INNOVATION WITHIN REACH

InvivoGen

2012 - 2013

CATALOG 2

Mammalian Cell Expression

INNOVATION WITHIN REACH

Welcome to this year's second catalog edition
Catalog 2: Mammalian Cell Expression 2012-2013

At InvivoGen, we strive to provide the bioresearch community with the very best in high quality products to foster cutting-edge research.

With our expertise in Innate Immunity we continue to innovate. Catalog 1: Innate Immunity 2012-2013 is devoted to the study of pattern recognition receptor (PRR) signaling pathways.

Catalog 2 on Mammalian Cell Expression is dedicated to build and support your research systems, featuring:

Anti-mycoplasma agents **Expression plasmids** Selective antibiotics
Custom Cloning CpG-free DNA plasmids **RNA interference**
Cell signaling Inhibitors **Reporter systems** Lucia™ reporter
Antibody Isotype collections Antibody Isotype switching
Engineering Fc fusions
Vaccine adjuvants **VacciGrade™ PRR ligands**

This year we introduce the Lucia™ reporter gene encoding for a novel secreted luciferase. Lucia™ is featured in a variety of expression vectors providing the means for monitoring multiple signaling pathways.

We have included new illustrations in the comprehensive section on inhibitors. Visualize inhibitor actions on different cell signaling pathways, epigenetic modifications and in autophagy.

Additionally, we are delighted to expand our collection of new antibodies and vaccine adjuvants.

For detailed information regarding all our current products and references,
Please visit us at **www.invivogen.com**
Find links to related products and downloadable mini-reviews and newsletters.

Once again, we hope you enjoy discovering our exciting innovations to advance your research!

From everyone at InvivoGen

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Shipping Information

All products are shipped via 2-3 day express air, unless specified otherwise. Shipments can be expedited to overnight service for an additional fee. 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. Charges will vary by package weight and destination. Orders received after 2:00 p.m. Pacific Time will be processed the next business day. All domestic shipments are shipped via InvivoGen's designated carrier. If another carrier is specified, a customer carrier account number must be provided and InvivoGen cannot guarantee delivery time. 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 through Wednesday.

To reduce shipping costs and delivery delays, all European orders are shipped from our affiliate in France, Cayla. European orders must be accompanied by the institution's VAT registration number.

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 at http://www.invivogen.com/quickorder.php. Orders can also be placed via e-mail to sales@invivogen.com for orders in the US and sales@invivogen.fr for orders in Europe. All orders online will receive e-mail confirmation when orders are shipped.

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1 CELL CULTURE & TRANSFECTION

CELL CULTURE & TRANSFECTION

Mycoplasma Detection & Elimination

Mycoplasma detection and elimination are essential to cell-based research. InvivoGen has developed an innovative mycoplasma detection kit exploiting the mammalian innate immune system and efficient antimycoplasma agents as well as other antimicrobial reagents.

➢ **PlasmoTest™ -** Mycoplasma detection

➢ **Plasmocin™ & Plasmocure™-** Mycoplasma elimination

➢ **Fungin, Normocin™ & Primocin™-** Antimicrobial reagents

Selective Antibiotics

InvivoGen offers a large choice of high-quality selective antibiotics provided as cell culture-tested, ready-to-use solutions.

➢ **Blasticidin**

➢ **G418 Sulfate**

➢ **Hygromycin B / HygroGold™**

➢ **Puromycin**

➢ **Phleomycin**

➢ **Zeocin™**

Transfection Reagent

InvivoGen's transfection reagent is designed to provide fast, easy and reliable introduction of plasmid DNA into mammalian cells. It combines consistent and highly efficient transfection of a wide variety of cell types with exceptionally low cytotoxicity.

➢ **LyoVec™**

Reporter Detection Reagents

InvivoGen provides a set of reagents for the detection of three different reporter gene systems. Among them, HEK-Blue™ Detection and QUANTI-Blue™ are becoming popular means to monitor the expression levels of secreted embryonic alkaline phosphatase (SEAP). Further, InvivoGen is introducing Lucia™, a novel optimized secreted luciferase, which coupled with QUANTI-Luc™ provides a powerful and easy-to-use new reporter system.

➢ **LacZ Reporter Gene System**

➢ **SEAP Reporter Gene System**

➢ **NEW ! Lucia® Reporter Gene System**

MYCOPLASMA DETECTION & ELIMINATION

Mycoplasma contamination remains a significant problem to the culture of mammalian cells. 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. 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.

Mycoplasma Detection

The only way to confirm mycoplasma contamination is by routine testing using special techniques. InvivoGen has developed PlasmoTest™, a breakthrough in mycoplasma detection for cell cultures. PlasmoTest™ is the first mycoplasma detection kit that uses engineered cells and therefore can be easily established as a routine procedure in the lab.

➢ **PlasmoTest™**

Mycoplasma Elimination

Once the mycoplasma contamination has been confirmed, it is usually recommended that the infected cell culture be immediately autoclaved to prevent the infection from spreading and to use only mycoplasma-free cultures. However, some cell lines are irreplaceable and require an effective eradication treatment. InvivoGen offers a choice of antimicrobial solutions designed to eliminate and prevent mycoplasma contaminations. Some are also active against bacteria and/or fungi.

➢ **Plasmocin™** - Elimination and Prevention of Mycoplasma

➢ **Plasmocure™** - Elimination of Mycoplasma

➢ **Normocin™** - Prevention Against Mycoplasma, Bacteria and Fungi

➢ **Primocin™** - Prevention Against Mycoplasma, Bacteria and Fungi for Primary Cells

➢ **Fungin™** - Prevention and Elimination of Fungi

PlasmoTest™ - Mycoplasma Detection

PlasmoTest™ provides a simple, rapid and reliable assay for the visual detection of mycoplasma contamination in cell cultures. This assay is the first to utilize cells to signal the presence of mycoplasma.

▸ **Simple -** Requires only basic cell culture knowledge. No need for specific lab equipment. Results are easily determined with the naked eye or quantified with a spectrophotometer.

▸ **Rapid -** Hands-on time less than 1 hour. Gives results after overnight incubation.

▸ **Versatile -** Detects all *Mycoplasma* and *Acholeplasma* species known to infect cell cultures, as well as other cell culture contaminants such as bacteria.

▸ **Sensitive -** Detects 5.10^2-5.10^5 cfu/ml mycoplasmas. No false positive: a positive result indicates the presence of a cell culture contaminant.

▸ **Complete -** Contains the Mycoplasma sensor cells and all the reagents needed to perform the assay, including positive and negative controls. Up to 500 samples can be tested with the kit. To perform further assays, only the reagents need to be reordered.

Principle

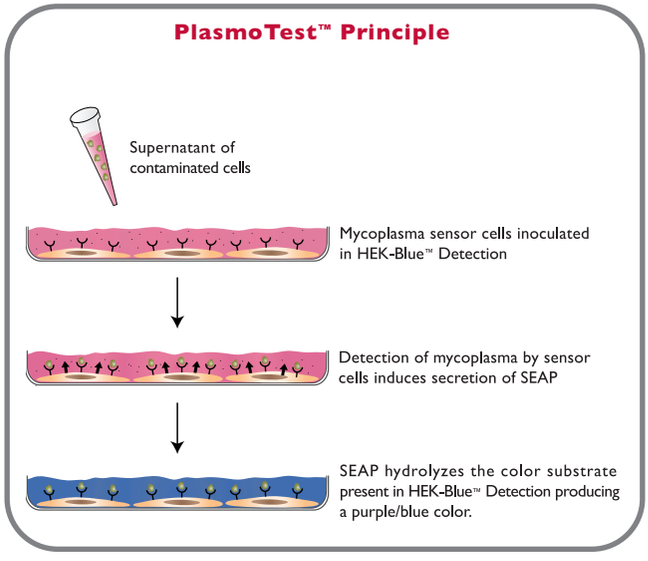
PlasmoTest™ features two major constituents: the Mycoplasma sensor cells and the HEK-Blue™ Detection medium. The Mycoplasma sensor cells detect the presence of mycoplasmas leading to a color change of the HEK-Blue™ Detection medium. The Mycoplasma sensor cells recognize mycoplasmas through Toll-Like 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 other transcription factors. 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.

Key Features

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 an optimized SEAP reporter gene, placed under the control of a promoter inducible by the transcription factors NF-κB and AP-1.

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. Furthermore, Normocin™ is included in the kit to protect HEK-Blue™-2 cells from any potential microbial contamination, whether caused by mycoplasmas, bacteria or fungi.

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 page 20).



Contents

PlasmoTest™ is composed of the HEK-Blue™-2 cells (3-5 x 10^6 cells) and the PlasmoTest™ Reagent Kit containing the following:
- HEK-Blue™ Selection (4 x 2 ml) - Antibiotic mix
- HEK-Blue™ Detection (2 pouches of 50 ml each)
- HEK-Blue™ water (2 x 60 ml)
- Normocin™ (200 mg - 4 x 1 ml)
- Positive control & negative control (1 vial each)

Buy PlasmoTest™ once then reorder only the PlasmoTest reagent kit or the reagents separately to perform further assays.

PRODUCT	QUANTITY	CAT. CODE
PlasmoTest™	1 kit	rep-pt2
PlasmoTest™ Reagent Kit	1 kit	rep-ptrk
HEK-Blue™ Detection	5 pouches	hb-det2
HEK-Blue™ Selection	5 x 2 ml	hb-sel
Normocin™	500 mg	ant-nr-1
PlasmoTest™ Controls	200 tests	pt-ctr2

Recent articles using PlasmoTest™

Bin Guan B. et al., 2011. ARID1A, a Factor That Promotes Formation of SWI/SNF-Mediated Chromatin Remodeling, Is a Tumor Suppressor in Gynecologic Cancers. Cancer Res., 71: 6718 - 6727.

Song L. et al., 2011. JAK1 Activates STAT3 Activity in Non-Small–Cell Lung Cancer Cells and IL-6 Neutralizing Antibodies Can Suppress JAK1-STAT3 Signaling. Mol. Cancer Ther.; 10: 481 - 494.

Voo KS. et al., 2009. Identification of IL-17-producing FOXP3+ regulatory T cells in humans. PNAS, 106: 4793 - 4798.

Plasmocin™ - The Mycoplasma Removal Agent

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 the 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.

In all animal cell lines tested to date, even at five times the working concentration, no apparent adverse effect on cellular metabolism has been observed. No resistance in liquid cultures of mycoplasmas has ever been identified in repeated experiments attempting to measure the mutation rate. Thus, development of resistant mycoplasma strains is virtually eliminated.

Plasmocin™ is also active at low concentrations on a broad range of Gram positive (Gram+) and Gram negative (Gram-) bacteria that are otherwise resistant to the mixture of streptomycin and penicillin, and exhibits no toxicity in eukaryotic cells.

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

Contents and Storage

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 6 months at 4°C and 2 years at -20°C.

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.

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

Recent articles using Plasmocin™

Colletti, GA. et al., 2012. Loss of Lysosomal Ion Channel Transient Receptor Potential Channel Mucolipin-1 (TRPML1) Leads to Cathepsin B-dependent Apoptosis. J. Biol. Chem., 287: 8082 - 8091.

Duan L. *et al.*, 2011. Prolylcarboxypeptidase regulates proliferation, autophagy, and resistance to 4-hydroxytamoxifen-induced cytotoxicity in estrogen receptor-positive breast cancer cells. J Biol Chem. 286(4):2864-76.

Haile ST. *et al.*, 2011. Tumor cell programmed death ligand 1-mediated T cell suppression is overcome by coexpression of CD80. J Immunol. 186(12):6822-9.

Pfisterer SG. *et al.*, 2011. Ca2+/calmodulin-dependent kinase (CaMK) signaling via CaMKI and AMP-activated protein kinase contributes to the regulation of WIPI-1 at the onset of autophagy. Mol Pharmacol. 80(6):1066-75.

Sastry KS. *et al.*, 2011. Targeting hepatitis B virus-infected cells with a T-cell receptor-like antibody. J Virol. 85(5):1935-42.

Saw NM. *et al.*, 2011. Vacuolar H(+)-ATPase subunits Voa1 and Voa2 cooperatively regulate secretory vesicle acidification, transmitter uptake, and storage. Mol Biol Cell. 22(18):3394-409.

Yi BR, et al., 2012. Synergistic effects of genetically engineered stem cells expressing cytosine deaminase and interferon-β via their tumor tropism to selectively target human hepatocarcinoma cells. Cancer Gene Ther. 19(9):644-51.

Plasmocure™ - The Alternative Mycoplasma Removal Agent

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 and Storage

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 6 months at 4°C and 2 years at -20°C.

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

Primocin™

The Antimicrobial Shield for Primary Cells

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. Primocin™ is the first formulation to offer complete protection against microbial contaminants. There is no need to add Pen/Strep.

Primocin™ is non-toxic to primary cells. It acts on targets found only in micro-organisms. Bacterial targets are the DNA gyrase and the prokaryotic ribosomal subunits, 30S and 50S. The fungal target is ergosterol, a molecule only found in the cell membrane of fungi and yeasts.
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 and Storage

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 3 months at 4°C and 18 months at -20°C.

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

Fungin™

The Solution for Fungal Contaminations

Description

Fungin™ is a new 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. The active concentration of Fungin™ can be stepped up from 10 µg/ml to 50 µg/ml if needed without displaying any negative effects.
Fungin™ is cell culture tested, and may be added to media containing commonly used antibacterial agents.

Contents and Storage

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 2 years at 4°C or -20°C.

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

Normocin™

The First Line of Defense for Your Cells

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 and Storage

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 2 years at 4°C or -20°C.

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

Recent articles using Normocin™, Primocin™ or Fungin™

Normocin™

Li YJ. *et al.*, 2012. Gold nanoparticles as a platform for creating a multivalent poly-SUMO chain inhibitor that also augments ionizing radiation. PNAS 109(11):4092-7.

Rank RG. *et al.*, 2012. Effect of Inflammatory Response on In Vivo Competition between Two Chlamydial Variants in the Guinea Pig Model of Inclusion Conjunctivitis. Infect. Immun., 80: 612 - 619.

Waisberg M. *et al.*, 2012. Plasmodium falciparum merozoite surface protein 1 blocks the proinflammatory protein S100P. PNAS. 109(14):5429-34.

Primocin™

Ross K. *et al.*, 2012. Polycomb group ring finger 1 cooperates with Runx1 in regulating differentiation and self-renewal of hematopoietic cells. Blood. 119(18):4152-61.

Shulga A. *et al.*, 2012. The loop diuretic bumetanide blocks posttraumatic p75NTR upregulation and rescues injured neurons. J Neurosci. 32(5):1757-70.

Yang X. *et al.*, 2012. Oestrogen upregulates L-type Ca^{2+} channels via oestrogen-receptor- by a regional genomic mechanism in female rabbit hearts. J Physiol. 590(Pt 3):493-508.

Fungin™

Armitage AE. *et al.*, 2011. Hepcidin regulation by innate immune and infectious stimuli. Blood. 118(15):4129-39.

Duan L. *et al.*, 2011. Prolylcarboxypeptidase regulates proliferation, autophagy, and resistance to 4-hydroxytamoxifen-induced cytotoxicity in estrogen receptor-positive breast cancer cells. J Biol Chem. 286(4):2864-76.

SELECTIVE ANTIBIOTICS

InvivoGen is a leader in the production of selective antibiotics. We manufacture the largest choice of antibiotics for 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, available from small quantities 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.

Large Choice of Antibiotics for 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 (see Chapter 3).

Also Available in Fast-Media®

All our selective antibiotics are available in our ready-made microwaveable *E. coli* Fast-Media®. The antibiotics are at the appropriate concentration in pre-mixed LB media for selection of *E. coli* transformants (see pages 48-49).

