AMP VacciGrade™ (1 mg)	vac-nacda	142
c-di-GMP (1 mg)	tlrl-nacdg	103
c-di-GMP Control (1 mg)	tlrl-napgpg	103
c-di-GMP Fluorinated (100 µg)	tlrl-nacdgf	103
c-di-GMP VacciGrade™ (1 mg)	vac-nacdg	142
c-di-IMP (1 mg)	tlrl-nacdi	103
Celastrol (1 mg)	ant-cls	115
CFA (10 ml)	vac-cfa-10	142
CFA (6 x 10 ml)	vac-cfa-60	142
ChemiComp GT115 (5 x 0.1 ml)	gt115-11	36
ChemiComp GT115 (5 x 0.2 ml)	gt115-21	36
ChemiComp GT116 (5 x 0.1 ml)	gt116-11	36
ChemiComp GT116 (5 x 0.2 ml)	gt116-21	36
Chitosan Ultrapure (100 mg)	tlrl-cht	98
Chitosan VacciGrade™ (100 mg)	vac-cht	143
Chloroquine (250 mg)	tlrl-chq	117
CL075 (500 µg)	tlrl-c75	92
CL075 (5 mg)	tlrl-c75-5	92
CL097 (500 µg)	tlrl-c97	92
CL097 (5 mg)	tlrl-c97-5	92
CL264 (500 µg)	tlrl-c264e	92
CL264 (5 mg)	tlrl-c264e-5	92
CL264 FITC (100 µg)	tlrl-fc264	92
CL264 Rhodamine (100 µg)	tlrl-rc264	92
CL307 (500 µg)	tlrl-c307	92
CL347 - AdiFectin™ (500 µg)	tlrl-c347	104
CL401 (500 µg)	tlrl-c401	104
CL401 VacciGrade™ (1 mg)	vac-c401	143
CL413 - Adilipoline™ (500 µg)	tlrl-c413	104
CL413 VacciGrade™ (1 mg)	vac-c413	143
CL419 (500 µg)	tlrl-c419	105
CL429 (5 mg)	tlrl-c429	97
CL429 VacciGrade™ (5 mg)	vac-c429	143
CL531 (500 µg)	tlrl-c531	104
CL553 - PamadiFectin (500 µg)	tlrl-c553	105
CL572 (500 µg)	tlrl-c572	104
CLI-095 (1 mg)	tlrl-cli95	115
Compound Dose Response	tlrl-test-2	108
Compound Profiling	tlrl-test-1	108
Concanavalin A (100 mg)	inh-cona	website
CP-690550 (5 mg)	tlrl-cp69	114
CPPD crystals (5 mg)	tlrl-cppd	98
Curdlan AL (1 g)	tlrl-cura	99
Cyclosporin A (100 mg)	tlrl-cyca	120
CYT387 (10 mg)	inh-cy87	114
Dexamethasone (100 mg)	tlrl-dex	115
DMXAA (5 mg)	tlrl-dmx	103
dsDNA-EC (200 µg)	tlrl-ecdna	102
***E. coli* ssDNA / LyoVec™** (200 µg)	tlrl-ssec	94

PRODUCT (QUANTITY)	CAT. CODE	PAGE
EndoFit™ Ovalbumin (10 mg)	vac-pova	145
EndoFit™ Ovalbumin (100 mg)	vac-pova-100	website
Everolimus (5 mg)	tlrl-eve	120
Fast-Media® Amp Agar (30 pouches)	fas-am-s	37
Fast-Media® Amp Agar (500 pouches)	fas-am-s500	37
Fast-Media® Amp LB (30 pouches)	fas-am-b	37
Fast-Media® Amp LB (500 pouches)	fas-am-b500	37
Fast-Media® Amp XGal Agar (20 pouches)	fas-am-x	37
Fast-Media® Base Agar (30 pouches)	fas-s	37
Fast-Media® Base Agar (500 pouches)	fas-s500	37
Fast-Media® Base LB (30 pouches)	fas-b	37
Fast-Media® Base LB (500 pouches)	fas-b500	37
Fast-Media® Blas Agar (20 pouches)	fas-bl-s	37
Fast-Media® Blas TB (20 pouches)	fas-bl-l	37
Fast-Media® Kan Agar (30 pouches)	fas-kn-s	37
Fast-Media® Kan Agar (500 pouches)	fas-kn-s500	37
Fast-Media® Kan LB (30 pouches)	fas-kn-b	37
Fast-Media® Kan LB (500 pouches)	fas-kn-b500	37
Fast-Media® Zeo Agar (20 pouches)	fas-zn-s	37
Fast-Media® Zeo TB (20 pouches)	fas-zn-l	37
Fast-Media® Zeo X-Gal Agar (20 pouches)	fas-zn-x	37
Fc-hDectin-1a (50 µg)	fc-hdec1a	138
Fc-mDectin-1a (50 µg)	fc-mdec1a	138
FITC-Geldanamycin (1 mg)	ant-fgl-1	123
FITC-Geldanamycin (5 mg)	ant-fgl-5	123
FK506 (10 mg)	tlrl-fk5	120
FLA-BS (100 µg)	tlrl-bsfla	91
FLA-BS Ultrapure (50 µg)	tlrl-pbsfla	91
FLA-PA Ultrapure (50 µg)	tlrl-pafla	91
Flagellin FliC VacciGrade™ (50 µg)	vac-fla	143
FLA-ST (100 µg)	tlrl-stfla	91
FLA-ST Ultrapure (10 µg)	tlrl-epstfla	91
FLA-ST Ultrapure (50 µg)	tlrl-epstfla-5	91
FLA-ST recombinant (10 µg)	tlrl-flic-10	92
FLA-ST recombinant (50 µg)	tlrl-flic-50	92
FSL-1 (100 µg)	tlrl-fsl	88
Fungin™ (75 mg)	ant-fn-1	11
Fungin™ (200 mg)	ant-fn-2	11
Furfurman (10 mg)	tlrl-ffm	101
G-ODN (200 µg)	tlrl-godn	95
G-ODN control (200 µg)	tlrl-godnc	95
G418 (1 g)	ant-gn-1	13
G418 (5 g)	ant-gn-5	13
Ganciclovir (250 mg)	sud-gcv	122
Gardiquimod (500 µg)	tlrl-gdqs	92
Gardiquimod (5 mg)	tlrl-gdq-5	92
Gardiquimod VacciGrade™ (5 mg)	vac-gdq	143
Gefitinib (10 mg)	tlrl-gef	117
Geldanamycin (5 mg)	ant-gl-5	123
Geldanamycin (25 mg)	ant-gl-25	123
Glybenclamide (1 g)	tlrl-gly	118
H-89 (5 mg)	tlrl-h89	118

PRODUCT INFORMATION

PRODUCT (QUANTITY)	CAT. CODE	PAGE
HCT116-Dual™ Cells (3-7 x 10^6 cells)	hctd-nfis	65
HEK-Blue™ CD40L Cells (3-7 x 10^6 cells)	hkb-cd40	57
HEK-Blue™ CLR Selection (10 x 1 ml)	hb-csm	45
HEK-Blue™ Detection (5 pouches)	hb-det2	16
HEK-Blue™ Detection (10 pouches)	hb-det3	16
HEK-Blue™ Endotoxin Standard (10 x 50 EU)	rep-hbes-10	9
HEK-Blue™ hDectin-1b Cells (3-7 x 10^6 cells)	hkb-hdect1b	45
HEK-Blue™ hNOD1 Cells (3-7 x 10^6 cells)	hkb-hnod1	44
HEK-Blue™ hNOD2 Cells (3-7 x 10^6 cells)	hkb-hnod2	44
HEK-Blue™ hTLR2 Cells (3-7 x 10^6 cells)	hkb-htlr2	43
HEK-Blue™ hTLR3 Cells (3-7 x 10^6 cells)	hkb-htlr3	43
HEK-Blue™ hTLR4 Cells (3-7 x 10^6 cells)	hkb-htlr4	43
HEK-Blue™ hTLR5 Cells (3-7 x 10^6 cells)	hkb-htlr5	43
HEK-Blue™ hTLR7 Cells (3-7 x 10^6 cells)	hkb-htlr7	43
HEK-Blue™ hTLR8 Cells (3-7 x 10^6 cells)	hkb-htlr8	43
HEK-Blue™ hTLR9 Cells (3-7 x 10^6 cells)	hkb-htlr9	43
HEK-Blue™ IFN-α/β Cells (3-7 x 10^6 cells)	hkb-ifnab	48
HEK-Blue™ IFN-γ Cells (3-7 x 10^6 cells)	hkb-ifng	49
HEK-Blue™ IL-1β Cells (3-7 x 10^6 cells)	hkb-il1b	50
HEK-Blue™ IL-1R Cells (3-7 x 10^6 cells)	hkb-il1r	51
HEK-Blue™ IL-4/IL-13 Cells (3-7 x 10^6 cells)	hkb-il4il13	54
HEK-Blue™ IL-6 Cells (3-7 x 10^6 cells)	hkb-hil6	55
HEK-Blue™ IL-12 Cells (3-7 x 10^6 cells)	hkb-il12	55
HEK-Blue™ IL-17 Cells (3-7 x 10^6 cells)	hkb-il17	53
HEK-Blue™ IL-18 Cells (3-7 x 10^6 cells)	hkb-hmil18	52
HEK-Blue™ IL-33 Cells (3-7 x 10^6 cells)	hkb-hil33	52
HEK-Blue™ ISG Cells (3-7 x 10^6 cells)	hkb-isg	59
HEK-Blue™ ISG-KO-STING Cells (3-7 x 10^6 cells)	hkb-kostg	68
HEK-Blue™ LPS Detection Kit 2	rep-lps2	9
HEK-Blue™ mDectin-2 Cells (3-7 x 10^6 cells)	hkb-mdect2	45
HEK-Blue™ mMincle Cells (3-7 x 10^6 cells)	hkb-mmcl	45
HEK-Blue™ mNOD1 Cells (3-7 x 10^6 cells)	hkb-mnod1	44
HEK-Blue™ mNOD2 Cells (3-7 x 10^6 cells)	hkb-mnod2	44
HEK-Blue™ mTLR2 Cells (3-7 x 10^6 cells)	hkb-mtlr2	43
HEK-Blue™ mTLR3 Cells (3-7 x 10^6 cells)	hkb-mtlr3	43
HEK-Blue™ mTLR4 Cells (3-7 x 10^6 cells)	hkb-mtlr4	43
HEK-Blue™ mTLR5 Cells (3-7 x 10^6 cells)	hkb-mtlr5	43
HEK-Blue™ mTLR7 Cells (3-7 x 10^6 cells)	hkb-mtlr7	43
HEK-Blue™ mTLR8 Cells (3-7 x 10^6 cells)	hkb-mtlr8	43
HEK-Blue™ mTLR9 Cells (3-7 x 10^6 cells)	hkb-mtlr9	43
HEK-Blue™ mTLR13 Cells (3-7 x 10^6 cells)	hkb-mtlr13	43
HEK-Blue™ Null1 Cells (3-7 x 10^6 cells)	hkb-null1	43
HEK-Blue™ Null1-k Cells (3-7 x 10^6 cells)	hkb-null1k	43
HEK-Blue™ Null1-v Cells (3-7 x 10^6 cells)	hkb-null1v	43
HEK-Blue™ Null2 Cells (3-7 x 10^6 cells)	hkb-null2	43
HEK-Blue™ Null2-k Cells (3-7 x 10^6 cells)	hkb-null2k	43
HEK-Blue™ Selection (10 x 1 ml)	hb-sel	8
HEK-Blue™ TNF-α Cells (3-7 x 10^6 cells)	hkb-tnfdmyd	56
Hemozoin (5 mg)	tlrl-hz	98
HKCA (10^9 cells) *(C. albicans)*	tlrl-hkca	100
HKEB (10^{10} cells) *(E. coli 0111:B4)*	tlrl-hkeb2	88
HKLM (10^{10} cells) *(L. monocytogenes)*	tlrl-hklm	88

PRODUCT (QUANTITY)	CAT. CODE	PAGE
HKLR (10^{10} cells) *(L. rhamnosus)*	tlrl-hklr	88
HKMT (10 mg) *(M. tuberculosis)*	tlrl-hkmt-1	88
HKMT (50 mg) *(M. tuberculosis)*	tlrl-hkmt-5	88
HKPA (10^{10} cells) *(P. aeruginosa)*	tlrl-hkpa	88
HKPG (10^{10} cells) *(P. gingivalis)*	tlrl-hkpg	88
HKSA (10^{10} cells) *(S. aureus)*	tlrl-hksa	88
HKSC (10^9 cells) *(S. cerevisiae)*	tlrl-hksc	100
HKSE (10^{10} cells) *(S. epidermidis)*	tlrl-hkse	88
HKSP (10^{10} cells) *(S. pneumoniae)*	tlrl-hksp	88
HKST (10^{10} cells) *(S. typhimurium)*	tlrl-hkst	88
HSV-60 Naked (200 µg)	tlrl-hsv60n	102
HSV-60c Naked (control) (200 µg)	tlrl-hsv60cn	102
HSV-60/LyoVec (100 µg)	tlrl-hsv60c	102
HSV-60c/LyoVec (control) (100 µg)	tlrl-hsv60cc	102
hTLR5-Fc (50 µg)	fc-htlr5	138
Human IgA2 Control (100 µg)	maba2-ctrl	134
Human TLR1-9 Agonist Kit (10 ligands)	tlrl-kit1hw	106
Human TLR3/7/8/9 Agonist Kit (14 ligands)	tlrl-kit3hw3	106
Human TLR9 Agonist Kit (6 ligands)	tlrl-kit9h	106
Human/mouse NOD1/2 Agonist Kit (10 ligands)	tlrl-nodkit2	107
Human/mouse TLR2 Agonist Kit (7 ligands)	tlrl-kit2hm	106
Hygromycin B Gold (1 g)	ant-hg-1	13
Hygromycin B Gold (5 g)	ant-hg-5	13
iE-DAP (5 mg)	tlrl-dap	96
iE-Lys (5 mg)	tlrl-lys	96
IFA (10 ml)	vac-ifa-10	142
IFA (6 x 10 ml)	vac-ifa-60	142
Imiquimod (R837) (500 µg)	tlrl-imqs	92
Imiquimod (R837) (5 mg)	tlrl-imq	92
Imiquimod VacciGrade™ (5 mg)	vac-imq	143
Ionomycin (1 mg)	inh-ion	website
ISD Naked (200 µg)	tlrl-isdn	102
ISD / LyoVec™ (100 µg)	tlrl-isdc	102
ISD Control Naked (200 µg)	tlrl-isdcn	102
ISD Control / LyoVec™ (100 µg)	tlrl-isdcc	102
Isoliquiritigenin (10 mg)	inh-ilg	118
J774-Dual™ Cells (3-7 x 10^6 cells)	j774d-nfis	65
Jurkat-Dual™ Cells (3-7 x 10^6 cells)	jktd-isnf	66
Jurkat-Lucia™ NFAT Cells (3-7 x 10^6 cells)	jktl-nfat	61
L18-MDP (1 mg)	tlrl-lmdp	97
LAM-MS (500 µg)	tlrl-lams	88
Laminarin (100 mg)	tlrl-lam	100
Leptomycin B (5 µg)	tlrl-lep	122
LL-37 (1 mg)	tlrl-l37	118
LM-MS (100 µg)	tlrl-lmms1	88
Loxoribine (50 mg)	tlrl-lox	92
LPS-B5 (5 mg)	tlrl-b5lps	90
LPS-B5 Ultrapure (5 mg)	tlrl-pb5lps	90