SELECTIVE ANTIBIOTIC	SELECTION TARGET	WORKING CONCENTRATION	RESISTANCE GENE	AVAILABLE QUANTITIES	PAGE
Blasticidin	Mammalian cells Bacteria	1 - 10 µg/ml 25 - 100 µg/ml	*Bsr*	100 mg, 500 mg (solution) 1 g (powder)	13
G418 Sulfate	Mammalian cells	400 - 1000 µg/ml	*Neo*	1 g, 5 g (solution)	13
Hygromycin B **HygroGold**	Mammalian cells Bacteria	50 - 200 µg/ml 100 µg/ml	*Hph*	1 g, 5 g (solution) 10 g (powder, HygroGold™)	14
Puromycin	Mammalian cells Bacteria	1 - 10 µg/ml 100 µg/ml	*Pac*	100 mg, 500 mg (solution)	14
Phleomycin	Fungi, yeasts	10 - 150 µg/ml	*Sh ble*	100 mg, 500 mg (solution) 250 mg, 500 mg, 1 g (powder)	15
Zeocin™	Mammalian cells Bacteria	50 - 300 µg/ml 25 µg/ml	*Sh ble*	1 g, 5 g (solution) 1 g, 5 g (powder)	15

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 and Storage

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 two years when stored at -20°C.

Related Products

pBLAST/pUNO, page 40
pMONO-blasti-mcs, page 30
pCpGfree-vitroB, page 54
psiRNA-h7SKblasti, page 62
Fast-Media® Blas, page 49
pFUSE2-CLIg, page 86
pSELECT-blasti, page 30
pSELECT-Tag, page 31
pVITRO-blasti, page 32

PRODUCT	QUANTITY	CAT. CODE
Blasticidin (solution)	100 mg (5 x 2 ml) 500 mg (25 x 2 ml) 500 mg (1 x 50 ml)	ant-bl-1 ant-bl-5 ant-bl-5b
Blasticidin (powder)	1 g	ant-bl-10p

Recent articles using InvivoGen's Blasticidin

Fréville A *et al.*, 2012. Plasmodium falciparum Inhibitor-3 Homolog Increases Protein Phosphatase Type 1 Activity and Is Essential for Parasitic Survival. J. Biol. Chem. 287: 1306 - 1321.

Le Floch R. *et al.*, 2011. CD147 subunit of lactate/H+ symporters MCT1 and hypoxia-inducible MCT4 is critical for energetics and growth of glycolytic tumors. PNAS. 108(40):16663-8.

Malykhina O. *et al.*, 2011. A Respiratory Syncytial Virus Replicon That Is Non-cytotoxic and Capable of Long-Term Foreign Gene Expression J.Virol., 85: 4792 - 4801.

Rorbach J. *et al.*, 2011. PDE12 removes mitochondrial RNA poly(A) tails and controls translation in human mitochondria. Nucleic Acids Res. 39: 7750 - 7763.

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 and Storage

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 two years when stored at -20°C.

Related Products

pMONO-neo-mcs, page 30
pCpGfree-vitroN, page 54
psiRNA-h7SKneo, page 62
pSELECT-neo, page 30
pVITRO-neo, page 32

PRODUCT	QUANTITY	CAT. CODE
G418 Sulfate	1 g (5 x 2 ml) 5 g (1 x 50 ml)	ant-gn-1 ant-gn-5

Recent articles using InvivoGen's G418

Carpenter S. *et al.*, 2011. Toll-like Receptor 3 (TLR3) Signaling Requires TLR4 Interactor with Leucine-rich Repeats (TRIL). J. Biol. Chem., 286: 38795 - 38804.

Genin EC. *et al.*, 2011. Substrate specificity overlap and interaction between adrenoleukodystrophy protein (ALDP/ABCD1) and adrenoleukodystrophy-related protein (ALDRP/ABCD2). J Biol Chem. 286(10):8075-84.

Maiguel D. *et al.*, 2011. Small molecule-mediated activation of the integrin CD11b/CD18 reduces inflammatory disease. Sci Signal. 4(189):ra57.

Myoung J & Ganem D., 2011. Infection of Lymphoblastoid Cell Lines by Kaposi's Sarcoma-Associated Herpesvirus: Critical Role of Cell-Associated Virus. J.Virol. 85: 9767 - 9777.

Takematsu H. *et al.*, 2011. Quantitative transcriptomic profiling of branching in a glycosphingolipid biosynthetic pathway. J Biol Chem. 286(31):27214-24.

Hygromycin B

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.

Two grades of Hygromycin B are available:
- **Hygromycin B** (purity >85%)
- **HygroGold™** (purity >98%)

Contents and Storage

Hygromycin B and HygroGold™ are provided as 100 mg/ml yellow solutions. HygroGold™ is also provided as a powder. Products are shipped at room temperature. Store at -20°C. Hygromycin B solutions are stable two years when stored at -20°C.

Related Products

pMONO-hygro-mcs, page 30
pCpGfree-vitroH, page 54
psiRNA-h7SKhygro page 62
Fast-Media® Hygro, page 49
pSELECT-hygro, page 30
pVITRO-hygro, page 32
pVIVO, page 34

PRODUCT	QUANTITY	CAT. CODE
Hygromycin B	1 g (5 x 2 ml) 5 g (1 x 50 ml)	ant-hm-1 ant-hm-5
HygroGold™	1 g (5 x 2 ml) 5 g (1 x 50 ml) 10 g (powder)	ant-hg-1 ant-hg-5 ant-hg-10p

Recent articles using InvivoGen's Antibiotics

Hygromycin B

Dantas TJ. *et al.*, 2011. Defective nucleotide excision repair with normal centrosome structures and functions in the absence of all vertebrate centrins. J Cell Biol. 193(2):307-18.

Leskelä TT. *et al.*, 2012. Cys-27 Variant of Human {delta}-Opioid Receptor Modulates Maturation and Cell Surface Delivery of Phe-27 Variant via Heteromerization. J. Biol. Chem., 287: 5008 - 5020.

Rorbach J. *et al.*, 2012. C7orf30 is necessary for biogenesis of the large subunit of the mitochondrial ribosome. Nucleic Acids Res. 40(9):4097-109.

HygroGold™

Bergson P. *et al.*, 2011. Verapamil Block of T-Type Calcium Channels. Mol. Pharmacol., 79: 411 - 419.

Bowen WS. *et al.*, 2012. Selective TRIF-dependent signaling by a synthetic toll-like receptor 4 agonist. Sci Signal. 5(211):ra13.

Taguchi K. *et al.*, 2011. Mechanosensitive EPLIN-dependent remodeling of adherens junctions regulates epithelial reshaping. J. Cell Biol., 194: 643 - 656.

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. Mammalian cells are generally sensitive to concentrations from 1 to 10 μg/ml.

Contents and Storage

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 two years when stored at -20°C.

Related Products

Fast-Media® Puro, page 49
pSELECT-puro, page 30

PRODUCT	QUANTITY	CAT. CODE
Puromycin	100 mg (5 x 2 ml) 500 mg (25 x 2 ml)	ant-pr-1 ant-pr-5

Recent articles using InvivoGen's Puromycin

Abe T. *et al.*, 2012. CD44 Participates in IP-10 Induction in Cells in Which Hepatitis C Virus RNA Is Replicating, through an Interaction with Toll-Like Receptor 2 and Hyaluronan. J Virol. 86(11):6159-70.

DeMars G. *et al.*, 2011. The extreme C-terminal region of Gαs differentially couples to the luteinizing hormone and beta2-adrenergic receptors. Mol Endocrinol. 25(8):1416-30.

Takahara K. *et al.*, 2012. Difference in Fine Specificity to Polysaccharides of Candida albicans Mannoprotein between Mouse SIGNR1 and Human DC-SIGN. Infect Immun. 80(5):1699-706.

Wortmann A. *et al.*, 2011. Cellular settings mediating Src Substrate switching between focal adhesion kinase tyrosine 861 and CUB-domain-containing protein 1 (CDCP1) tyrosine 734. J Biol Chem. 286(49):42303-15.

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 and Storage

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 two years when properly stored.

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

Recent articles using InvivoGen's Phleomycin

Abbà S. *et al.*, 2011. A PLAC8-containing protein from an endomycorrhizal fungus confers cadmium resistance to yeast cells by interacting with Mlh3p. Nucleic Acids Res. 39(17):7548-63.

Ong HB. *et al.*, 2011. Dissecting the metabolic roles of pteridine reductase 1 in Trypanosoma brucei and Leishmania major. J Biol Chem. 286(12):10429-38.

Rosowski EE. *et al.*, 2011. Strain-specific activation of the NF-kappaB pathway by GRA15, a novel Toxoplasma gondii dense granule protein. J Exp Med. 208(1):195-212.

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 and Storage

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 two years at -20°C.

Related Products

pFUSE-CHIg, page 86
pMONO-zeo-mcs, page 30
pSELECT-Tag, page 31
pBOOST, page 102
pCpGfree-siRNA, page 55
psiRNA-h7SKGFPzeo, page 62
Fast-Media® Zeo, page 49
pFUSE-Fc, page 27
pSELECT-zeo, page 30
pDRIVE, page 41
pVAC, page 103
pCpGfree, page 52
psiRNA-h7SKzeo, page 62

PRODUCT	QUANTITY	CAT. CODE
Zeocin™ (solution)	1 g (5 x 2 ml) 5 g (25 x 2 ml) 5 g (1 x 50 ml)	ant-zn-1 ant-zn-5 ant-zn-5b
Zeocin™ (powder)	1 g 5 g	ant-zn-1p ant-zn-5p

Recent articles using InvivoGen's Zeocin™

Sandström AG. *et al.*, 2012. Combinatorial reshaping of the Candida antarctica lipase A substrate pocket for enantioselectivity using an extremely condensed library. PNAS. 109(1):78-83.

Liu KC. *et al.*, 2012. Myosin-X functions in polarized epithelial cells. Mol Biol Cell. 23(9):1675-87.

Minczuk M. *et al.*, 2011. TEFM (c17orf42) is necessary for transcription of human mtDNA. Nucleic Acids Res. 39(10):4284-99.

Berger R. *et al.*, 2011. NF-κB is required for Smac mimetic-mediated sensitization of glioblastoma cells for γ-irradiation-induced apoptosis. Mol Cancer Ther. 10(10):1867-75.

Hankins JL. *et al.*, 2011. Exogenous ceramide-1-phosphate reduces lipopolysaccharide (LPS)-mediated cytokine expression. J Biol Chem. 286(52):44357-66.

TRANSFECTION REAGENT - LyoVec™

LyoVec™ is the first lyophilized cationic lipid-based transfection reagent. 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™.

Features and Benefits

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

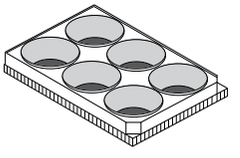
Increased Stability
- Stable over one year when lyophilized
- Stable up to six months when rehydrated

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.

LyoVec™ Procedure

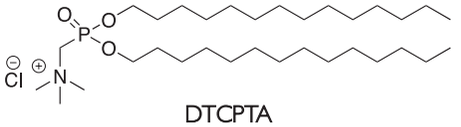
STEP 1 - LyoVec™ Reconstitution
Reconstitute LyoVec™ with 2 ml sterile H_2O or PBS
Note: Reconstituted LyoVec™ can be stored at 4°C for 6 months.

STEP 2 - Transfection of Adherent and Suspension Cells
1-10 µl pDNA (1-3 µg) + 100 µl LyoVec™ -> 20' at room temperature
Note: Bulk quantities of pDNA/LyoVec™ complexes can be prepared for multiple wells.



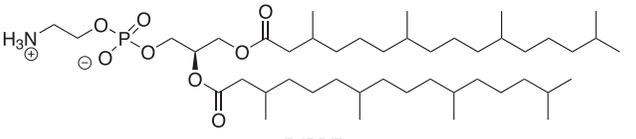
1 ml cell suspension (0.25-1.10^6 cells) + 100 µl pDNA/LyoVec™ complexes -> mix gently. Incubate at 37°C in 5% CO_2 for 24-72 hours before testing transgene expression or applying antibiotic selection.

Structure



DTCPTA

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



DiPPE

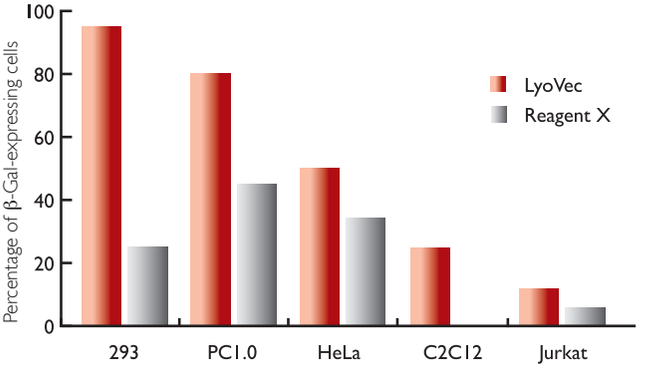
1,2-Diphytanoyl-sn-Glycero-3-Phosphoethanolamine

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

Contents and Storage

LyoVec™ is provided as a lyophilized powder, sterile and packaged in sealed 2 ml glass vials (4 or 9 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. Each order of LyoVec™ comes with 1 vial of control LyoVec™/GFP-LacZ complex. LyoVec™ is shipped at room temperature. Upon receipt, store at 4°C. LyoVec™ is stable up to 1 year when properly stored.

PRODUCT	QUANTITY	CAT. CODE
LyoVec™	8 ml (160 reactions) 18 ml (360 reactions)	lyec-12 lyec-22



Transfection efficiencies of LyoVec™: 2 to 8 x 10^5 cells per 6-well plates were transfected with 1 µg pVITRO2-GFP/LacZ complexed with 100 µl LyoVec™ in 2 ml of culture medium containing 10% FBS. LacZ expression was assessed 48h post-transfection. Note: Reagent X is one of the most utilized transfection reagents on the market. Transfection was performed according to the manufacturer's protocol.

REPORTER DETECTION REAGENTS

Gene reporter systems are extensively used for the study of eukaryotic gene expression and cellular events coupled to gene expression. Typically the reporter gene of choice is cloned with a promoter sequence of interest into an expression vector that is then tranferred into cells. Cells are subsequently assayed for the presence of the reporter by directly measuring the reporter protein itself or the enzymatic activity of the reporter protein, either *in vitro* or *in vivo*. An ideal reporter system has no background activity and quantitative measurements are made using assays that are easy, sensitive and reliable. InvivoGen provides three reporter gene systems:

➢ **β-Galactosidase (LacZ) reporter gene system**

➢ **Secreted embryonic alkaline phosphatase (SEAP) reporter gene system**

➢ **Secreted luciferase, Lucia®, reporter gene system**

Introducing **Lucia™** - a NEW secreted luciferase

InvivoGen's Lucia® is 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® is a secreted coelenterazine-utilizing luciferase that generates a 1000-fold higher bioluminescent signal in comparison to the commonly used Firefly and *Renilla* luciferases. Lucia® is designed for high and prolonged expression in mammalian cells.

Lucia® has several advantages over currently available luciferases and reporter systems, which are highlighted below:

- Lucia® is efficiently secreted into the cell culture supernatant.
- Reporter activity is rapidely and easily determined in real-time without disturbing cells in one simple endpoint assay.
- Stable expression of Lucia® makes this reporter system ideal for use in cell based assays and high-throughput applications.

The Lucia® reporter system provides an improvement in the sensitivity, reliability and ease of your live-cell reporter gene assays. Lucia® products include:

➢ **Lucia® Gene** (see page 22)

➢ **Recombinant Lucia® Protein** (see page 22)

➢ **Lucia® Antibody** (see page 22)

➢ **QUANTI-Luc™,** a Lucia® detection reagent (see page 23)

➢ **Lucia®-expressing cell lines** (see Catalog 1 - Innate Immunity)

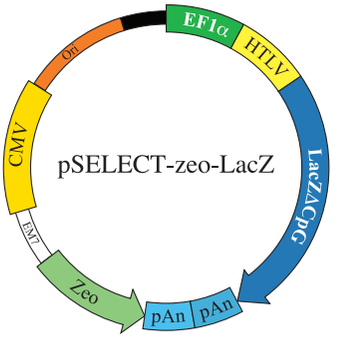
➢ **Lucia®-expressing Plasmids** (see page 25)

LacZ Reporter Gene System

The *E. coli lacZ* gene encoding β-galactosidase is the classical histochemical reporter gene. β-Galactosidase catalyzes the hydrolysis of X-Gal producing a blue precipitate that can be easily visualized under a microscope. The LacZ Staining Kits provide simple and convenient methods for the visual detection of LacZ expression within cells or tissues.