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PRODUCT (QUANTITY)	CAT. CODE	PAGE
LPS-EB (5 mg)	tlrl-eblps	90
LPS-EB Biotin (500 µg)	tlrl-bblps	90
LPS-EB Ultrapure (5 x 10^6 EU)	tlrl-3pelps	90
LPS-EB VacciGrade™ (5 x 10^6 EU)	vac-3pelps	143
LPS-EK (5 mg)	tlrl-eklps	90
LPS-EK Ultrapure (1 mg)	tlrl-peklps	90
LPS-PG (1 mg)	tlrl-pglps	88
LPS-PG Ultrapure (1 mg)	tlrl-ppglps	90
LPS-RS (5 mg)	tlrl-rslps	91
LPS-RS Ultrapure (1 mg)	tlrl-prslps	91
LPS-SM Ultrapure (5 mg)	tlrl-smlps	91
LTA-BS (5 mg)	tlrl-lta	88
LTA-SA (5 mg)	tlrl-slta	88
LTA-SA Purified (5 mg)	tlrl-pslta	88
LumiKine™ hIFN-α (5 plates)	lumi-hifna	135
LumiKine™ hIFN-β (5 plates)	lumi-hifnb	135
LumiKine™ mIFN-α (5 plates)	lumi-mifna	135
LumiKine™ mIFN-β (5 plates)	lumi-mifnb	135
LumiKine™ Xpress hIFN-α (5 plates)	luex-hifna	135
LumiKine™ Xpress hIFN-β (5 plates)	luex-hifnb	135
LumiKine™ Xpress mIFN-α (5 plates)	luex-mifna	135
LumiKine™ Xpress mIFN-β (5 plates)	luex-mifnb	135
LY294002 (5 mg)	tlrl-ly29	120
LyoVec™ (10 ml, 200 reactions)	lyec-1	15
LyoVec™ (20 ml, 400 reactions)	lyec-2	15
LyoVec™ (8 ml, 160 reactions)	lyec-12	15
LyoVec™ (18 ml, 360 reactions)	lyec-22	15
M-TriDAP (1 mg)	tlrl-mtd	96
M-TriLYS (1 mg)	tlrl-mtl	97
MAb-hDC-SIGN (100 µg)	mab-hdcsg	132
MAb-hMD2 (100 µg)	mab-hmd2	133
MAb-hMR (100 µg)	mab-hmr	133
MAb-hTLR2 (100 µg)	mab-htlr2	133
MAb-hTLR3 (100 µg)	mab-htlr3	133
MAb2-hTLR4 (100 µg)	mab2-htlr4	133
MAb-hTLR4 (100 µg)	mab-htlr4	133
MAb-hTLR4/MD2 (100 µg)	mab-htlr4md2	133
MAb-mTLR2 (100 µg)	mab-mtlr2	133
MAb-mTLR4/MD2 (100 µg)	mab-mtlr4md2	133
MAb-mTLR9 (100 µg)	mab-mtlr9	133
MDP (5 mg)	tlrl-mdp	97
MDP Biotin (500 µg)	tlrl-bmdp	97
MDP control (5 mg)	tlrl-mdpc	97
MDP FITC (500 µg)	tlrl-fmdp	97
MDP Rhodamine (500 µg)	tlrl-rmdp	97
Metformin (1 mg)	tlrl-metf	120
MG-132 (5 mg)	tlrl-mg132	115
Mouse IgG1 Control (100 µg)	mabg1-ctrlm	134
Mouse IgG2a Control (100 µg)	mabg2a-ctrlm	134
Mouse IgG2b Isotype Control (100 µg)	mabg2b-ctrlm	134
Mouse TLR1-9 Agonist Kit (9 ligands)	tlrl-kit1mw	106
Mouse TLR9 Agonist Kit (6 ligands)	tlrl-kit9m	107

PRODUCT (QUANTITY)	CAT. CODE	PAGE
MPLA-SM (1 mg)	tlrl-mpla	91
MPLA-SM VacciGrade™ (1 mg)	vac-mpla	143
MPLAs (1 mg)	tlrl-mpls	91
MPLAs VacciGrade™ (1 mg)	vac-mpls	143
MSU Crystals (5 mg)	tlrl-msu	98
Murabutide (5 mg)	tlrl-mbt	97
Murabutide Control (5 mg)	tlrl-mbtc	97
N-Glycolyl-MDP (5 mg)	tlrl-gmdp	97
N-Glycolyl-MDP VacciGrade™ (5 mg)	vac-gmdp	143
Nano-SiO2 (10 mg)	tlrl-sio	98
Nigericin (10 mg)	tlrl-nig	98
Nigericin (50 mg)	tlrl-nig-5	98
Normocin™ (500 mg)	ant-nr-1	11
Normocin™ (1 g)	ant-nr-2	11
Normocure™ (50 mg)	ant-noc	10
ODN 1585 (200 µg)	tlrl-1585	94
ODN 1585 (1 mg)	tlrl-1585-1	94
ODN 1585 (5 mg)	tlrl-1585-5	94
ODN 1585 control (200 µg)	tlrl-1585c	94
ODN 1585 control (1 mg)	tlrl-1585c-1	94
ODN 1585 control (5 mg)	tlrl-1585c-5	94
ODN 1585 VacciGrade (1 mg)	vac-1585-1	143
ODN 1668 (200 µg)	tlrl-1668	94
ODN 1668 (1 mg)	tlrl-1668-1	94
ODN 1668 (5 mg)	tlrl-1668-5	94
ODN 1668 control (200 µg)	tlrl-1668c	94
ODN 1668 control (1 mg)	tlrl-1668c-1	94
ODN 1826 (200 µg)	tlrl-1826	94
ODN 1826 (1 mg)	tlrl-1826-1	94
ODN 1826 (5 mg)	tlrl-1826-5	94
ODN 1826 Biotin (50 µg)	tlrl-1826b	94
ODN 1826 control (ODN 2138) (200 µg)	tlrl-1826c	94
ODN 1826 control (ODN 2138) (1 mg)	tlrl-1826c-1	94
ODN 1826 control (ODN 2138) (5 mg)	tlrl-1826c-5	94
ODN 1826 FITC (50 µg)	tlrl-1826f	94
ODN 1826 VacciGrade (1 mg)	vac-1826-1	143
ODN 2006 (ODN 7909) (200 µg)	tlrl-2006	94
ODN 2006 (ODN 7909) (1 mg)	tlrl-2006-1	94
ODN 2006 (ODN 7909) (5 mg)	tlrl-2006-5	94
ODN 2006 Biotin (50 µg)	tlrl-2006b	94
ODN 2006 control (ODN 2137) (200 µg)	tlrl-2006c	94
ODN 2006 control (ODN 2137) (1 mg)	tlrl-2006c-1	94
ODN 2006 control (ODN 2137) (5 mg)	tlrl-2006c-5	94
ODN 2006 FITC (50 µg)	tlrl-2006f	94
ODN 2006-G5 (200 µg)	tlrl-2006g5	94
ODN 2006-G5 (1 mg)	tlrl-2006g5-1	94
ODN 2006-G5 (5 mg)	tlrl-2006g5-5	94
ODN 2006-G5 control (200 µg)	tlrl-2006g5c	94
ODN 2006 VacciGrade (1 mg)	vac-2006-1	143
ODN 2007 (200 µg)	tlrl-2007	94
ODN 2007 (1 mg)	tlrl-2007-1	94
ODN 2007 (5 mg)	tlrl-2007-5	94

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PRODUCT (QUANTITY)	CAT. CODE	PAGE
ODN 2007 Control (200 µg)	tlrl-2007c	94
ODN 2007 Control (1 mg)	tlrl-2007c-1	94
ODN 2007 Control (5 mg)	tlrl-2007c-5	94
ODN 2088 (200 µg)	tlrl-2088	95
ODN 2088 (1 mg)	tlrl-2088-1	95
ODN 2088 (5 mg)	tlrl-2088-5	95
ODN 2088 control (200 µg)	tlrl-2088c	95
ODN 2088 control (1 mg)	tlrl-2088c-1	95
ODN 2088 control (5 mg)	tlrl-2088c-5	95
ODN 2216 (200 µg)	tlrl-2216	94
ODN 2216 (1 mg)	tlrl-2216-1	94
ODN 2216 (5 mg)	tlrl-2216-5	94
ODN 2216 Biotin (50 µg)	tlrl-2216b	94
ODN 2243 (ODN 2216 control) (200 µg)	tlrl-2243	94
ODN 2243 (ODN 2216 control) (1 mg)	tlrl-2243-1	94
ODN 2243 (ODN 2216 control) (5 mg)	tlrl-2243-5	94
ODN 2216 FITC (50 µg)	tlrl-2216f	94
ODN 2336 (200 µg)	tlrl-2336	94
ODN 2336 (1 mg)	tlrl-2336-1	94
ODN 2336 (5 mg)	tlrl-2336-5	94
ODN 2336 control (200 µg)	tlrl-2336c	94
ODN 2336 control (1 mg)	tlrl-2336c-1	94
ODN 2336 control (5 mg)	tlrl-2336c-5	94
ODN 2395 (200 µg)	tlrl-2395	94
ODN 2395 (1 mg)	tlrl-2395-1	94
ODN 2395 (5 mg)	tlrl-2395-5	94
ODN 2395 control (200 µg)	tlrl-2395c	94
ODN 2395 control (1 mg)	tlrl-2395c-1	94
ODN 2395 control (5 mg)	tlrl-2395c-5	94
ODN 2395 FITC (50 µg)	tlrl-2395f	94
ODN 2395 VacciGrade (1 mg)	vac-2395-1	143
ODN 4084-F (200 µg)	tlrl-4084	95
ODN BW006 (ODN 684) (200 µg)	tlrl-bw006	94
ODN BW007 (200 µg)	tlrl-bw007	94
ODN D-SL01 (200 µg)	tlrl-dsl01	94
ODN D-SL01 (200 µg)	tlrl-dsl01	94
ODN D-SL03 (200 µg)	tlrl-dsl03	94
ODN INH-1 (200 µg)	tlrl-inh1	95
ODN INH-18 (200 µg)	tlrl-inh18	95
ODN INH-47 (200 µg)	tlrl-inh47	95
ODN M362 (200 µg)	tlrl-m362	94
ODN M362 (1 mg)	tlrl-m362-1	94
ODN M362 (5 mg)	tlrl-m362-5	94
ODN M362 control (200 µg)	tlrl-m362c	94
ODN M362 control (1 mg)	tlrl-m362c-1	94
ODN M362 control (5 mg)	tlrl-m362c-5	94
ODN M362 FITC (50 µg)	tlrl-m362f	94
ODN TTAGGG (ODN A151) (200 µg)	tlrl-ttag151	95
ODN TTAGGG (ODN A151) (1 mg)	tlrl-ttag151-1	95
ODN TTAGGG control (200 µg)	tlrl-ttagc	95
ODN TTAGGG control (1 mg)	tlrl-ttagc-1	95
ORN02 / LyoVec™ (4 x 25 µg)	tlrl-orn2	92

PRODUCT (QUANTITY)	CAT. CODE	PAGE
ORN06 / LyoVec™ (4 x 25 µg)	tlrl-orn6	92
ORN Sa19 (200 µg)	tlrl-orn19	95
ORN Sa19 control (200 µg)	tlrl-orn19c	95
OSU 03012 (10 mg)	inh-os03	121
OVA 257-264 (1 mg)	vac-sin	145
OVA 323-339 (1 mg)	vac-isq	145
Ovalbumin (1 g)	vac-ova	145
Ovalbumin EndoFit™ (10 mg)	vac-pova	145
Ovalbumin EndoFit™ (100 mg)	vac-pova-100	145
OxPAPC (1 mg)	tlrl-oxp1	118
PAb Control (200 µg)	pab-sctr	134
PAb-hTLR1 (200 µg)	pab-hstlr1	133
PAb-hTLR2 (200 µg)	pab-hstlr2	133
PAb-hTLR4 (200 µg)	pab-hstlr4	133
PAb-hTLR5 (200 µg)	pab-hstlr5	133
PAb-hTLR6 (200 µg)	pab-hstlr6	133
Pam2CSK4 (1 mg)	tlrl-pm2s-1	88
Pam2CSK4 Biotin (50 µg)	tlrl-bpam2	88
Pam2CSK4 Rhodamine (50 µg)	tlrl-rpam2	88
Pam3CSK4 (1 mg)	tlrl-pms	88
Pam3CSK4 Biotin (50 µg)	tlrl-bpms	88
Pam3CSK4 Rhodamine (50 µg)	tlrl-rpms	88
Pam3CSK4 VacciGrade™ (1 mg)	vac-pms	143
PamadiFectin (CL553) (500 µg)	tlrl-c553	105
Parthenolide (50 mg)	inh-ptd	118
pCpGfree-basic (mSEAP) (20 µg)	pcpgf-bas	website
pCpGfree-basic-Lucia (20 µg)	pcpgf-baslc	28
pCpGfree-Lucia (20 µg)	pcpgf-lucia	website
pCpGfree-mcs (20 µg)	pcpgf-mcs	28
pCpGfree-mSEAP (20 µg)	pcpgf-mseap	website
pCpGfree-OVA (20 µg)	pcpgf-ova	28, 145
pCpGfree-promoter (mSEAP) (20 µg)	pcpgf-prom	website
pCpGfree-promoter-Lucia (20 µg)	pcpgf-promlc	28
pCpGfree-vitroBLacZ (20 µg)	pcpgvtb-lz	website
pCpGfree-vitroBmcs (20 µg)	pcpgvtb-mcsg2	website
pCpGfree-vitroHLacZ (20 µg)	pcpgvth-lz	website
pCpGfree-vitroHmcs (20 µg)	pcpgvth-mcsg2	website
pCpGfree-vitroNLacZ (20 µg)	pcpgvtn-lz	website
pCpGfree-vitro-Nmcs (20 µg)	pcpgvtn-mcsg2	website
pCpGrich-mcs (20 µg)	pcpgr-mcs	28
PD0325901 (2 mg)	inh-pd32	115
PD98059 (10 mg)	tlrl-pd98	115
pDRIVE-custom (20 µg)	p-custom	website
pDRIVE5-Lucia-<Native Prom> (20 µg)	pdrive5lc-<prom>	33
pDRIVE5-Lucia-<Composite Prom> (20 µg)	pdrive5lc-<prom>	33
pDRIVE5-SEAP-<Native Prom> (20 µg)	pdrive5s-<prom>	33
pDRIVE5-SEAP-<Composite Prom> (20 µg)	pdrive5s-<prom>	33
Pepinh-MYD (2 mg)	tlrl-pimyd	118
Pepinh-TRIF (2 mg)	tlrl-pitrif	118
Peptide M / Agarose (2 ml)	gel-pdm-2	129
Peptide M / Agarose (5 ml)	gel-pdm-5	129
Perifosine (5 mg)	tlrl-peri	121