LacZ Reporter Gene

InvivoGen provides the LacZΔCpG gene, a humanized and CpG-free allele of the LacZ gene. This CpG-free gene is ten times more active than the wild-type gene in mammalian cells. It can be used for *in vitro* or *in vivo* applications. The LacZΔCpG gene is provided in the pSELECT-zeo-LacZ plasmid under the control of the EF-1α/HTLV composite promoter. The pSELECT-zeo-LacZ plasmid is selectable with Zeocin™ in mammalian cells and in bacteria.



Contents and Storage

The LacZΔCpG gene is provided as 20 µg of lyophilized DNA. It is supplied with 4 pouches of *E. coli* Fast-Media® Zeo (2 TB and 2 Agar; see page 49). Plasmid is shipped at room temperature. Store at -20°C for up to 12 months.

PRODUCT	QUANTITY	CAT. CODE
pSELECT-zeo-LacZ	20 µg	psetz-lacz

LacZ Staining Kits

InvivoGen provides two LacZ staining kits:

- LacZ Cell Staining Kit allows you to determine the percentage of transfected cells expressing the *lacZ* gene. All reagents are provided to prepare the fixative, staining and rinse solutions. The assay can be completed in 30 minutes, and the blue precipitate can take from 30 minutes to overnight to appear. The LacZ Cell Staining Kit contains sufficient reagent to stain one hundred 35 mm plates.

- LacZ Tissue Staining Kit allows the detection of transduced cells expressing the *lacZ* gene within fresh or frozen tissues. All reagents are provided to prepare the buffer, fixative and staining solutions. The staining of the tissues can be observed in 5 to 24 hours. The LacZ Tissue Staining Kit contains sufficient reagent to prepare 100 ml solutions.

Contents and Storage

The LacZ Cell Staining Kit contains: 10X PBS, X-Gal, Potassium Ferrocyanide, Potassium Ferricyanide, MgCl2 and 10X Fixative Solution.
The LacZ Tissue Staining Kit contains: 10X PBS, X-Gal, Potassium Ferrocyanide, Potassium Ferricyanide, MgCl2, Glutaraldehyde, Igepal and Na deoxycholate.
Products are shipped at room teperature. Store at 4°C or -20°C according to the product label. All reagents are stable for 6 months.

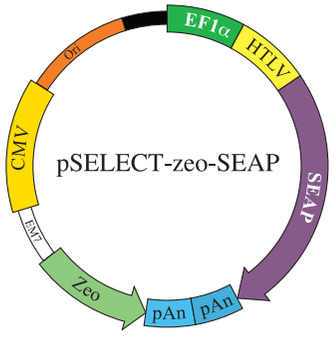
PRODUCT	QUANTITY	CAT. CODE
LacZ Cell Staining Kit	1 kit	rep-lz-c
LacZ Tissue Staining Kit	1 kit	rep-lz-t

SEAP Reporter Gene System

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 to determine reporter activity without disturbing the cells, does not require the preparation of cell lysates and can be used for kinetic studies.

SEAP Reporter Gene

InvivoGen provides the secreted embryonic alkaline phosphatase (SEAP) gene in the pSELECT-zeo-SEAP plasmid. It can be used *in vivo* and *in vitro* to transfect mammalian cells stably or transiently. The SEAP gene expression is driven by the EF-1α/HTLV composite promoter that combines the elongation factor 1 alpha core promoter and the 5'untranslated region of the Human T-cell Leukemia Virus. The pSELECT-zeo-SEAP plasmid is selectable with Zeocin™ in both mammalian cells and bacteria.



Contents and Storage

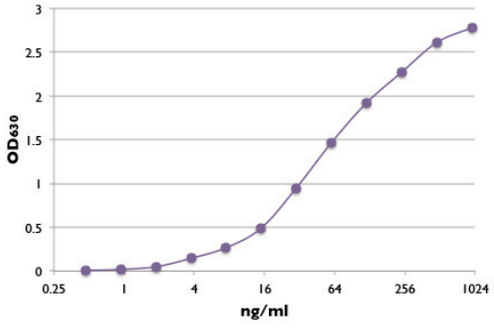
SEAP reporter gene is provided as 20 μg of lyophilized DNA. It is supplied with 1 pouch of QUANTI-Blue™ and 4 pouches of *E. coli* Fast-Media® Zeo (2 TB and 2 Agar, see page 49). Plasmid is shipped at room temperature. Store at -20°C for up to 12 months.

PRODUCT	QUANTITY	CAT. CODE
pSELECT-zeo-SEAP	20 μg	psetz-seap

NEW! 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 purified by affinity chromatography. It is formulated in 91 mM glycine, 91 mM TRIS and 5% w/v saccharose.

Application: Positive control for SEAP reporter assays.
A dilution series of the recombinant SEAP protein can also be used to determine the linear range of the assays.



Activity of recombinant SEAP protein determined by measuring the OD at 630 nm using QUANTI-Blue™. Values were measured after 1h in QUANTI-Blue™.

Contents and Storage

Recombinant SEAP protein is provided as a 10 μg lyophilizate. Product is shipped at room temperature. Store at -20°C for 12 months.

PRODUCT	QUANTITY	CAT. CODE
Recombinant SEAP Protein	10 μg	rec-hseap

SEAP Reporter Assay Kit

The SEAP Reporter Assay Kit provides a rapid and convenient method to determine the levels of SEAP in the culture medium of transfected cells expressing the *seap* gene. SEAP catalyzes the hydrolysis of p-Nitrophenyl phosphate producing a yellow end product that can be read spectrophotometrically at 405 nm.

- **Hands-on-time:** less than 1 hour
- **Obtention of results:** 10 to 40 minutes
- **Sensitivity:** as little as 10^{-10} g/ml of SEAP
- **Concentration range:** 1 ng - 1 μg/ml

Contents and Storage

The SEAP Reporter Assay Kit contains all reagents necessary to measure SEAP activity in 150 samples: 50X Dilution Buffer, 5X Assay Buffer, 100 mM L-Homoarginine, tablets of SEAP substrate. Store 50X Dilution Buffer and 5X Assay Buffer at 4°C. Store all other components at -20°C. All reagents are stable for 6 months when properly stored.

PRODUCT	QUANTITY	CAT. CODE
SEAP Reporter Assay Kit	1 kit	rep-sap

QUANTI-Blue™ & HEK-Blue™ Detection

QUANTI-Blue™ - Detection and quantification of SEAP

HEK-Blue™ Detection - Real-time detection of SEAP

QUANTI-Blue™ is a detection reagent developed to determine the levels of SEAP in biological samples, such as cell supernatants and mouse plasma. 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™.

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.

	QUANTI-Blue™	HEK-Blue™ Detection
Description	Detection reagent	Cell culture medium / detection reagent
Applications	• Detection of SEAP in biological samples • Kinetic studies of SEAP	• Real-time detection of SEAP produced by cells • Applicable to high-throughput screening
Readout Method	• Naked eye (purple/blue color) • Spectrophotometry (620 - 655 nm)	• Naked eye (purple/blue color) • Spectrophotometry (620 - 655 nm)
Procedure	1. Resuspend QUANTI-Blue™ 2. Transfer to multi-well plate 3. Add biological samples (cell supernatants, mouse plasma) 4. Incubate 15 mins to 24 hours at 37°C 5. Assess SEAP levels	1. Resuspend HEK-Blue™ Detection 2. Prepare a suspension of SEAP-expressing cells 3. Add cells to multi-well plate with SEAP inducers 4. Incubate 6 to 24 hours at 37°C, 5% CO_2 5. Assess SEAP levels

Contents and Storage

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 room temperature. After preparation, product is stable 2 weeks at 4°C and 2 months at -20°C.

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 room temperature. After preparation, product is stable 2 weeks at 4°C and 2 months at -20°C.

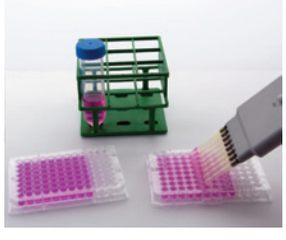
PRODUCT	QUANTITY*	CAT. CODE
QUANTI-Blue™	5 pouches (5 x 100 ml) 10 pouches (10 x 100 ml)	rep-qb1 rep-qb2
HEK-Blue™ Detection	5 pouches (5 x 50 ml) 10 pouches (10 x 50 ml)	hb-det2 hb-det3

*** Bulk quantities readily available**

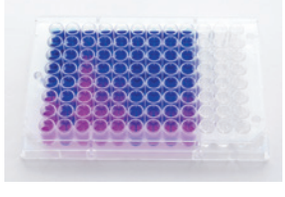
QUANTI-Blue™ Procedure

1. Prepare QUANTI-Blue™ by adding 100 ml water to the contents of one pouch.

2. Aliquote 200 µl QUANTI-Blue™ per well of a multiwell plate.



3. Add 20 µl supernatant of SEAP-expressing cells.

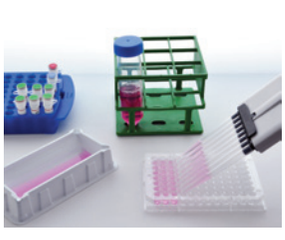


4. After 15 minutes to 24 hours incubation, assess SEAP activity with the naked eye or with a microplate reader at 620-655 nm.

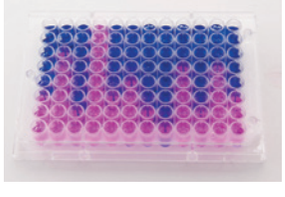
HEK-Blue™ Procedure

1. Prepare HEK-Blue™ Detection by adding 50 ml water to the contents of one pouch.

2. Prepare a suspension of SEAP-expressing cells using HEK-Blue™ Detection.



3. Combine SEAP inducers and cell suspension in a multiwell plate. Incubate 6 to 24 hours at 37°C, 5% CO_2.



4. Assess SEAP activity with the naked eye or with a microplate reader at 620-655 nm.

Recent articles using QUANTI-Blue™ or HEK-Blue™ Detection

QUANTI-Blue™

Abdulkhalek S. *et al.*, 2011. Neu1 Sialidase and Matrix Metalloproteinase-9 Cross-talk Is Essential for Toll-like Receptor Activation and Cellular Signaling. J. Biol. Chem., 286: 36532 - 36549.

Naka T. *et al.*, 2011. Structure and Host Recognition of Serotype 13 Glycopeptidolipid from Mycobacterium intracellulare. J. Bacteriol., 193: 5766 - 5774.

Lu H. *et al.*, 2012. VTX-2337 Is a Novel TLR8 Agonist That Activates NK Cells and Augments ADCC. Clin. Cancer Res., 18: 499 - 509.

Tsai CY. *et al.*, 2012. Size-Dependent Attenuation of TLR9 Signaling by Gold Nanoparticles in Macrophages .J. Immunol., 188: 68 - 76.

Grover RK. *et al.*, 2012. The costimulatory immunogen LPS induces the B-Cell clones that infiltrate transplanted human kidneys. Proc Natl Acad Sci U S A. [Epub ahead of print]

HEK-Blue™ Detection

Shiose S. *et al.*, 2011. Toll-like Receptor 3 Is Required for Development of Retinopathy Caused by Impaired All-trans-retinal Clearance in Mice. J. Biol. Chem., 286: 15543 - 15555.

Satta N. *et al.*, 2011. Toll-like receptor 2 mediates the activation of human monocytes and endothelial cells by antiphospholipid antibodies. Blood, 117: 5523 - 5531.

Luke JM. *et al.*, 2011. Coexpressed RIG-I Agonist Enhances Humoral Immune Response to Influenza Virus DNA Vaccine. J. Virol., 85: 1370 - 1383.

Xu J. *et al.*, 2011. Extracellular Histones Are Mediators of Death through TLR2 and TLR4 in Mouse Fatal Liver Injury. J. Immunol., 187: 2626 - 2631.

Choi H. *et al.*, 2011. Anti-inflammatory protein TSG-6 secreted by activated MSCs attenuates zymosan-induced mouse peritonitis by decreasing TLR2/NF-{kappa}B signaling in resident macrophages. Blood, 118: 330 - 338.

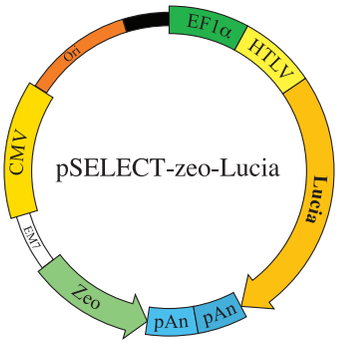
NEW! Lucia® Reporter Gene System

Lucia® Reporter Gene

InvivoGen provides the Lucia® reporter gene in the pSELECT-zeo plasmid. It can be used *in vivo* and *in vitro* to transfect mammalian cells stably or transiently. Lucia® gene expression is driven by the EF-1α/HTLV composite promoter that combines the elongation factor 1 alpha core promoter and the 5'untranslated region of the Human T-cell Leukemia Virus. The pSELECT-zeo-Lucia plasmid contains the zeocin resistance marker for selection in both mammalian cells and bacteria.

Contents and Storage

Lucia® reporter gene is provided as 20 µg of lyophilized DNA. It is supplied with 1 pouch of QUANTI-Luc™ and 4 pouches of *E. coli* Fast-Media® Zeo (2 TB and 2 Agar, see page 49). Plasmid is shipped at room temperature. Store at -20°C for up to one year.



PRODUCT	QUANTITY	CAT. CODE
pSELECT-zeo-Lucia	20 µg	psetz-lucia

Recombinant Lucia® Protein

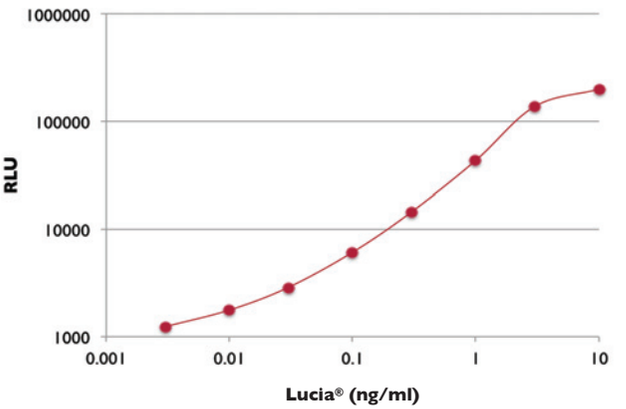
Recombinant Lucia® Protein is a monomeric protein expressed in CHO cells. The mature protein is composed of 192 amino acids and has an estimated molecular weight of 21 kDa. Recombinant Lucia® protein is provided in FBS-containing culture medium.

Application: Positive control for QUANTI-Luc™, a Lucia® reporter assay reagent. A dilution series of the recombinant Lucia™ protein can also be used to determine the linear range of the assay.

Contents and Storage

Recombinant Lucia® Protein is provided lyophilized. Product is shipped at room temperature. Store at -20°C for 12 months.

PRODUCT	QUANTITY	CAT. CODE
Recombinant Lucia® Protein	1 µg	rec-lucia



Activity of recombinant Lucia® protein determined by measuring Relative Light Units (RLU) in a luminometer using QUANTI-Luc™.

Anti-Lucia-IgG

Anti-Lucia-IgG is a monoclonal mouse IgG1 antibody against Lucia®. It has been generated by immunizing mice with the recombinant Lucia® protein and screened for neutralization activity. Anti-Lucia-IgG is purified from the sera by Protein G affinity chromatography.