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pFUSE(ss)-CHIg-hA1 (20 µg)	pfuse(ss)-hcha1	127
pFUSE(ss)-CHIg-hA2m1 (20 µg)	pfuse(ss)-hcha2m1	127
pFUSE(ss)-CHIg-hD (20 µg)	pfuse(ss)-hchd	127
pFUSE(ss)-CHIg-hE (20 µg)	pfuse(ss)-hche	127
pFUSE(ss)-CHIg-hG1 (20 µg)	pfuse(ss)-hchg1	127
pFUSE(ss)-CHIg-hG1e1 (20 µg)	pfuse(ss)-hchg1e1	127
pFUSE(ss)-CHIg-hG1e2 (20 µg)	pfuse(ss)-hchg1e2	127
pFUSE(ss)-CHIg-hG1e3 (20 µg)	pfuse(ss)-hchg1e3	127
pFUSE(ss)-CHIg-hG1e4 (20 µg)	pfuse(ss)-hchg1e4	127
pFUSE(ss)-CHIg-hG1e5 (20 µg)	pfuse(ss)-hchg1e5	127
pFUSE(ss)-CHIg-hG1e6 (20 µg)	pfuse(ss)-hchg1e6	127
pFUSE(ss)-CHIg-hG1e7 (20 µg)	pfuse(ss)-hchg1e7	127
pFUSE(ss)-CHIg-hG1e9 (20 µg)	pfuse(ss)-hchg1e9	127
pFUSE(ss)-CHIg-hG2 (20 µg)	pfuse(ss)-hchg2	127
pFUSE(ss)-CHIg-hG3 (20 µg)	pfuse(ss)-hchg301	127
pFUSE(ss)-CHIg-hG4 (20 µg)	pfuse(ss)-hchg4	127
pFUSE(ss)-CHIg-hG4e1 (20 µg)	pfuse(ss)-hchg4e1	127
pFUSE(ss)-CHIg-hM (20 µg)	pfuse(ss)-hchm	127
pFUSE(ss)-CHIg-mA (20 µg)	pfuse(ss)-mcha	127
pFUSE(ss)-CHIg-mD (20 µg)	pfuse(ss)-mchd	127
pFUSE(ss)-CHIg-mE (20 µg)	pfuse(ss)-mche	127
pFUSE(ss)-CHIg-mG1 (20 µg)	pfuse(ss)-mchg1	127
pFUSE(ss)-CHIg-mG2a (20 µg)	pfuse(ss)-mchg2a	127
pFUSE(ss)-CHIg-mG2b (20 µg)	pfuse(ss)-mchg2b	127
pFUSE(ss)-CHIg-mG3 (20 µg)	pfuse(ss)-mchg3	127
pFUSE(ss)-CHIg-mM (20 µg)	pfuse(ss)-mchm	127
pFUSE(ss)-CHIg-ratG1 (20 µg)	pfuse(ss)-rtchg1	127
pFUSE(ss)-CHIg-ratG2a (20 µg)	pfuse(ss)-rtchg2a	127
pFUSE(ss)-CHIg-ratG2b (20 µg)	pfuse(ss)-rtchg2b	127
pFUSE(ss)-CHIg-ratG2c (20 µg)	pfuse(ss)-rtchg2c	127
pFUSE(ss)-CHIg-rG (20 µg)	pfuse(ss)-rchg	127
pFUSE(ss)-CHIg-rhG1 (20 µg)	pfuse(ss)-rhchg1	127
pFUSE(ss)-CHIg-rhG2 (20 µg)	pfuse(ss)-rhchg2	127
pFUSE(ss)-CHIg-rhG3 (20 µg)	pfuse(ss)-rhchg3	127
pFUSE(ss)-CHIg-rhG4 (20 µg)	pfuse(ss)-rhchg4	127
pFUSE2(ss)-CLIg-hk (20 µg)	pfuse2(ss)-hclk	128
pFUSE2(ss)-CLIg-hl2 (20 µg)	pfuse2(ss)-hcll2	128
pFUSE2(ss)-CLIg-mk (20 µg)	pfuse2(ss)-mclk	128
pFUSE2(ss)-CLIg-ml1 (20 µg)	pfuse2(ss)-mcll1	128
pFUSE2(ss)-CLIg-ml2 (20 µg)	pfuse2(ss)-mcll2	128
pFUSE2(ss)-CLIg-ratK (20 µg)	pfuse2(ss)-rtclk	128
pFUSE2(ss)-CLIg-ratL1 (20 µg)	pfuse2(ss)-rtcll1	128
pFUSE2(ss)-CLIg-rhK (20 µg)	pfuse2(ss)-rhclk	128
pFUSE2(ss)-CLIg-rk1 (20 µg)	pfuse2(ss)-rclk1	128
pFUSE2(ss)-CLIg-rk2 (20 µg)	pfuse2(ss)-rclk2	128
pFUSE-Lucia-CHIg-hG1 (20 µg)	pfuselc-hchg1	128
pFUSE-Lucia-CHIg-hG2 (20 µg)	pfuselc-hchg2	128
pFUSE-Lucia-CHIg-hG3 (20 µg)	pfuselc-hchg3	128
pFUSE-Lucia-CHIg-hG4 (20 µg)	pfuselc-hchg4	128
pFUSE-Lucia-CHIg-mG1 (20 µg)	pfuselc-mchg1	128
pFUSE-Lucia-CHIg-mG2a (20 µg)	pfuselc-mchg2a	128

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pFUSE-Lucia-CHIg-mG2b (20 µg)	pfuselc-mchg2b	128
pFUSE-Lucia-CHIg-mG3 (20 µg)	pfuselc-mchg3	128
pFUSE-hIgG1-Fc(1/2) (20 µg)	pfuse-hg1fc(1/2)	22
pFUSE-hIgG2-Fc(1/2) (20 µg)	pfuse-hfc(1/2)	22
pFUSE-hIgG3-Fc(1/2) (20 µg)	pfuse-hg3fc(1/2)	22
pFUSE-hIgG4-Fc(1/2) (20 µg)	pfuse-hg4fc(1/2)	22
pFUSE-mIgG1-Fc(1/2) (20 µg)	pfuse-mg1fc(1/2)	22
pFUSE-mIgG2a-Fc(1/2) (20 µg)	pfuse-mg2afc(1/2)	22
pFUSE-mIgG2b-Fc(1/2) (20 µg)	pfuse-mg2bfc(1/2)	22
pFUSE-mIgG3-Fc(1/2) (20 µg)	pfuse-mg3fc(1/2)	22
pFUSE-rIgG-Fc(1/2) (20 µg)	pfuse-rfc(1/2)	22
pFUSE-rtIgG2b-Fc(1/2) (20 µg)	pfuse-rtg2bfc(1/2)	22
pFUSE-hIgG1e1-Fc(1/2) (20 µg)	pfc(1/2)-hg1e1	22
pFUSE-hIgG1e2-Fc(1/2) (20 µg)	pfc(1/2)-hg1e2	22
pFUSE-hIgG1e3-Fc(1/2) (20 µg)	pfc(1/2)-hg1e3	22
pFUSE-hIgG1e4-Fc(1/2) (20 µg)	pfc(1/2)-hg1e4	22
pFUSE-hIgG1e5-Fc(1/2) (20 µg)	pfc(1/2)-hg1e5	22
pFUSE-hIgG1e6-Fc(1/2) (20 µg)	pfc(1/2)-hg1e6	22
pFUSE-hIgG1e7-Fc(1/2) (20 µg)	pfc(1/2)-hg1e7	22
pFUSE-hIgG1e9-Fc(1/2) (20 µg)	pfc(1/2)-hg1e9	22
pFUSE-hIgG1e11-Fc(1/2) (20 µg)	pfc(1/2)-hg1e11	22
pFUSE-hIgG1e12-Fc(1/2) (20 µg)	pfc(1/2)-hg1e12	22
pFUSE-hIgG1e13-Fc(1/2) (20 µg)	pfc(1/2)-hg1e13	22
pFUSE-hIgG2e1-Fc(1/2) (20 µg)	pfc(1/2)-hg2e1	22
pFUSE-hIgG4e1-Fc(1/2) (20 µg)	pfc(1/2)-hg4e1	22
pFUSE-mIgG2ae1-Fc(1/2) (20 µg)	pfc(1/2)-mg2ae1	22
pFUSE-Lucia-hG1Fc (20 µg)	pfuse-hg1lc	23
pFUSE-Lucia-mG2aFc (20 µg)	pfuse-mg2alc	23
pFUSE-SEAP-hG1Fc (20 µg)	pfuse-hg1sp	23
pFUSE-SEAP-hG2Fc (20 µg)	pfuse-hg2sp	23
pFUSE-SEAP-hG3Fc (20 µg)	pfuse-hg3sp	23
pFUSE-SEAP-hG4Fc (20 µg)	pfuse-hg4sp	23
pFUSE-SEAP-mG1Fc (20 µg)	pfuse-mg1sp	23
pFUSE-SEAP-mG2aFc (20 µg)	pfuse-mg2asp	23
pFUSE-SEAP-mG2bFc (20 µg)	pfuse-mg2bsp	23
pFUSE-SEAP-mG3Fc (20 µg)	pfuse-mg3sp	23
pFUSE-SEAP-rFc (20 µg)	pfuse-rsp	23
pFUSE-SEAP-rtFc (20 µg)	pfuse-rtsp	23
pFUSEN-hG1e2Fc (20 µg)	pfcn-hg1e2	23
pFUSEN-hG1Fc (20 µg)	pfcn-hg1	23
pFUSEN-hG2Fc (20 µg)	pfcn-hg2	23
pFUSEN-Lucia-hG1e2Fc (20 µg)	pfcn-lchg1e2	23
pFUSEN-Lucia-hG1Fc (20 µg)	pfcn-lchg1	23
pFUSEN-Lucia-hG2Fc (20 µg)	pfcn-lchg2	23
pFUSEN-Lucia-mG2aFc (20 µg)	pfcn-lcmg2a	23
pFUSEN-mG2aFc (20 µg)	pfcn-mg2	23
PGN-BS (5 mg)	tlrl-pgnb3	89
PGN-EB (1 mg)	tlrl-pgneb	89
PGN-ECndi ultrapure, insoluble (5 mg)	tlrl-kipgn	96
PGN-ECndss ultrapure, soluble (1 mg)	tlrl-ksspgn	96
PGN-EK (1 mg)	tlrl-pgnek	89