Application: Neutralization of Lucia® used in a dual luciferase reporter assay employing the *Renilla* luciferase, another coelenterazine luciferase.

Contents and Storage

Anti-Lucia IgG is provided lyophilized from a 0.2 µm filtered solution in PBS. Product is shipped at room temperature. Store at -20°C for 12 months.

PRODUCT	QUANTITY	CAT. CODE
Anti-Lucia-IgG	100 µg	mabg-lucia

QUANTI-Luc™ - Coelenterazine-utilizing luciferase detection reagent

InvivoGen's NEW and original lyophilized product, QUANTI-Luc™, is an assay reagent containing all the components required to quantitatively measure the activity of Lucia® and other coelenterazine-utilizing luciferases. QUANTI-Luc™ is optimized for use with Lucia® 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.

- **Ready to use -** Just add water
- **Cost effective -** One pouch prepares 5 x 96 well plates
- **Practical -** Working reagent stable for up to a month

Key Features

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. Amenable 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.

Applications: Use with Lucia®

Exceptional sensitivity and reproducibility
Optimized for the detection of Lucia®, a luciferase producing 1000-fold higher bioluminescent signal compared to the commonly used Firefly and *Renilla* luciferases. Lucia® is one log more sensitive than SEAP. InvivoGen has developed new reporter cell lines providing you with a choice of using SEAP or Lucia® as the reporter:
- single promoter reporter cells, HEK-Dual™ IFN-γ and HEK-Dual™ TNF-α (see Catalog 1 - Innate Immunity),
- double promoter reporter cells, THP1-Dual™ (NF-κB, ISG) and Jurkat-Dual™ (NF-κB, ISG) (see Catalog 1 - Innate Immunity).

No cell lysis required
Lucia® is secreted into the cell culture media. Small sample volumes of 20 µl are sufficient.

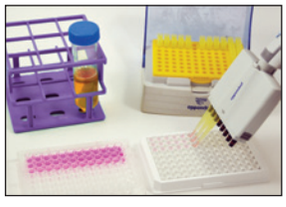
Rapid acquistion of results
The signal stability of the reaction with Lucia® allows for a single endpoint reading after addition of QUANTI-Luc™ to samples, which shortens time-to-results by half compared to other coelenterazine-utilizing luciferases.

Related Products

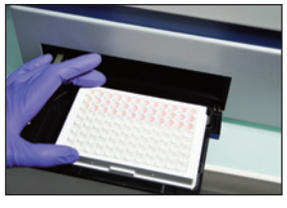
Lucia® Reporter Cell Lines, see Catalog 1 - Innate Immunity
Lucia® Expression Plasmids, page 25

QUANTI-Luc™ 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. Set up the luminometer prior to addition of 50 µl QUANTI-Luc™ reagent to each well either manually or by automated injection. Measure luminosity in endpoint mode when using Lucia® or in kinetic mode depending on the coelenterazine-luciferase used.

Contents and Storage

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 up to 12 months. After preparation, product is stable 1 week at 4°C and 1 month at -20°C.

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

*** Bulk quantities readily available**

2 MAMMALIAN EXPRESSION VECTORS

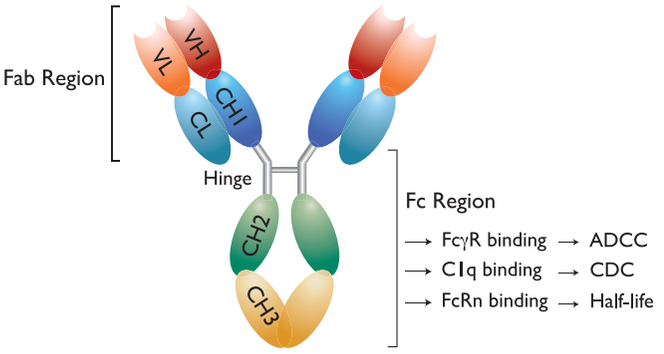
CLONING VECTORS

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, 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
pFUSE-Fc	**Generation of Fc-Fusion Proteins**	- Zeocin™	- IgG Fc regions of human, mouse, rabbit or rat origin - Fc regions with or without introns - Native or engineered Fc regions - Lucia®-tagged Fc regions	p. 27
pMONO	**Expression of One Gene of Interest**	- Blasticidin - Hygromycin B - Kanamycin / G418 - Zeocin™	- Single transcription unit - Strong and constitutive promoter	p. 30
pSELECT	**Expression of One Gene of Interest**	- Blasticidin - Hygromycin B - Kanamycin / G418 - Puromycin - Zeocin™ - GFP-Zeocin™	- Two transcription units - Strong and constitutive promoters	p. 30
pSELECT-Tag	**Expression of a Tagged Gene**	- Blasticidin - Hygromycin B	- GFP, HA, His or Lucia® tag - Two transcription units - Strong and constitutive promoters	p. 31
pVITRO	**Expression of Two Genes of Interest *in vitro***	- Blasticidin - Hygromycin B - Kanamycin / G418	- Two transcription units - Choice of strong and constitutive promoters - With 2 MCS or choice of 2 reporters (GFP/LacZ, GFP/SEAP, Lucia®/SEAP)	p. 32
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/LacZ, GFP/SEAP, Lucia®/SEAP)	p. 34
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 - Lucia®-tagged heavy chains	p. 86 (Chap 6)
pCpGfree	**Sustained *in vivo* gene or shRNA expression or promoter CpG methylation studies**	- Zeocin™	- CpG-free plasmid backbone - With MCS or reporter (LacZ, mSEAP, Lucia®)	p. 52 (Chap 3)
psiRNA	**Expression of shRNA(s)**	- Zeocin™	- Human 7SK RNA Pol III promoter - White and blue selection	p. 62 (Chap 4)

ENGINEERING Fc REGIONS

Immunoglobulin G (IgG) antibodies are large molecules composed of two heavy chains γ and two light chains, either κ or λ. They can be separated in two regions: the Fab (fragment-antigen binding) that contains the variable domain responsible for the antibody specificity, and the Fc (fragment crystalline) that binds specific proteins to induce immune responses such as opsonization and cell lysis. The IgG class is divided in four isotypes: IgG1, IgG2, IgG3 and IgG4 in humans, and IgG1, IgG2a, IgG2b and IgG3 in mice. They share more than 95% homology in the amino acid sequences of the Fc regions but show major differences in the amino acid composition and structure of the hinge region. The Fc region mediates its serum half-life and effector functions, such as complement-dependent cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cell phagocytosis (ADCP). IgG isoforms exert different levels of effector functions increasing in the order of IgG4<IgG2<IgG1≤IgG3. Human IgG1 displays high ADCC and CDC, and is the most suitable for therapeutic use against pathogens and cancer cells. Engineering the Fc region of a therapeutic monoclonal antibody or Fc fusion protein allows the generation of molecules that are better suited to the pharmacology 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 FcγRs 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 resulting 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].

1. Strohl WR., 2009. Optimization of Fc-mediated effector functions of monoclonal antibodies. Curr Opin Biotechnol. 20(6):685-91. 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.

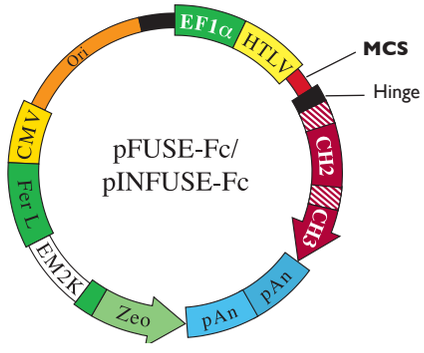
Antibody Effector Activities and Affinities

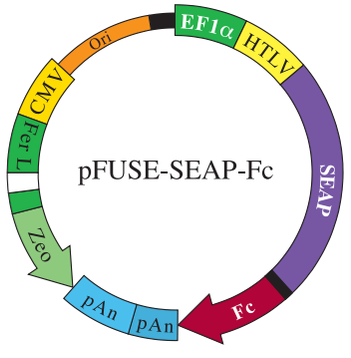
Isotype	Species	ADCC	CDC	Protein A binding	Protein G binding
IgG1	Human	+++	+++	++++	++++
IgG2	Human	+/-	+	++++	++++
IgG3	Human	+++	++++	-	++++
IgG4	Human	+/-	-	++++	++++
IgG1	Mouse	-	+/-	+	++++
IgG2a	Mouse	+++	+++	++++	++++
IgG2b	Mouse	+++	+++	+++	+++
IgG3	Mouse	+++	+	++	+++
IgG	Rabbit	N/A	+++	++++	+++
IgG2b	Rat	++	++	-	++

pFUSE-Fc - Fc-Fusion Proteins

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 at its N terminus 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
- Fc regions without intron (pFUSE-Fc) or with introns (pINFUSE-Fc)
- Native Fc regions or engineered Fc regions with altered effector functions





Description

An Expanding Family of pFUSE-Fc

pFUSE-Fc plasmids contain the composite EF-1α/HTLV promoter for high level expression of the fc-fusion protein, a multiple cloning site (MCS) located upstream of the Fc region to facilitate the cloning of the functional domain of a given protein, and a large choice of Fc regions to generate fc-fusion proteins with different characterisitics (species, isotypes, altered effector functions). pFUSE-Fc plasmids are selectable with Zeocin™ in *E. coli* and mammalian cells. They can be used for transient or stable transfection.

Secretion of Fc-Fusion Proteins

To allow their secretion, Fc-Fusion proteins require a signal peptide that is either native or added through cloning into a pFUSE-Fc or pINFUSE plasmid. Two versions of these vectors are available, either without or with the IL2 signal sequence (IL2ss).

• **No IL2ss:** without the IL2ss for the secretion of an Fc-fusion protein containing the functional domain of a protein that is naturally secreted and thus contains a native signal sequence.

• **With IL2ss:** contains the IL2ss for the generation of Fc-Fusion proteins derived from proteins that are not naturally secreted.

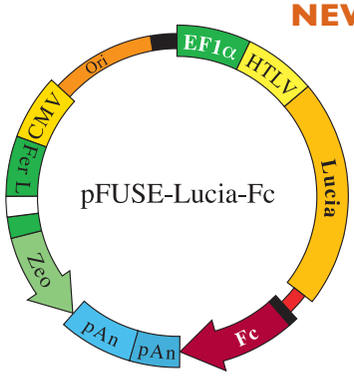
Ease of Detection and Purification

Fc-Fusion proteins can be easily detected in the supernatant of pFUSE-Fc-transfected cells by SDS-PAGE. Their functional domains can be identified by immunoblotting and ligand blotting. Furthermore, Fc-Fusion proteins can be easily purified by single-step protein A or protein G affinity chromatography.

A Large Choice of pFUSE-Fc Plasmids

Plasmid	Cloning site/Gene	Fc Region	Isotypes Available
pFUSE-Fc	MCS	- Native - No introns	- Human IgG1, 2, 3, 4 - Mouse IgG1, 2a, 2b, 3 - Rabbit IgG - Rat IgG2b
pINFUSE-Fc	MCS	- Native - With introns	- Human IgG1, 2, 3, 4 - Mouse IgG2b
pFUSE-Fc engineered	MCS	- Engineered - No introns	- Human IgG1, 2, 4 - Mouse IgG2a
pFUSE-Lucia-Fc NEW	Lucia-MCS	- Native - No introns	- Human IgG1 - Mouse IgG2a
pFUSE-SEAP-Fc	SEAP	- Native - No introns	- Human IgG1, 2, 3, 4 - Mouse IgG2a, 2b, 3 - Rabbit IgG - Rat IgG2b

NEW



Lucia®-Tagged Fc-Fusion Proteins

Lucia® is a novel secreted luciferase reporter protein with 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.

APPLICATIONS

Choose a pFUSE-Fc plasmid accordingly 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
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
pFUSE-Lucia-Fc - Lucia®-Tagged Immunoadhesins				
pFUSE-Lucia-hG1Fc **NEW**	Human IgG1	20 µg	pfuse-hg1lc	-
pFUSE-Lucia-mG2aFc **NEW**	Mouse IgG2a	20 µg	pfuse-mg2alc	-
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-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).

PRODUCT	ISOTYPE	MUTATIONS (ref.)	EFFECTOR FUNCTION	QTY	CAT. CODE (No IL2ss)	CAT. CODE (With IL2ss)
pFUSE-Fc - Engineered Fc Regions						
pFUSE-hIgG1e1-Fc	Human IgG1	T250Q/M428L (1)	Increased binding to FcRn Increased half-life	20 µg	pfc1-hg1e1	pfc2-hg1e1
pFUSE-hIgG1e2-Fc	Human IgG1	M252Y/S254T/T256E + H433K/N434F (2)	Increased binding to FcRn Increased half-life	20 µg	pfc1-hg1e2	pfc2-hg1e2
pFUSE-hIgG1e3-Fc**	Human IgG1	E233P/L234V/L235A/ΔG236 + A327G/A330S/P331S (3, 4)	Reduced binding to FcγRI Reduced ADCC and CDC	20 µg	pfc1-hg1e3	pfc2-hg1e3
pFUSE-hIgG1e4-Fc	Human IgG1	E333A (5, 6)	Increased binding to FcγRIIIa Increased ADCC and CDC	20 µg	pfc1-hg1e4	pfc2-hg1e4
pFUSE-hIgG1e5-Fc	Human IgG1	S239D/I332E/A330L (7, 8)	Increased binding to FcγRIIIa Increased ADCC	20 µg	pfc1-hg1e5	pfc2-hg1e5
pFUSE-hIgG1e6-Fc	Human IgG1	P257I/Q311I (9)	Increased binding to FcRn Unchanged half-life	20 µg	pfc1-hg1e6	pfc2-hg1e6
pFUSE-hIgG1e7-Fc	Human IgG1	K326W/E333S (10)	Increased binding to C1q Increased CDC	20 µg	pfc1-hg1e7	pfc2-hg1e7
pFUSE-hIgG1e9-Fc	Human IgG1	S239D/I332E/G236A (11)	Increased FcγRIIa/FcγRIIb ratio Increased macrophage phagocytosis	20 µg	pfc1-hg1e9	pfc2-hg1e9
pFUSE-hIgG2e1-Fc	Human IgG2	K322A (5)	Reduced binding to C1q Reduced CDC	20 µg	pfc1-hg2e1	pfc2-hg2e1
pFUSE-hIgG4e1-Fc	Human IgG4	S228P (12)	Reduced Fab-arm exchange	20 µg	pfc1-hg4e1	pfc2-hg4e1
pFUSE-mIgG2ae1-Fc	Mouse IgG2a	L235E + E318A/K320A/K322A (10)	Reduced binding to FcγRI and C1q Reduced ADCC and CDC	20 µg	pfc1-mg2ae1	pfc2-mg2ae1

** 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).

1. Hinton PR. *et al.*, 2004. Engineered human IgG antibodies with longer serum half-lives in primates. J Biol Chem. 279(8):6213-6. **2. Vaccaro C. *et al.*, 2005.** Engineering the Fc region of immunoglobulin G to modulate in vivo antibody levels. Nat Biotechnol. 23(10):1283-8. **3. 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. **4. 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. **5. 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. **6. Idusogie EE. *et al.*, 2001.** Engineered antibodies with increased activity to recruit complement. J Immunol. 166(4):2571-5. **7. Lazar GA. *et al.*, 2006.** Engineered antibody Fc variants with enhanced effector function. PNAS 103(11): 4005–4010. **8. Ryan MC. *et al.*, 2007.** Antibody targeting of B-cell maturation antigen on malignant plasma cells. Mol. Cancer Ther., 6: 3009 - 3018. **9. 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. **10. 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. **11. Richards JO,. *et al.*, 2008.** Optimization of antibody binding to FcgammaRIIa enhances macrophage phagocytosis of tumor cells. Mol Cancer Ther. 7(8):2517-27. **12. 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.

Contents and Storage

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 up to one year when properly stored. Each plasmid is provided with 4 pouches of *E. coli* Fast-Media® Zeo (2 TB and 2 Agar, see pages 48-49).

Recent articles using InvivoGen's pFUSE-Fc

Garcia-Alles LF. *et al.*, 2011. Crystal structure of human CD1e reveals a groove suited for lipid-exchange processes. PNAS, 108: 13230 - 13235.

Jepson S. *et al.*, 2012. LINGO-1, a Transmembrane Signaling Protein, Inhibits Oligodendrocyte Differentiation and Myelination through Intercellular Self-interactions J. Biol. Chem., 287: 22184 - 22195.

Kawabata A. *et al.*, 2011. Analysis of a Neutralizing Antibody for Human Herpesvirus 6B Reveals a Role for Glycoprotein Q1 in Viral Entry. J. Virol., 85: 12962 - 12971.

Lenormand C. *et al.*, 2012. HLA-DQA2 and HLA-DQB2 Genes Are Specifically Expressed in Human Langerhans Cells and Encode a New HLA Class II Molecule. J. Immunol., 188: 3903 - 3911.

Lisboa FA. *et al.*, 2011. Pregnancy-specific Glycoprotein 1 Induces Endothelial Tubulogenesis through Interaction with Cell Surface Proteoglycans. J. Biol. Chem., 286: 7577 - 7586.

Matsushita H. *et al.*, 2011. Differential but Competitive Binding of Nogo Protein and Class I Major Histocompatibility Complex (MHCI) to the PIR-B Ectodomain Provides an Inhibition of Cells. J. Biol. Chem., 286: 25739 - 25747.

Rosner C. *et al.*, 2011. Rhesus Macaque Inhibitory and Activating KIR3D Interact with Mamu-A–Encoded Ligands. J. Immunol., 186: 2156 - 2163.

Sharma A. *et al.*, 2012. A Monoclonal Antibody against Human Notch1 Ligand–Binding Domain Depletes Subpopulation of Putative Breast Cancer Stem–like Cells. Mol. Cancer Ther., 11: 77 - 86.

Yoshida S. *et al.*, 2012. Involvement of an NKG2D Ligand H60c in Epidermal Dendritic T Cell-Mediated Wound Repair. J. Immunol., 188: 3972 - 3979.

pMONO & pSELECT - Expression of One Gene of Interest

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 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.

• **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.

Features and Benefits

Strong and Ubiquitous Expression of the Transgene

- pMONO plasmids feature the SV40/FerH/mEF-1α promoter 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.

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

Variety of Selection Options

- 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 of 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.

- 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.

Multiple Cloning Site or Reporter Gene

- 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.

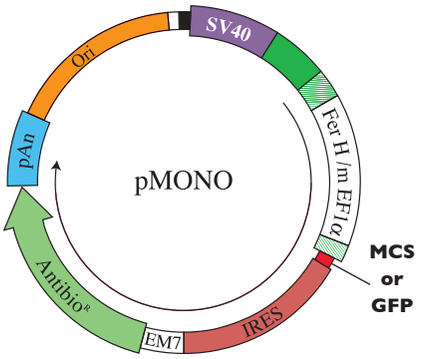
- pSELECT plasmids contain an MCS downstream of the EF-1α/HTLV promoter or the LacZ reporter gene as a control.

Contents and Storage

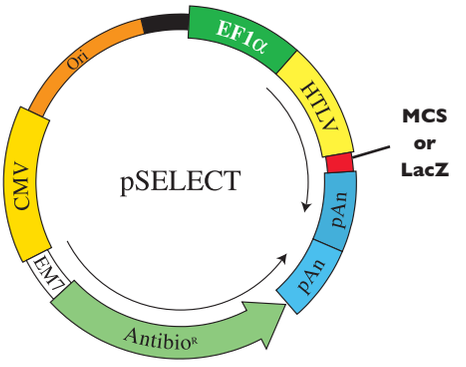
pMONO and pSELECT plasmids are provided as 20 µg of lyophilized DNA. Product is shipped at room temperature and should be stored at -20°C. Plasmid is stable up to one year when properly stored.
pMONO and pSELECT plasmids are provided with 4 pouches of *E. coli* Fast-Media® containing the appropriate selective antibiotic (2 TB and 2 Agar).

Related Products

Blasticidin, page 13
Puromycin, page 14
Fast-Media®, pages 48-49
HygroGold™, see page 14
Zeocin™, page 15



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



PRODUCT	QTY	CAT. CODE (MCS)	CAT. CODE (LacZ)
pSELECT-blasti	20 µg	psetb-mcs	psetb-lacz
pSELECT-hygro	20 µg	pseth-mcs	pseth-lacz
pSELECT-neo	20 µg	psetn-mcs	psetn-lacz
pSELECT-puro	20 µg	psetp-mcs	psetp-lacz
pSELECT-zeo	20 µg	psetz-mcs	psetz-lacz
pSELECT-GFPzeo	20 µg	psetgz-mcs	psetgz-lacz

pSELECT-Tag - Expression of a GFP-, HA-, His- or Lucia®-Tagged Gene

pSELECT-Tag is a new family of expression plasmids designed to generate tagged proteins in order to facilitate their detection and/or purification. pSELECT-Tag features three well-known tags: the green fluorescent protein (GFP) gene, the human influenza hemagglutinin (HA) epitope and the polyhistidine (His) tag, and a new innovative tag, Lucia®, a novel secreted luciferase. pSELECT-Tag plasmids allow the addition of the tag at either the N or C terminus of the protein of interest.

Features and Benefits

Three Commonly-Used Tags
- GFP-Tag
- HA-Tag
- His-Tag

And One New Innovative Tag
• **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 page 23).

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 a multiple cloning site and followed by a Stop codon.

Many Selectable Markers Available
pSELECT plasmids come with a variety of selectable markers that confer antibiotic resistance in both *E. coli* and mammalian cells.

Applications

• **GFP-Tag:** Visualization of the spatial and temporal localization of the tagged protein by fluorescence microscopy

• **HA-Tag:** Detection of the tagged protein by immunocytochemistry, immunoprecipitation or Western blotting, or purification of the tagged protein by affinity chromatography

• **His-Tag:** Purification of the tagged protein using an NI-NTA column

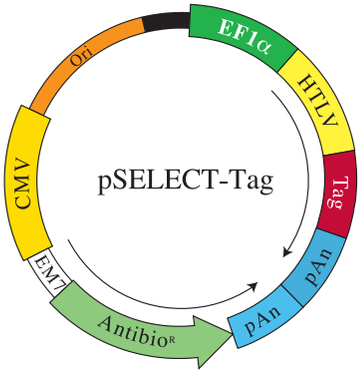
• **Lucia®-Tag:** Detection and quantification of the tagged protein by bioluminescence assay

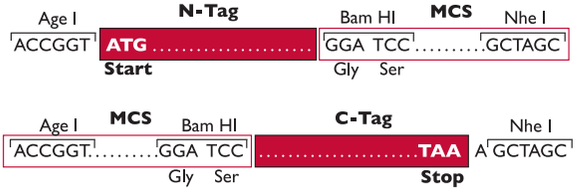
Contents and Storage

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 up to one year when properly stored.
pSELECT-Tag plasmids are provided with 4 pouches of *E. coli* Fast-Media® containing the appropriate selective antibiotic (2 TB and 2 Agar).

Related Products

Blasticidin, page 13
Zeocin™, page 15
Anti-HA Tag Antibody, page 94
Fast-Media®, pages 48-49





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

pVITRO - Expression of Two Genes of Interest *in vitro*

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

Features and Benefits

Gene Combinations in a Single Plasmid

Co-expression of two or more genes from a single vector is more efficient and convenient than using two separate vectors. pVITRO are multigenic plasmids that contain two distinct transcription units (TU).

Strong and Constitutive Expression of Two Transgenes

Each pVITRO plasmid features two constitutive promoters that drive high levels of expression from two separate TU in a large number of mammalian cell lines. Transcriptional interference is minimized by using promoters of different origins or promoters that are coordinately activated (i.e. ferritin promoters), and strong polyadenylation signals (polyA).

- **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 (blasticidin resistance), hph (hygromycin resistance), neo (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 3' of the Ori, as a polycistronic mRNA and translated through the IRES of the Foot and Mouth Disease Virus.

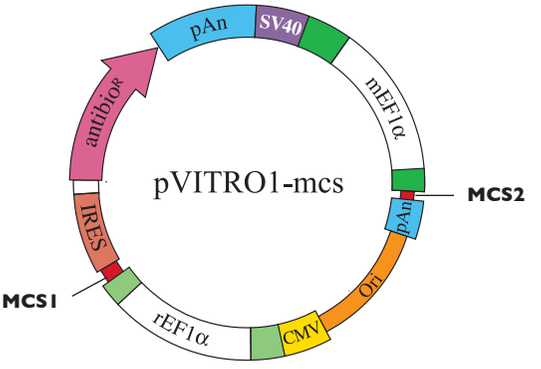
Available with Two MCS or Two Reporter Genes

- **pVITRO1-mcs & pVITRO2-mcs** plasmids contain two multiple cloning sites (MCS) for the convenient cloning of cDNAs.
- **pVITRO1-rep & pVITRO2-rep** express two reporter genes:
  - GFP and LacZ
  - **NEW !** GFP and SEAP
  - **NEW !** Lucia® and SEAP

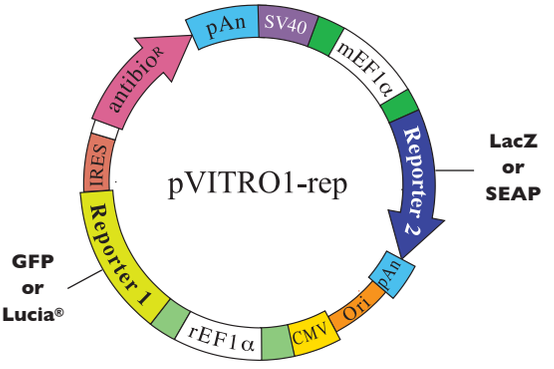
GFP (green fluorescent protein), LacZ (β-galactosidase) and SEAP (secreted embryonic alkaline phosphatase) are commonly used reporter proteins. Lucia® is a novel secreted luciferase with strong bioluminescent activity. The Lucia® reporter gene is ideal for promoter activity and gene expression studies. Lucia® luciferase activity can be detected and quantified directly in the culture medium of transfected cells using InvivoGen's QUANTI-Luc™ detection reagent (see page 23).

pVITRO1-rep and pVITRO2-rep plasmids can be used as control plasmids or cloning vectors. The reporter genes are flanked by unique restriction sites and can be easily replaced by open reading frames chosen from InvivoGen's Gene A-List (see page 39 or visit our website www.invivogen.com/orfs).

Plasmid	Promoters	Selection	Cloning sites/Genes
pVITRO1-mcs	CMV/rEF1α & SV40/mEF1α	- Blasticidin - Hygromycin B - Kanamycin/G418	MCS1 & MCS2
pVITRO1-rep	CMV/rEF1α & SV40/mEF1α	- Blasticidin - Hygromycin B - Kanamycin/G418	- GFP & LacZ - GFP & SEAP - Lucia® & SEAP
pVITRO2-mcs	SV40/hFerH/ mEF1α & CMV/hFerL/chEF1α	- Blasticidin - Hygromycin B - Kanamycin/G418	MCS1 & MCS2
pVITRO2-rep	SV40/hFerH/ mEF1α & CMV/hFerL/chEF1α	- Blasticidin - Hygromycin B - Kanamycin/G418	- GFP & LacZ - GFP & SEAP - Lucia® & SEAP



MCS 1 5'- Bsp EI, Bst 1107I, Bam HI, Bsi WI, Avr II -3'
MCS 2 5'- Age I, Eco RV, Bgl II, Bsr GI, Nhe I -3'



Recent articles using pVITRO plasmids

Kluza J. *et al.*, 2011. Exploiting mitochondrial dysfunction for effective elimination of imatinib-resistant leukemic cells. PLoS One. 6(7):e21924.

Sissons JR. *et al.*, 2012. Cutting Edge: MicroRNA Regulation of Macrophage Fusion into Multinucleated Giant Cells. J Immunol. 189(1):23-7.

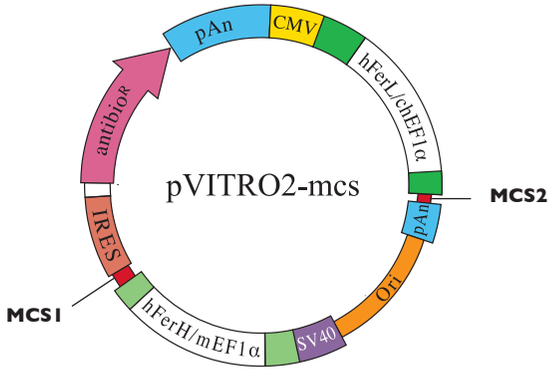
Wilber A. *et al.*, 2011. Efficient non-viral integration and stable gene expression in multipotent adult progenitor cells. Stem Cells Int. 2011:717069.

Senigl F. *et al.*, 2012. Transcriptional provirus silencing as a crosstalk of de novo DNA methylation and epigenomic features at the integration site. Nucleic Acids Res. 40(12):5298-312.

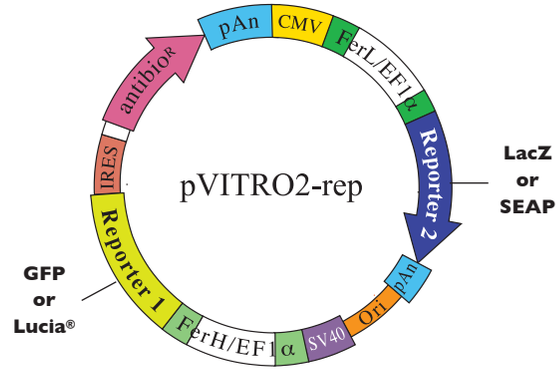
Cheng X. *et al.*, 2012. Surface glycoproteins of influenza A H3N2 virus modulate virus replication in the respiratory tract of ferrets. Virology. 2012 Jun 26. [Epub ahead of print]

Joha S. *et al.*, 2012. GILZ inhibits the mTORC2/AKT pathway in BCR-ABL(+) cells. Oncogene. 31(11):1419-30.

Schaeffer C. *et al.*, 2012. Urinary secretion and extracellular aggregation of mutant uromodulin isoforms. Kidney Int. 81(8):769-78.



MCS 1 5'- Age I, Eco RV, Bam HI, Mlu I, Cla I, Sal I, Avr II -3'
MCS 2 5'- Sgr AI, Fsp I, Bgl II, Eco RI, Bst BI, Xho I, Nhe I-3'



PRODUCT	QTY	CAT. CODE
pVITRO1 Plasmids		
pVITRO1-blasti-mcs	20 µg	pvitro1-bmcs
pVITRO1-blasti-GFP/LacZ	20 µg	pvitro1-bgfplacz
pVITRO1-blasti-GFP/SEAP NEW	20 µg	pvitro1-bgfpsp
pVITRO1-blasti-Lucia/SEAP NEW	20 µg	pvitro1-blucsp
pVITRO1-hygro-mcs	20 µg	pvitro1-mcs
pVITRO1-hygro-GFP/LacZ	20 µg	pvitro1-gfplacz
pVITRO1-hygro-GFP/SEAP NEW	20 µg	pvitro1-gfpsp
pVITRO1-hygro-Lucia/SEAP NEW	20 µg	pvitro1-lucsp
pVITRO1-neo-mcs	20 µg	pvitro1-nmcs
pVITRO1-neo-GFP/LacZ	20 µg	pvitro1-ngfplacz
pVITRO1-neo-GFP/SEAP NEW	20 µg	pvitro1-ngfpsp
pVITRO1-neo-Lucia/SEAP NEW	20 µg	pvitro1-nlucsp
pVITRO2 Plasmids		
pVITRO2-blasti-mcs	20 µg	pvitro2-bmcs
pVITRO2-blasti-GFP/LacZ	20 µg	pvitro2-bgfplacz
pVITRO2-blasti-GFP/SEAP NEW	20 µg	pvitro2-bgfpsp
pVITRO2-blasti-Lucia/SEAP NEW	20 µg	pvitro2-blucsp
pVITRO2-hygro-mcs	20 µg	pvitro2-mcs
pVITRO2-hygro-GFP/LacZ	20 µg	pvitro2-gfplacz
pVITRO2-hygro-GFP/SEAP NEW	20 µg	pvitro2-gfpsp
pVITRO2-hygro-Lucia/SEAP NEW	20 µg	pvitro2-lucsp
pVITRO2-neo-mcs	20 µg	pvitro2-nmcs
pVITRO2-neo-GFP/LacZ	20 µg	pvitro2-ngfplacz
pVITRO2-neo-GFP/SEAP NEW	20 µg	pvitro2-ngfpsp
pVITRO2-neo-Lucia/SEAP NEW	20 µg	pvitro2-nlucsp

Contents and Storage

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 up to one year when properly stored.
Each pVITRO is provided with 4 pouches of the appropriate *E. coli* Fast-Media® (2 TB and 2 Agar).