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PGN-SA (5 mg)	tlrl-pgns2	89
PGN-SAndi ultrapure, insoluble (5 mg)	tlrl-sipgn	96
Phleomycin (100 mg; 5 x 1 ml)	ant-ph-1	14
Phleomycin (500 mg; 25 x 1 ml)	ant-ph-5	14
Phleomycin (500 mg; 1 x 25 ml)	ant-ph-5b	14
Phleomycin (250 mg powder)	ant-ph-2p	14
Phleomycin (500 mg powder)	ant-ph-5p	14
Phleomycin (1 g powder)	ant-ph-10p	14
PI-103 (5 mg)	inh-pi10	121
Piceatannol (5 mg)	tlrl-pct	118
pINFUSE-hIgG1-Fc(1/2) (20 µg)	pfc(1/2)-hgin1	22
pINFUSE-hIgG2-Fc(1/2) (20 µg)	pfc(1/2)-hgin2	22
pINFUSE-hIgG3-Fc(1/2) (20 µg)	pfc(1/2)-hgin3	22
pINFUSE-hIgG4-Fc(1/2) (20 µg)	pfc(1/2)-hgin4	22
pINFUSE-mIgG2b-Fc(1/2) (20 µg)	pfc(1/2)-mgin2	22
Plasmocin™ prophylactic (25 mg)	ant-mpp	10
Plasmocin™ treatment (50 mg)	ant-mpt	10
Plasmocure™ (100 mg)	ant-pc	10
PlasmoTest™ (kit; test up to 250 samples)	rep-pt1	8
PlasmoTest™ Controls (200 tests)	pt-ctr2	8
PlasmoTest™ Reagent Kit (500 samples)	rep-ptrk	8
PLX4032 (20 mg)	inh-pl40	115
PLX4720 (10 mg)	inh-pl47	116
PMA (5 mg)	tlrl-pma	website
pMONO-blasti-GFP (20 µg)	pmonob-gfp	24
pMONO-blasti-mcs (20 µg)	pmonob-mcs	24
pMONO-hygro-GFP (20 µg)	pmonoh-gfp	24
pMONO-hygro-mcs (20 µg)	pmonoh-mcs	24
pMONO-neo-GFP (20 µg)	pmonon-gfp	24
pMONO-neo-mcs (20 µg)	pmonon-mcs	24
pMONO-zeo-GFP (20 µg)	pmonoz-gfp	24
pMONO-zeo-mcs (20 µg)	pmonoz-mcs	24
pNiFty-Luc (20 µg)	pnifty-luc	29
pNiFty-SEAP (20 µg)	pnifty-seap	29
pNiFty2-56K-SEAP (20 µg)	pnf2-56ksp	29
pNiFty2-Luc (20 µg)	pnifty2-luc	29
pNiFty2-SEAP (20 µg)	pnifty2-seap	29
pNiFty3-Lucia (20 µg)	pnf3-lc1	website
pNiFty3-SEAP (20 µg)	pnf3-sp1	website
pNiFty3-A-Lucia (20 µg)	pnf3-lc3	website
pNiFty3-A-SEAP (20 µg)	pnf3-sp3	website
pNiFty3-AN-Lucia (20 µg)	pnf3-lc6	website
pNiFty3-AN-SEAP (20 µg)	pnf3-sp6	website
pNiFty3-I-Lucia (20 µg)	pnf3-lc4	website
pNiFty3-I-SEAP (20 µg)	pnf3-sp4	website
pNiFty3-IAN-Lucia (20 µg)	pnf3-lc7	website
pNiFty3-IAN-SEAP (20 µg)	pnf3-sp7	website
pNiFty3-N-Lucia (20 µg)	pnf3-lc2	website
pNiFty3-N-SEAP (20 µg)	pnf3-sp2	website
pNiFty3-T-Lucia (20 µg)	pnf3-lc5	website
pNiFty3-T-SEAP (20 µg)	pnf3-sp5	website