Related Products

Blasticidin, page 13
HygroGold™, see page 14
Zeocin™, page 15
LyoComp GT116, page 47
LacZ staining kits, page 18
HEK-Blue™ Detection, page 20
G418, page 13
Puromycin, page 14
Fast-Media®, pages 48-49
ChemiComp GT116, page 47
QUANTI-Luc™, page 23
QUANTI-Blue™, page 20

pVIVO - Expression of Two Genes of Interest *in vivo*

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 a pair of multiple cloning sites or a pair of reporter genes.

Features and Benefits

Concomitant Expression of Two Genes of Interest

pVIVO plasmids contain two separate transcription units (TU), each consisting of a strong promoter and an efficient polyadenylation signal. Both promoters in each pVIVO plasmid were chosen for their ability to work simultaneously, eliminating the potential risk of transcriptional interferences between the two TUs.

• **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 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. The resulting SV40/hFerH/mEF1a and CMV/hFerL/chEF1a promoters confer high constitutive levels of transgene expression.

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, were removed from the plasmid backbone. These specific motifs, found in *E. coli* hygromycin resistance (*hph*) and β-galactosidase (*lacZ*) genes, were eliminated by chemically synthesizing a new allele of these genes without altering the protein sequence (see page 56).

Available with Two MCS or Two Reporter Genes

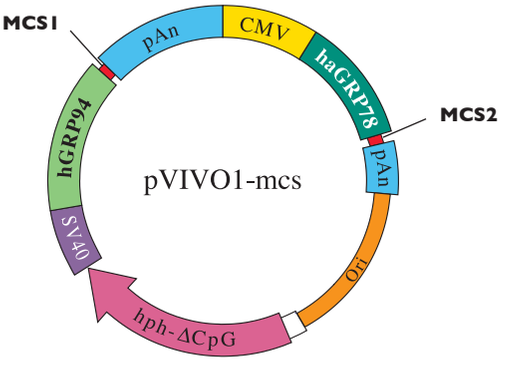
• **pVIVO1-mcs & pVIVO2-mcs** plasmids contain two multiple cloning sites (MCS) for the convenient cloning of cDNAs.

• **pVIVO1-rep & pVIVO2-rep** express two reporter genes:
- GFP and LacZ
- **NEW !** GFP and SEAP
- **NEW !** Lucia® and SEAP

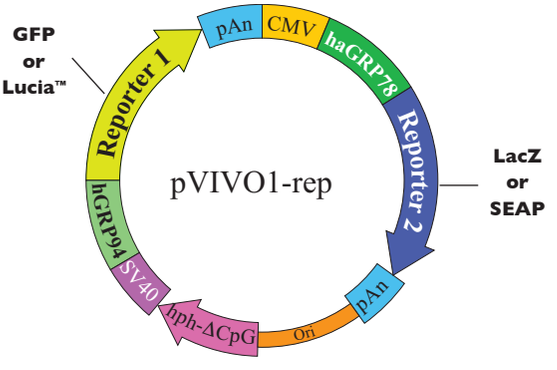
GFP (green fluorescent protein), LacZ (β-galactosidase) and SEAP (secreted embryonic alkaline phosphatase) are commonly used reporter proteins. Lucia® is a novel secreted luciferase with strong bioluminescent activity. The Lucia® reporter gene is ideal for promoter activity and gene expression studies. Lucia® luciferase activity can be detected and quantified directly in the culture medium of transfected cells using InvivoGen's QUANTI-Luc™ detection reagent (see page 23).

pVIVO1-rep and pVIVO2-rep plasmids can be used as control plasmids or cloning vectors. The reporter genes are flanked by unique restriction sites and can be easily replaced by open reading frames chosen from InvivoGen's Gene A-List (see page 39 or visit our website www.invivogen.com/orfs).

Plasmid	Promoters	Expression	Cloning sites/Genes
pVIVO1-mcs	SV40/hGRP94 & CMV/haGRP78	Stress inducible	MCS1 & MCS2
pVIVO1-rep	SV40/hGRP94 & CMV/haGRP78	Stress inducible	- GFP & LacZ - GFP & SEAP - Lucia™ & SEAP
pVIVO2-mcs	SV40/hFerH/ mEF1α & CMV/hFerL/chEF1α	Constitutive	MCS1 & MCS2
pVIVO2-rep	SV40/hFerH/ mEF-1α & CMV/hFerL/chEF1α	Constitutive	- GFP & LacZ - GFP & SEAP - Lucia™ & SEAP



MCS 1 5'- Age I, Bsp HI, Xba I, Hind III, Bst 1107I, Avr II -3'
MCS 2 5'- Ngo MI, Nco I, Bgl II, Eco RI, Nhe I-3'



Recent articles using pVIVO

Augier S. *et al.*, 2010. Inflammatory blood monocytes contribute to tumor development and represent a privileged target to improve host immunosurveillance. J Immunol. 185(12):7165-73.

Charoensit P. *et al.*, 2010. Enhanced growth inhibition of metastatic lung tumors by intravenous injection of ATRA-cationic liposome/IL-12 pDNA complexes in mice. Cancer Gene Ther. 17(7):512-22.

Collins SA. *et al.*, 2010. AAV2-mediated in vivo immune gene therapy of solid tumours. Genet Vaccines Ther. 8:8.

Kumagai M. *et al.*, 2012. Effective transgene expression without toxicity by intraperitoneal administration of PEG-detachable polyplex micelles in mice with peritoneal dissemination. J Control Release. 160(3):542-51.

Mandke R & Singh J., 2012. Cationic nanomicelles for delivery of plasmids encoding interleukin-4 and interleukin-10 for prevention of autoimmune diabetes in mice. Pharm Res. 29(3):883-97.

She K. *et al.*, 2012. Glutamate Binding to GluN2B Controls Surface Trafficking of N-Methyl-D-aspartate (NMDA) Receptors. J Biol Chem. [Epub ahead of print]

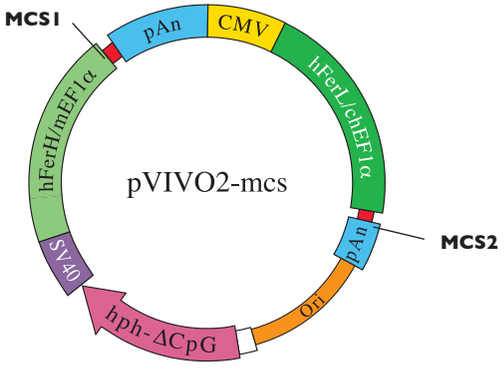
Wiesener N. *et al.*, 2011. Analysis of different DNA vaccines for protection of experimental influenza A virus infection. Viral Immunol. 24(4):321-30.

Yamauchi J. *et al.*, 2011. The atypical Guanine-nucleotide exchange factor, dock7, negatively regulates schwann cell differentiation and myelination. J Neurosci. 31(35):12579-92.

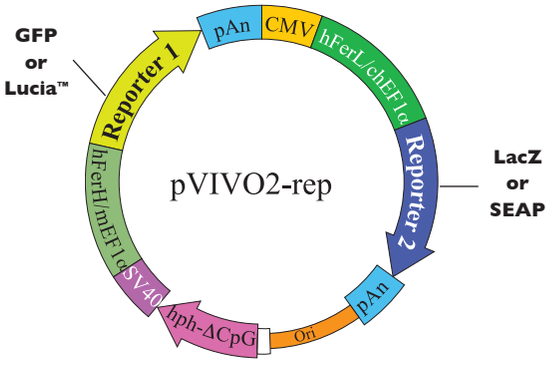
PRODUCT		QTY	CAT. CODE
pVIVO1 Plasmids			
pVIVO1-mcs		20 µg	pvivo1-mcs
pVIVO1-GFP/LacZ		20 µg	pvivo1-gfplacz
pVIVO1-GFP/SEAP	**NEW**	20 µg	pvivo1-gfpsp
pVIVO1-Lucia/SEAP	**NEW**	20 µg	pvivo1-lucsp
pVIVO2 Plasmids			
pVIVO2-mcs		20 µg	pvivo2-mcs
pVIVO2-GFP/LacZ		20 µg	pvivo2-gfplacz
pVIVO2-GFP/SEAP	**NEW**	20 µg	pvivo2-gfpsp
pVIVO2-Lucia/SEAP	**NEW**	20 µg	pvivo2-lucsp

Contents and Storage

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 up to one year when properly stored.
Each pVIVO is provided with 4 pouches of *E. coli* Fast-Media® Hygro (2 TB and 2 Agar).



MCS 1 5'- Bsp HI, Xba I, Bsr GI, Bst 1107I, Avr II -3'
MCS 2 5'- Nco I, Bam HI, Eco RI, Eco RV, Nhe I-3'



Related Products

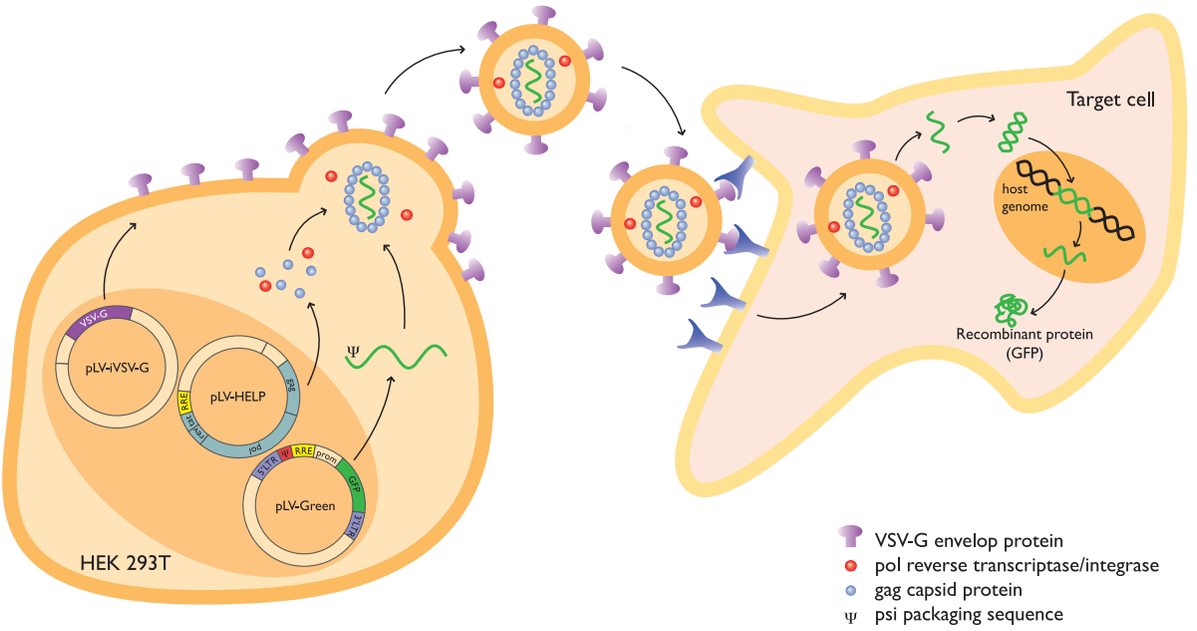
Hygromycin B, see page 14
LyoComp GT116, page 47
LacZ staining kits, page 18
HEK-Blue™ Detection, page 20
Fast-Media®, pages 48-49
ChemiComp GT116, page 47
QUANTI-Luc™, page 23
QUANTI-Blue™, page 20

LENTIVIRAL VECTORS - LENTI-Smart™

Lentiviral vectors derived from the human immunodeficiency virus (HIV-1) have become major tools for gene delivery in mammalian cells. The primary feature of lentivirus vectors is the ability to mediate potent transduction and stable expression into dividing and non-dividing cells, both *in vitro* and *in vivo*. Currently, production of high titers of lentiviral vectors is a time consuming, multi-step procedure with low reproducibility. LENTI-Smart™ has been designed to solve these problems, by creating a lyophilizate of optimized packaging plasmids combined with a DNA transfection reagent. Upon rehydration, this lyophilizate serves as a "carrier" for your preferred lentiviral expression plasmid. Depending on your preference, LENTI-Smart™ kits are available for the production of either **integrating or non-integrating lentiviral (NIL) vectors**.

- **Consistent high titers -** 1-5 x 10^6 transducing units (TU) per ml (unconcentrated) are commonly achieved.
- **Fast -** Lentiviral production time reduced and no need to produce the packaging and pseudotyping plasmids
- **Simple -** Everything is ready for your experiment, just add your favorite lentiviral expression plasmid.
- **Versatile -** Can be used with most commercially available lentiviral expression plasmids.
- **Reproducible -** Fluctuating parameters are kept to a minimum.

Lentiviral Vector Production and Cell Transduction



Lentiviral vectors are typically produced in HEK 293T cells. Essential lentiviral (HIV-1) genes must be expressed in these cells to allow the generation of lentiviral particles. These genes are usually expressed by several plasmids: (i) a lentiviral expression plasmid, such as pLV-Green, containing the *psi* (Ψ) packaging sequence and the transgene gene inserted between the lentiviral LTRs for target cell integration, (ii) a packaging plasmid, such as pLV-HELP, encoding the *pol, gag, rev* and *tat* viral genes and containing the *rev*-response element (RRE); and (iii) a pseudotyping plasmid, such as pLV-iVSV-G, encoding the G protein of the Vesicular Stomatitis Virus (VSV-G) envelope gene. Unlike the HIV envelope, the VSV-G envelope has a broad cell host range extending the cell types that can be transduced by VSV-G-expressing lentiviruses. Two days after transfection of HEK 293T cells, the cell supernatant contains recombinant lentiviral vectors, which can be used to transduce the target cells. Once in the target cells, the viral RNA is reverse-transcribed, imported into the nucleus and stably integrated into the host genome. One or two days after the integration of the viral RNA, the expression of the recombinant protein can be detected.

LENTI-Smart™ (INT) - Generation of Integrating Lentiviruses

The unique formulation of LENTI-Smart™ allows the preparation of lentiviral expression plasmid / packaging plasmids complexes by simply rehydrating the lyophilizate with the lentiviral expression plasmid solution. There is no need for a transfection reagent as it is included in the LENTI-Smart™ lyophilizate. Transfection of HEK 293T cells is readily performed by adding the complexes to the cells. Lentiviral particles can be collected 2 days after transfection.

Description

LENTI-Smart™ (INT) is a ready-to-use product that allows for rapid and reliable production of high titers of second generation lentiviral particles in HEK 293T cells. LENTI-Smart™ (INT) combines a mix of optimized packaging plasmids, pLV-iVSV-G and pLV-HELP, precomplexed to a transfection reagent, LyoVec™, selected for its high transfection efficiency and low cell toxicity. This lyophilized complex is provided with a control lentiviral expression plasmid, pLV-Green.

Packaging Plasmids

The two packaging plasmids forming the LENTI-Smart™ (INT) lyophilizate provide the structural and replication proteins *in trans* that are required for the production of the lentiviral particles (see page 36).

• **pLV-iVSV-G** expresses the G glycoprotein gene from Vesicular Stomatitis Virus (VSV-G) to allow production of a pseudotyped lentiviral vector with a broad host range.

• **pLV-HELP** contains the viral *gag*, *pol*, *rev* and *tat* genes and the *rev*-responsive element (RRE).

- *gag* encodes the membrane associated and capsid proteins.
- *pol* encodes viral enzymes, including the reverse transcriptase and integrase, required for replication and integration.
- *tat* encodes the virus transactivator protein.
- *rev* encodes the Rev protein which interacts with the RRE to induce expression of the *gag* and *pol* genes.
- RRE permits Rev-dependent expression of the *gag* and *pol* genes.

Control Lentiviral Expression Plasmid

LENTI-Smart™ (INT) is provided with a control lentiviral expression plasmid designed to optimize virus production and cell transduction.

• **pLV-Green** expresses a green fluorescent protein (GFP) gene and contains key viral elements for lentivirus production and safety:

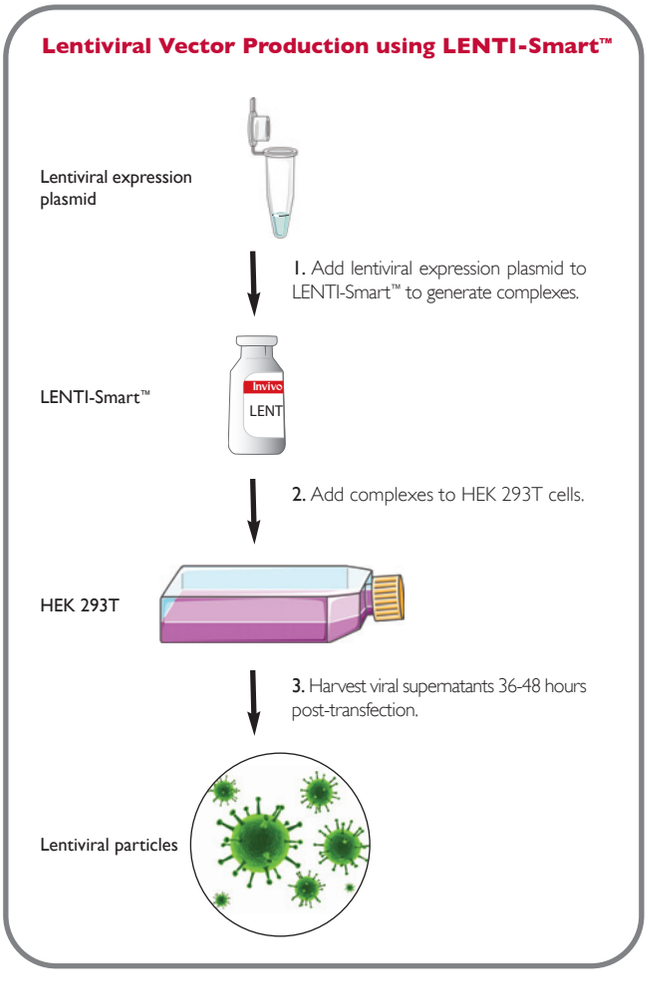
- *psi* encodes the packaging signal.
- Long terminal repeats containing a deletion in the U3 region to ensure self-inactivation of the lentiviral vector.
- *rev* and RRE (see above).

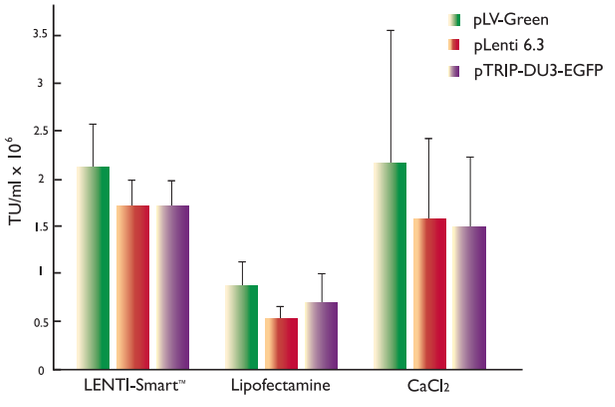
Contents and Storage

LENTI-Smart™ (INT) is available in 2 sizes, either 5 vials or 10 vials. Each vial allows the transfection of HEK 293T cells with a lentiviral expression plasmid in a 10-cm culture plate or a 75 cm^2 flask..

LENTI-Smart™ (INT) is provided with a vial of the control lentiviral expression plasmid. The LENTI-Smart™ vials are provided lyophilized, the control plasmid is provided as a liquid. Products are shipped at room temperature and should be stored at -20°C.

PRODUCT	QTY	CAT. CODE
LENTI-Smart™ (INT)	5 vials 10 vials	ltsint-5 ltsint-10





Viral titers obtained using LENTI-Smart™ and other transfection reagents with different commercially available lentiviral expression plasmids.

LENTI-Smart™ NIL - Generation of Non-Integrating Lentiviruses

Integration of a transgene into the host genome poses safety concerns due to the potential risk of insertional mutagenesis. To overcome this problem, non-integrating lentiviral vectors have been designed that combine the advantageous features of lentiviral vectors with episomal replication alleviating the risk of insertional mutagenesis. InvivoGen provides LENTI-Smart™ NIL, the first kit designed for the generation of non-integrating lentiviral vectors.

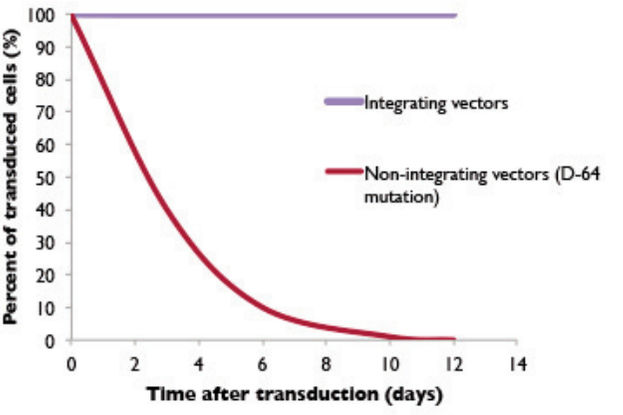
Description

Non-integrating lentiviral vectors are produced through the use of a mutated integrase protein as circular vector episomes. They are gradually lost by dilution in dividing cells providing transient transgene expression, but are stable in in non-dividing cells allowing long-term transgene expression.
Similar to LENTI-Smart™ (INT), LENTI-Smart™ NIL combines two packaging plasmids precomplexed to the transfection reagent, LyoVec™, and is provided with a control lentiviral plasmid. The packaging plasmids are:
• **pLV-iVSV-G** (see page 37)
• **pLV-HELP-NIL D64** which expresses a mutant integrase (D64V) resulting in the generation of lentiviral vectors that are integration defective[1,2].
The control lentiviral plasmid is:
• **pLV-Green** which expresses a GFP gene (see page 37).

1. Leavitt AD. ***et al.,*** **1996.** Human immunodeficiency virus type 1 integrase mutants retain in vitro integrase activity yet fail to integrate viral DNA efficiently during infection. J Virol. 70(2):721-8. **2. Nightingale SJ.** ***et al.*****, 2006.** Transient gene expression by nonintegrating lentiviral vectors. Mol Ther. 13(6):1121-32.

Contents and Storage

LENTI-Smart™ NIL is available in 2 sizes, either 5 vials or 10 vials. Each vial allows the transfection of HEK 293T cells with a lentiviral expression plasmid in a 10-cm culture plate or a 75 cm^2 flask.
LENTI-Smart™ NIL is provided with a vial of the control lentiviral expression plasmid. The LENTI-Smart™ NIL vials are provided lyophilized, the control plasmid is provided as a liquid. Products are shipped at room temperature and should be stored at -20°C.



Duration of GFP expression using integrating or non-integrating (NIL) lentiviral vectors. Percentage of GFP positive HCT116 cells was assessed by FACS analysis, following transduction with LENTI-Smart™ (INT)- or LENTI-Smart™ NIL-derived lentiviral vectors.

PRODUCT	QTY	CAT. CODE
LENTI-Smart™ NIL Kit	5 vials 10 vials	ltsnil-5 ltsnil-10

LENTI-Smart™ Starter Kit

Generation of Integrating and Non-Integrating Lentiviruses

Description

LENTI-Smart™ Starter Kit allows the generation of integrating and non-integrating lentiviral vectors to compare their potential.
LENTI-Smart™ Starter Kit contains LENTI-Smart™ (INT) and LENTI-Smart™ NIL vials. It also contains a vial of the control lentiviral expression plasmid, pLV-Green.

PRODUCT	QTY	CAT. CODE
LENTI-Smart™ Starter Kit	10 vials	lts-str

Contents and Storage

The LENTI-Smart™ Starter Kit contains 5 vials of LENTI-Smart™ (INT), 5 vials of LENTI-Smart™ NIL and one vial of the control lentiviral expression plasmid. The LENTI-Smart™ vials are provided lyophilized, the control plasmid is provided as a liquid. Products are shipped at room temperature and should be stored at -20°C.

GENES - Gene A-List™ - Human and Murine ORFs

Gene A-List™ is a collection of high quality, full-length sequenced human and mouse open reading frames (ORFs) available in an expression vector ready-to-use for protein expression. All ORFs are cloned downstream of a strong and ubiquitous mammalian promoter which makes these clones suitable for expression and functional studies in various mammalian cell lines.

Features and Benefits

Full-length Sequenced Open Reading Frames

InvivoGen provides a large collection of human and murine genes, fully sequenced. 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. Furthermore for cloning convenience, a neutral amino acid is sometimes added immediately after the Start codon in order to create a suitable cloning site.
Within the Gene A-List™, some genes 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.
The ORFs are supplied in two different plasmid backbones:

- **pORF**, a plasmid selectable only in *E. coli* with ampicillin
- **pBLAST/pUNO**, a plasmid selectable in mammalian cells and in *E. coli* with blasticidin.

Search the Gene A-List™ online

You can easily find your ORF of interest in the Gene A-List™ 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.

MAJOR GENE FAMILIES	EXAMPLES
Adaptor Genes	MyD88, TIRAP, TRAM, TRIF/TICAM1
Angiogenic & Angiostatic Genes	ANGPT-1, NRP1, ANGIO, ENDO, FLK1, FLT1
Apoptotic & Anti-Apoptotic Genes	BAD, Bcl-XS, BIK, Bcl-XL, Bcl-2, Survivin
Autophagy Genes	Atg5, Beclin-1, LC3b
Cellular Matrix Genes	Caveolin-1, Decorin
Chemokine & Chemokine Receptor Genes	IP-10, LIX, MIG, MIP-1, PF4, SDF1, CCR4, CCR5, CXCR3
Chromatin-Remodeling Genes	DNMT1, HDAC1, KAISO, SUV39H1
Connexin Genes	Cx26, Cx32, Cx43
Co-Receptor Genes	CD14, LBP, MD1, MD2
Co-Stimulatory / CD Genes	4-1BB, B7.1, B7.2, OX40L, CD4, CD8, CD40, CD70
Cytokine & Cytokine Suppressor Genes	GM-CSF, IL-2, IL-10, IL-12, LIF, TNF-α, SOCS-1, SOCS-2
Cytolytic Genes	Granulysin, Granzyme, Perforin
Cytotoxic / Suicide Genes	CD, UPRT, HSV1-TK
DAMP & DAMP Receptor Genes	HMGB1, S100A8, S100A9, SAA1, Mincle, RAGE, TREM-1
Growth Factors	EGF, FGF, HGF, IGF, TGF-β, VEGF
Hematopoietic Genes	EPO, HOXB4, TPO
Immune Receptors	FcγR, IL-1R, IL-6R, IL-10R, TNFRSF
Inhibitors of Differentiation	Id1, Id2, Id3, Id4
Interferon & Interferon Signaling Genes	IFN-α, IFN-β, IFN-γ, IRF1, IRF3, IRF5, IRF7
Pattern Recognition Receptors	TLR1-13, NOD1, NOD2, NLRP3, MDA-5, RIG-I, Dectin-1
Reprogramming Factors (IPSC)	Klf4, Lin28, c-Myc, Nanog, Oct4, Sox2
Signaling Genes	AKT, FADD, IPS-1, IRAK, RIP1, STAT, TAK1, TBK1, TRAF6
Signaling Inhibitors	A20, Bcl-3, IRAK-M, SIGGIR, SIKE, Tollip
Transcription Factors	c-REL, IRF, NF-κB, SMAD, STAT
Tumor Antigens	MAGE
Tumor Suppressors	HRAS, KRAS, p21, p53, PTEN

pORF

Description

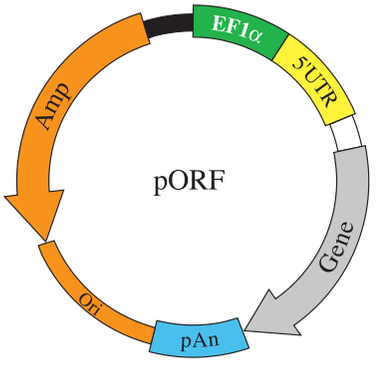
pORF plasmids feature a large collection of native and fusion genes as open reading frames (ORF). The expression of each ORF is under the control of a strong and ubiquitous mammalian promoter. This promoter consists of the elongation factor 1 alpha (EF-1α) core promoter combined to the 5'UTR of either the human T cell leukemia virus (HTLV) type 1 long terminal repeat or the human eukaryotic initiation factor 4g (eIF-4g). These two composite promoters yield similar expression levels.

pORF plasmids are selectable in bacteria with ampicillin. The bacterial selection cassette can be replaced upon request with a selection cassette that functions both in bacteria and mammalian cells. This cassette subcloned from the pSELECT can express the resistance gene of your choice.

pORF plasmids containing a multiple cloning site (MCS) in place of an ORF were designed to serve as controls and/or cloning vectors. The MCS features commonly used restriction sites to allow convenient insertion of an ORF.

Contents and Storage

Each pORF plasmid is provided as a lyophilized transformed *E. coli* strain on a paper disk. Transformed strains are shipped at room temperature and should be stored at -20°C. Lyophilized *E. coli* cells are stable for at least one year when properly stored.
Each pORF is provided with 4 pouches of *E. coli* Fast-Media® Amp (2 TB and 2 Agar).



PRODUCT	QTY	CAT. CODE
pORF-<Gene>	*E. coli* disk	porf-<gene>
pORF-mcs	*E. coli* disk	porf-mcs

*Visit www.invivogen.com/ORF.php for complete ordering information

Related Products

Fast-Media® Amp, page 48 — pSELECT, page 30

pBLAST & pUNO

Description

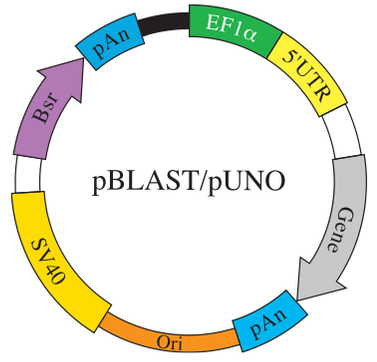
pBLAST/pUNO plasmids feature a wide choice of native and fusion genes, mainly involved in innate immunity. The gene of interest is under the control of the same promoter as the pORF plasmids (EF-1α/HTLV or EF-1α/eIF-4g promoter), allowing for strong expression in mammalian cells.

pBLAST/pUNO plasmids contain the blasticidin resistance gene (*bsr*) under the control of a mammalian promoter in tandem with a bacterial promoter. This allows the amplification of the plasmid in *E. coli* and the selection of stable clones in mammalian cells. Blasticidin is a potent antibiotic that permits the selection of transfectants in only a few days.

pBLAST-mcs and pUNO-mcs plamids were designed to serve as control and/or cloning vectors. They carry a multiple cloning site (MCS) that features commonly used restriction sites to allow convenient insertion of an open reading frame.