PRODUCT (QUANTITY)	CAT. CODE	PAGE
pNiFty3-TAN-Lucia (20 µg)	pnf3-lc8	website
pNiFty3-TAN-SEAP (20 µg)	pnf3-sp8	website
Poly(A:U) (10 mg)	tlrl-pau	90
Poly(dA) (200 µg)	tlrl-pan	102
Poly(dA) / LyoVec (100 µg)	tlrl-pac	102
Poly(dA:dT) / LyoVec (100 µg)	tlrl-patc	102
Poly(dA:dT) Naked (200 µg)	tlrl-patn	102
Poly(dA:dT) Naked (1 mg)	tlrl-patn-1	102
Poly(dA:dT) Rhodamine (10 µg)	tlrl-patr	102
Poly(dG:dC) / LyoVec (100 µg)	tlrl-pgcc	102
Poly(dG:dC) Naked (200 µg)	tlrl-pgcn	102
Poly(dT) (100 nmol)	tlrl-pt17	92
Poly(I:C) (HMW) (10 mg)	tlrl-pic	90
Poly(I:C) (HMW) (50 mg)	tlrl-pic-5	90
Poly(I:C) (HMW) / LyoVec (100 µg)	tlrl-piclv	99
Poly(I:C) (HMW) Biotin (10 µg)	tlrl-picb	90
Poly(I:C) (HMW) Fluorescein (10 µg)	tlrl-picf	90
Poly(I:C) (HMW) Rhodamine (10 µg)	tlrl-picr	90
Poly(I:C) (HMW) VacciGrade™ (10 mg)	vac-pic	143
Poly(I:C) (LMW) (25 mg)	tlrl-picw	90
Poly(I:C) (LMW) (250 mg)	tlrl-picw-250	90
Poly(I:C) (LMW) / LyoVec™ (100 µg)	tlrl-picwlv	99
Poly(I:C) (LMW) Rhodamine (10 µg)	tlrl-piwr	90
Polymyxin B (100 mg)	tlrl-pmb	118
Primocin™ (500 mg)	ant-pm-1	11
Primocin™ (1 g)	ant-pm-2	11
PromTest (10 x 5 µg)	prom-test	website
Protein G / Agarose (2 ml)	gel-agg-2	129
Protein G / Agarose (5 ml)	gel-agg-5	129
Protein L / Agarose (2 ml)	gel-protl-2	129
Protein L / Agarose (10 ml)	gel-protl-10	129
pSELECT-blasti/mcs (20 µg)	psetb-mcs	24
pSELECT-CGFP-blasti (20 µg)	psetb-cgfp	25
pSELECT-CGFP-zeo (20 µg)	psetz-cgfp	25
pSELECT-CHA-blasti (20 µg)	psetb-cha	25
pSELECT-CHA-zeo (20 µg)	psetz-cha	25
pSELECT-CHis-blasti (20 µg)	psetb-chis	25
pSELECT-CHis-zeo (20 µg)	psetz-chis	25
pSELECT-GFPzeo-mcs (20 µg)	psetgz-mcs	24
pSELECT-hygro-mcs (20 µg)	psetn-mcs	24
pSELECT-neo-mcs (20 µg)	pseth-mcs	24
pSELECT-NGFP-blasti (20 µg)	psetb-ngfp	25
pSELECT-NGFP-zeo (20 µg)	psetz-ngfp	25
pSELECT-NHA-blasti (20 µg)	psetb-nha	25
pSELECT-NHA-zeo (20 µg)	psetz-nha	25
pSELECT-NHis-blasti (20 µg)	psetb-nhis	25
pSELECT-NHis-zeo (20 µg)	psetz-nhis	25
pSELECT-NLucia-blasti (20 µg)	psetb-nlucia	25
pSELECT-NLucia-zeo (20 µg)	psetz-nlucia	25
pSELECT-puro-mcs (20 µg)	psetp-mcs	24
pSELECT-zeo-Lucia (20 µg)	psetz-lucia	website

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pSELECT-zeo-mcs (20 µg)	psetz-mcs	24
pSELECT-zeo-seap (20 µg)	psetz-seap	website
pUNO1-<Gene> (20 µg)	puno1-<gene>	32
pUNO1-mcs (20 µg)	puno1-mcs	website
Puromycin (100 mg; 10 x 1 ml)	ant-pr-1	14
Puromycin (500 mg; 50 x 1 ml))	ant-pr-5	14
Puromycin (500 mg; 1 x 50 ml)	ant-pr-5b	14
Pustulan (100 mg)	tlrl-pst	100
pVITRO1-blasti-GFP/SEAP (20 µg)	pvitro1-bgfpsp	website
pVITRO1-blasti-Lucia/SEAP (20 µg)	pvitro1-blucsp	website
pVITRO1-blasti-mcs (20 µg)	pvitro1-bmcs	26
pVITRO1-hygro-GFP/SEAP (20 µg)	pvitro1-gfpsp	website
pVITRO1-hygro-Lucia/SEAP (20 µg)	pvitro1-lucsp	website
pVITRO1-hygro-mcs (20 µg)	pvitro1-mcs	26
pVITRO1-neo-GFP/SEAP (20 µg)	pvitro1-ngfpsp	website
pVITRO1-neo-Lucia/SEAP (20 µg)	pvitro1-nlucsp	website
pVITRO1-neo-mcs (20 µg)	pvitro1-nmcs	26
pVITRO2-blasti-GFP/SEAP (20 µg)	pvitro2-bgfpsp	website
pVITRO2-blasti-Lucia/SEAP (20 µg)	pvitro2-blucsp	website
pVITRO2-blasti-mcs (20 µg)	pvitro2-bmcs	26
pVITRO2-hygro-GFP/SEAP (20 µg)	pvitro2-gfpsp	website
pVITRO2-hygro-Lucia/SEAP (20 µg)	pvitro2-lucsp	website
pVITRO2-hygro-mcs (20 µg)	pvitro2-mcs	26
pVITRO2-neo-GFP/SEAP (20 µg)	pvitro2-ngfpsp	website
pVITRO2-neo-Lucia/SEAP (20 µg)	pvitro2-nlucsp	website
pVITRO2-neo-mcs (20 µg)	pvitro2-nmcs	26
pVIVO1-GFP/SEAP (20 µg)	pvivo1-gfpsp	website
pVIVO1-Lucia/SEAP (20 µg)	pvivo1-lucsp	website
pVIVO2-Lucia/SEAP (20 µg)	pvivo2-lucsp	website
pVIVO1-mcs (20 µg)	pvivo1-mcs	27
pVIVO2-GFP/SEAP (20 µg)	pvivo2-gfpsp	website
pVIVO2-Lucia/SEAP (20 µg)	pvivo2-lucsp	website
pVIVO2-mcs (20 µg)	pvivo2-mcs	27
QUANTI-Blue™ (5 pouches)	rep-qb1	16
QUANTI-Blue™ (10 pouches)	rep-qb2	16
QUANTI-Luc™ (2 pouches)	rep-qlc1	17
QUANTI-Luc™ (5 pouches)	rep-qlc2	17
Quil-A® adjuvant (1 g)	vac-quil	142
R406 (5 mg)	inh-r406	118
R848 (500 µg)	tlrl-r848	92
R848 (5 mg)	tlrl-r848-5	92
R848 VacciGrade™ (5 mg)	vac-r848	143
Ramos-Blue™ Cells (3-7 x 10^6 cells)	rms-sp	62
Ramos-Blue™ KD-MyD Cells (3-7 x 10^6 cells)	rms-kdmyd	68
Rapamycin (5 mg)	tlrl-rap	121
Rat IgG1 Control (100 µg)	mabg1-ctlrt	134
Rat IgG2a Control (100 µg)	mabg2a-ctlrt	134
Rat IgG2b Control (100 µg)	mabg2b-ctlrt	134
RAW-Blue™ Cells (3-7 x 10^6 cells)	raw-sp	63
RAW-Blue™ ISG Cells (3-7 x 10^6 cells)	raw-isg	website
RAW-Lucia™ ISG Cells (3-7 x 10^6 cells)	rawl-isg	59
RAW-Lucia™ ISG-KO-cGAS Cells (3-7 x 10^6 cells)	rawl-kocgas	69

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RAW-Lucia™ ISG-KO-IFI16 Cells (3-7 x 10^6 cells)	rawl-koif16	69
RAW-Lucia™ ISG-KO-IRF3 Cells (3-7 x 10^6 cells)	rawl-koirf3	69
RAW-Lucia™ ISG-KO-IRF7 Cells (3-7 x 10^6 cells)	rawl-koirf7	69
RAW-Lucia™ ISG-KO-MAVS Cells (3-7 x 10^6 cells)	rawl-komavs	69
RAW-Lucia™ ISG-KO-MDA5 Cells (3-7 x 10^6 cells)	rawl-komda5	69
RAW-Lucia™ ISG-KO-RIG-I Cells (3-7 x 10^6 cells)	rawl-korigi	69
RAW-Lucia™ ISG-KO-STING Cells (3-7 x 10^6 cells)	rawl-kostg	69
RAW-Lucia™ ISG-KO-TBK1 Cells (3-7 x 10^6 cells)	rawl-kotbk	69
RAW-Lucia™ ISG-KO-TREX1 Cells (3-7 x 10^6 cells)	rawl-kotrex	69
RAW-Lucia™ ISG-KO-TRIF Cells (3-7 x 10^6 cells)	rawl-kotrif	69
RecFLA-ST (10 µg)	tlrl-flic-10	92
RecFLA-ST (50 µg)	tlrl-flic-50	92
Recombinant human CD40L (10 µg)	rcyec-hcd40l	137
Recombinant human IFN-γ (20 µg)	rcyec-hifng	137
Recombinant human IL-1β (10 µg)	rcyec-hil1b	137
Recombinant human IL-4 (10 µg)	rcyec-hil4	137
Recombinant human IL-6 (10 µg)	rcyec-hil6	137
Recombinant human IL-12 (10 µg)	rcyc-hil12	137
Recombinant human IL-13 (10 µg)	rcyec-hil13	137
Recombinant human IL-17a (10 µg)	rcyc-hil17a	137
Recombinant human IL-18 (10 µg)	rcyec-hil18	137
Recombinant human IL-33 (10 µg)	rcyec-hil33	137
Recombinant human TNF-α (20 µg)	rcyc-htnfa	137
Recombinant Lucia Luciferase Protein (1 µg)	rec-lucia	137
Recombinant SEAP Protein (10 µg)	rec-hseap	137
Resveratrol (100 mg)	tlrl-resv	116
Ruxolitinib (5 mg)	tlrl-rux	114
SAHA (25 mg)	inh-saha	122
SB202190 (5 mg)	tlrl-sb90	116
SB203580 (5 mg)	tlrl-sb20	116
SB431542 (5 mg)	inh-sb43	116
Schizophyllan (100 mg)	tlrl-spg	100
Scleroglucan (100 mg)	tlrl-scg	100
SEAP Reporter Assay Kit	rep-sap	website
SP600125 (10 mg)	tlrl-sp60	116
ssPolyU / LyoVec (100 µg)	tlrl-lpu	93
ssPolyU Naked (10 mg)	tlrl-sspu	93
ssRNA40 / LyoVec (100 µg)	tlrl-lrna40	93
ssRNA41 / LyoVec (100 µg)	tlrl-lrna41	93
ssRNA-DR / LyoVec (100 µg)	tlrl-ssdr	93
Streptavidin-Lucia (2 x 500 µl)	rep-strlc	134
TDB (2 x 1 mg)	tlrl-tdb	101
TDB HS-15 (2 x 1 mg)	tlrl-stdb	101
TDB VacciGrade (2 mg)	vac-tdb	144
Temsirolimus (10 mg)	inh-tems	121
THP1-Blue™ ISG Cells (3-7 x 10^6 cells)	thp-isg	60
THP1-defASC Cells (3-7 x 10^6 cells)	thp-dasc	46
THP1-defCASP1 Cells (3-7 x 10^6 cells)	thp-dcasp1	46
THP1-defNLRP3 Cells (3-7 x 10^6 cells)	thp-dnlp	46
THP1-Dual™ (NF-κB, ISG) Cells (3-7 x 10^6 cells)	thpd-nfis	66
THP1-Dual™ KO-cGAS Cells (3-7 x 10^6 cells)	thpd-kocgas	70
THP1-Dual™ KO-IFI16 Cells (3-7 x 10^6 cells)	thpd-koifi16	70

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THP1-Dual™ KO-MyD Cells (3-7 x 10^6 cells)	thpd-komyd	70
THP1-Dual™ KO-STING Cells (3-7 x 10^6 cells)	thpd-kostg	70
THP1-Dual™ KO-TREX1 Cells (3-7 x 10^6 cells)	thpd-kotrex	70
THP1-Lucia™ NF-κB Cells (3-7 x 10^6 cells)	thpl-nfkb	63
THP1-Null Cells (3-7 x 10^6 cells)	thp-null	46
TL8-506 (500 µg)	tlrl-tl8506	93
TLR1-9 Agonist Kit-Human (10 ligands)	tlrl-kit1hw	106
TLR1-9 Agonist Kit-Mouse (9 ligands)	tlrl-kit1mw	106
TLR2 Agonist Kit (7 ligands)	tlrl-kit2hm	106
TLR3/7/8/9 Agonist Kit (14 ligands)	tlrl-kit3hw3	106
TLR9 Agonist Kit-Human (6 ODNs)	tlrl-kit9h	106
TLR9 Agonist Kit-Mouse (6 ODNs)	tlrl-kit9m	107
TLR9 Agonist Kit-B-Class (6 ODNs)	tlrl-kit9b	107
TLR9 Agonist Kit-A&C-Class (6 ODNs)	tlrl-kit9ac	107
TLR9 Antagonist Kit (6 ODNs)	tlrl-kit9i	107
Tri-DAP (1 mg)	tlrl-tdap	96
Tri-Lys (1 mg)	tlrl-tlys	96
Trichostatin A (1 mg)	met-tsa-1	122
Trichostatin A (5 mg)	met-tsa-5	122
Triptolide (1 mg)	ant-tpl	116
U0126 (5 mg)	tlrl-u0126	116
VACV-70 / LyoVec (100 µg)	tlrl-vav70c	102
VACV-70 Naked (200 µg)	tlrl-vav70n	102
VACV-70c / LyoVec (control) (100 µg)	tlrl-vav70cc	102
VACV-70c Naked (control) (200 µg)	tlrl-vav70cn	102
VX-765 (10 mg)	inh-vx765-1	118
VX-765 (0 mg)	inh-vx765-5	118
WGP Dispersible (50 mg)	tlrl-wgp	100
WGP Soluble (50 mg)	tlrl-wgps	100
Wortmannin (5 mg)	tlrl-wtm	121
YM201636 (5 mg)	inh-ym20	118
Z-VAD-FMK (1 mg)	tlrl-vad	118
Zeocin™ (1 g; 10 x 1 ml)	ant-zn-1	14
Zeocin™ (1 g powder)	ant-zn-1p	14
Zeocin™ (5 g; 50 x 1 ml)	ant-zn-5	14
Zeocin™ (5 g 1 x 50 ml)	ant-zn-5b	14
Zeocin™ (5 g powder)	ant-zn-5p	14
Zymosan (100 mg)	tlrl-zyn	100
Zymosan Depleted (10 mg)	tlrl-dzn	100

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CONTACT INFORMATION

INVIVOGEN USA

General Information
Toll-free: 888 457 5873
Fax: 858 457 5843
Email: info@invivogen.com

Customer Service
Email: sales@invivogen.com

Technical Support
Email: tech@invivogen.com

INVIVOGEN EUROPE

General Information
Tel: +33 (0)5.62.71.69.39
Fax: +33 (0)5.62.71.69.30
Email: info@invivogen.fr

Customer Service
Email: orders@invivogen.fr

Technical Support
Email: tech@invivogen.fr

INVIVOGEN ASIA

General Information
Toll-free: +852-36223480
Fax: +852-36223483
Email: info.hk@invivogen.com

Customer Service
Email: sales.hk@invivogen.com

Technical Support
Email: tech.hk@invivogen.com

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www.invivogen.com

InvivoGen

INVIVOGEN USA

3950 Sorrento Valley Blvd,
Suite IOO
San Diego, California 92121, USA
Toll-Free: 888 457 5873
Fax: 858 457 5843
info@invivogen.com

INVIVOGEN EUROPE

Z.I. Montaudran
5 rue Jean Rodier
3 1400 Toulouse, FRANCE
Tel: +33 562 71 69 39
Fax: +33 562 71 69 30
info@invivogen.fr

INVIVOGEN ASIA

Unit 709A, Bio-Informatics Center
2 Science Park West Avenue,
HKSTP, Shatin, HONG KONG
Toll-free: +852-36223480
Fax: +852-36223483
info.hk@invivogen.com