Contents and Storage

pBLAST plasmids are provided as 20 µg of lyophilized DNA. pUNO plasmids are provided as lyophilized transformed *E. coli* strains on a paper disk. Products are shipped at room temperature. Store at -20°C. Plasmids and lyophilized *E. coli* cells are stable up to one year when properly stored.
Each pBLAST and pUNO is provided with 4 pouches of *E. coli* Fast-Media® Blas (2 TB and 2 Agar).



PRODUCT	QTY	CAT. CODE
pBLAST-<Gene>	20 µg	pbla-<gene>
pBLAST-mcs	20 µg	pbla-mcs
pUNO-<Gene>	*E. coli* disk	puno-<gene>
pUNO-mcs	*E. coli* disk	puno-mcs

*Visit www.invivogen.com/gene.php for complete ordering information

Related Products

Fast-Media® Blas, page 48 — Blasticidin, page 13

CELLULAR PROMOTERS - Prom A-List™

Prom A-List™ is an expanding collection of native and composite promoters provided in the pDRIVE plasmid. Promoters are valuable tools to study the expression of a given gene both *in vitro* and *in vivo*. Now with pDRIVE, 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.

Features and Benefits

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 a specific type of cells or tissues.
• **Tumor Specific Promoters,** active specifically in tumor cells.

Easy Subcloning of the Promoters

In every pDRIVE, several unique restriction sites flank the promoter (a multiple cloning site at the 5' end and Nco I or Bsp HI at the 3' end) 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*

pDRIVE 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*.

Two Backbones with Different Reporter Genes

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

• LacZ in pDRIVE

pDRIVE plasmids contain the LacZ reporter gene which expression can be determined using chromogenic (see page 18), luminescent or histochemical detection.

• SEAP in pDRIVE5

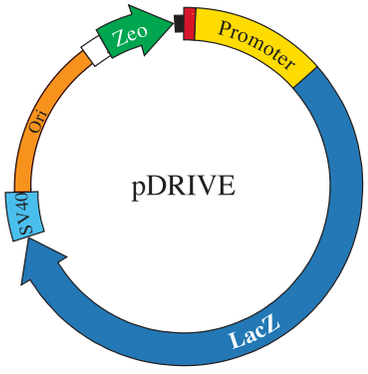
pDRIVE5-SEAP plasmids express the secreted embryonic alkaline phosphatase (SEAP) reporter gene. SEAP expression levels can be readily assessed using chromogenic assays, such as QUANTI-Blue™, or luminescence assays.

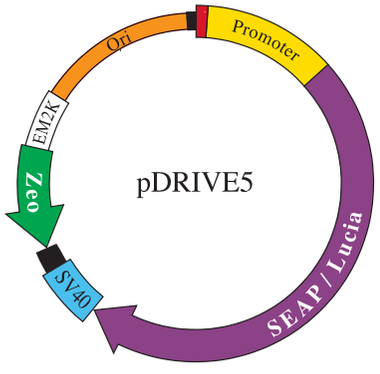
• **NEW!** Lucia® in pDRIVE5

pDRIVE5-Lucia plasmids feature the Lucia® gene, encoding a new 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® can be determined by measuring the luminescence using coelenterazine-based reagents such as QUANTI-Luc™.

Related Products

Custom-pDRIVE, page 45
QUANTI-Blue™, page 20
Fast-Media® Zeo, page 49
QUANTI-Luc™, page 23





Contents and Storage

pDRIVE plasmids are provided as transformed *E. coli* strains lyophilized on a paper disk. Products are shipped at room temperature. Store at -20°C. pDRIVE-transformed *E. coli* strains are stable for at least 1 year when properly stored. Each pDRIVE plasmid is provided with 4 pouches of *E. coli* Fast-Media® Zeo (2 TB and 2 Agar).

PRODUCT	QTY	CAT. CODE*
pDRIVE (LacZ)-<promoter>	*E. coli* disk	pdrive-<prom>
pDRIVE5-SEAP-<promoter>	*E. coli* disk	pdrive5s-<prom>
pDRIVE5-Lucia-<promoter> **NEW**	*E. coli* disk	pdrive5lc-<prom>

* Complete list available at www.invivogen.com/prom-a-list

PROMOTER	GENE	TISSUE DISTRIBUTION	SPECIES	SIZE	CAT. CODE*
Ubiquitous Native Promoters					
β-Act	Beta-Actin	Ubiquitous	Human	2387 bp	pdrive-hbact
EF-1α	Elongation Factor 1 alpha	Ubiquitous	Chimpanzee Mouse Rat	1365 bp 1315 bp 1314 bp	pdrive-chef1 pdrive-mef1 pdrive-ref1
EGR1	Early Growth Response 1	Ubiquitous, inducible by radiation	Human	1062 bp	pdrive-hegr1
eIF4A1	Eukaryotic Initiation Factor 4A1	Ubiquitous	Human Mouse	523 bp 482 bp	pdrive-heif4a1 pdrive-meif4a1
FerH	Ferritin Heavy Chain	Ubiquitous	Human	370 bp	pdrive-hferh
FerL	Ferritin Light Chain	Ubiquitous	Human	429 bp	pdrive-hferl
GAPDH	Glyceraldehyde 3-phosphate Dehydrogenase	Ubiquitous	Human	794 bp	pdrive-hgapdh
GRP78	Glucose-Regulated Protein 78	Ubiquitous, inducible by stress	Human Mouse Rat	531 bp 545 bp 679 bp	pdrive-hgrp78 pdrive-mgrp78 pdrive-rgrp78
GRP94	Glucose-Regulated Protein 94	Ubiquitous, inducible by stress	Human Mouse Rat	701 bp 696 bp 731 bp	pdrive-hgrp94 pdrive-mgrp94 pdrive-rgrp94
HSP70	Heat Shock Protein 70	Ubiquitous, inducible by heat	Human Mouse	464 bp 1041 bp	pdrive-hhsp70 pdrive-mhsp70
β-Kin	Beta-Kinesin	Ubiquitous	Human	539 bp	pdrive-hbkin
PGK1	Phosphoglycerate Kinase 1	Ubiquitous	Mouse	1428 bp	pdrive-mpgk
ROSA	Rosa	Ubiquitous	Human Mouse	2815 bp 1926 bp	pdrive-hrosa pdrive-mrosa
Ubi B	Ubiquitin B	Ubiquitous	Human	1092 bp	pdrive-hubiquitinb
Ubiquitous Composite Promoters					
β-Act/RU5'	Beta-Actin prom / HTLV 5'UTR	Ubiquitous	Human	1785 bp	pdrive-hbactru5
EF-1α/RU5'	Elongation Factor 1 prom / HTLV 5'UTR	Ubiquitous	Chimpanzee Rat	672 bp 630 bp	pdrive-chef1ru5 pdrive-ref1ru5
FerH/RU5'	Ferritin H prom / HTLV 5'UTR	Ubiquitous	Human	457 bp	pdrive-hferhru5
SV40/FerH	SV40 enh / Ferritin H prom / EF-1α 5'UTR	Ubiquitous	Human / mouse	1423 bp	pdrive-sv40ferhef1
FerL/RU5'	Ferritin L prom / HTLV 5'UTR	Ubiquitous	Human	537 bp	pdrive-hferlru5
CMV/FerL	CMV enh / Ferritin L prom / EF-1α 5'UTR	Ubiquitous	Human / chimp.	1666 bp	pdrive-cmvferlef1
Tissue-Specific Native Promoters					
B29	Immunoglobulin Beta	B cells	Human Mouse	1219 bp 1235 bp	pdrive-hb29 pdrive-mb29
CD14	Monocyte Receptor for Bacterial LPS	Monocytic cells	Human	612 bp	pdrive-hcd14
CD43	Leukosialin, Sialophorin	Leukocytes and platelets	Human	775 bp	pdrive-hcd43
CD45	Leukocyte Common Antigen (LCA)	Hematopoietic cells	Human	856 bp	pdrive-hcd45
CD68	Human Homolog of Macrosialin	Macrophages	Human Mouse	658 bp 811 bp	pdrive-hcd68 pdrive-mcd68
Desmin	Desmin	Muscle	Mouse	983 bp	pdrive-mdesmin
Elastase	Elastase1	Pancreatic acinar cells	Rat	264 bp	pdrive-relastase
Endoglin	Endoglin	Endothelial cells	Human	886 bp	pdrive-hendoglin
FN	Fibronectin	Differentiating cells, healing tissues	Human	1037 bp	pdrive-hfibronectin
Flt-1	VEGF Receptor 1	Endothelial cells	Human	786 bp	pdrive-hflt1
GFAP	Glial Fibrillary Acidic Protein	Astrocytes	Human Mouse Rat	1675 bp 1679 bp 1588 bp	pdrive-hgfap pdrive-mgfap pdrive-rgfap
GPIIb	Integrin alpha IIb	Megakaryocytes	Human	928 bp	pdrive-hgp2b
ICAM-2	Intercellular Adhesion Molecule 2	Endothelial cells	Human	397 bp	pdrive-hicam2

***Check our website for more information on the catalog codes at www.invivogen.com/prom-a-list**

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

Recent articles using pDRIVE plasmids

pDRIVE-hB29 - Taylor CA. *et al.*, 2012. Modulation of eIF5A Expression Using SNS01 Nanoparticles Inhibits NF-κB Activity and Tumor Growth in Murine Models of Multiple Myeloma. Mol Ther. 20(7):1305-14.

pDRIVE-mCD68 - Gautier EL. *et al.*, 2009. Macrophage apoptosis exerts divergent effects on atherogenesis as a function of lesion stage. Circulation. 119(13):1795-804.

pDRIVE-hHSP70 - Kobelt D. *et al.*, 2010. Activation of the CMV-IE promoter by hyperthermia in vitro and in vivo: biphasic heat induction of cytosine deaminase suicide gene expression. Mol Biotechnol. 46(2):197-205.

pDRIVE-mOG2 - Feichtinger GA. *et al.*, 2011. Enhanced reporter gene assay for the detection of osteogenic differentiation. Tissue Eng Part C Methods. 17(4):401-10.

pDRIVE-SV40/AFP - Zhang KJ. *et al.*, 2012. Targeting Gene-Viro-Therapy with AFP driving Apoptin gene shows potent antitumor effect in hepatocarcinoma. J Biomed Sci. 19:20.

pDRIVE-PSA/hPSA - Mashima T. *et al.*, 2010. Pharmacological targeting of constitutively active truncated androgen receptor by nigericin and suppression of hormone-refractory prostate cancer cell growth. Mol Pharmacol. 78(5):846-54.

PromTest™ - Ten Promoters to Choose From

Promoters regulate differently gene expression depending on the cellular context. To help you determine easily and rapidly the best promoter for your cell line, InvivoGen offers PromTest™, a collection of ten ubiquitous promoters driving the GFP reporter gene.

- Ten ready-to-use promoters with different levels of expression
- Drive GFP expression for convenient monitoring of promoter strength
- Cost-effective

Description

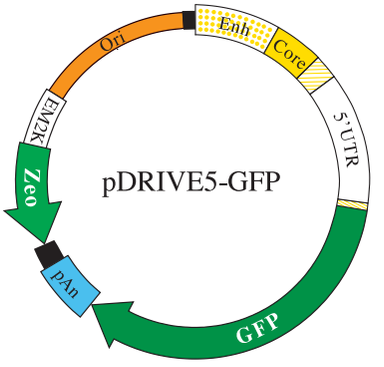
PromTest™ is a collection of ten ubiquitous composite promoters provided in the pDRIVE5-GFP plasmid. These composite promoters were generated by assembling enhancers, core promoters and 5'UTRs of different origins. The activity of each combination depends on the cellular context (see graphs).

pDRIVE5-GFP features a GFP reporter gene for convenient monitoring of promoter activity. The strength of each promoter can be assessed qualitatively by fluorescence microscopy and quantitatively using a fluorometer or flow cytometry.

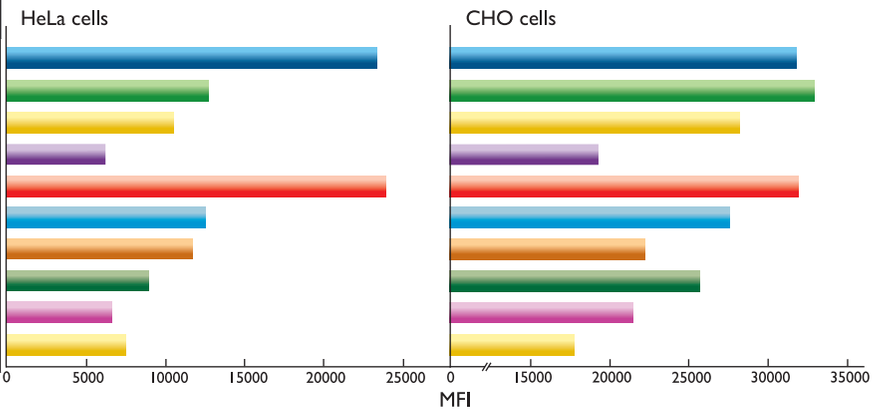
pDRIVE5-GFP plasmids are selectable in *E. coli* with Zeocin™.

PromTest™ contains 5 µg of each plasmid, enough for multiple transfections using your favorite reagent or technique. pDRIVE5-GFP plasmids can be amplified in any common *E. coli* laboratory strains. They are also available individually as 20 µg high-quality endotoxin-free DNA.

Once you have determined the best promoter for your cell line and application, you can either replace the GFP gene with your gene of interest, or subclone the promoter into another plasmid with mammalian selection, such as InvivoGen's pSELECT.



Plasmid	Enhancer	Core Promoter	5' UTR
pDRIVE5-GFP-1	hCMV	hEF1	HTLV
pDRIVE5-GFP-2	mCMV	hEF1	HTLV
pDRIVE5-GFP-3	SV40	hEF1	HTLV
pDRIVE5-GFP-4	mTyr	hEF1	HTLV
pDRIVE5-GFP-5	hCMV	hCMV	HTLV
pDRIVE5-GFP-6	SV40	hCMV	HTLV
pDRIVE5-GFP-7	hCMV	hFerL	chEF1
pDRIVE5-GFP-8	mCMV	hFerL	chEF1
pDRIVE5-GFP-9	SV40	hFerL	chEF1
pDRIVE5-GFP-10	hAldA	hFerL	chEF1



Evaluation of PromTest™ in different cell lines: HeLa (human cervical cancer) and CHO (chinese hamster ovary) cells were transiently transfected with each of the 10 pDRIVE5-GFP plasmids of PromTest™ using LyoVec™ (cat. code: lyec-12). The strength of the various promoters was analyzed by flow-cytometry 48h after transfection.

Abbreviations: chEF1, chimpanzee elongation factor 1 alpha; hAldA, human aldolase A; hCMV, human cytomegalovirus; hEF1, human elongation factor 1 alpha; hFerL, human ferritin light chain; HTLV, human T lymphocyte virus; mCMV, mouse cytomegalovirus; mTyr, mouse tyrosinase; SV40, simian virus 40.

Contents and Storage

PromTest™ contains 10 plasmids, each provided as 5 µg lyophilized DNA. Each plasmid can also be purchased individually as 20 µg high-quality endotoxin-free DNA. Plasmids are shipped at room temperature. Store at -20°C. Plasmids are stable up to one year when properly stored.

PRODUCT	QTY	CAT. CODE
PromTest™	10 x 5 µg	prom-test
pDRIVE5-GFP-n	20 µg	pdv5-gfp<n>

Custom-Made pDRIVE - Create Your Own Composite Promoter

You can create your own composite promoter by combining the enhancer, core promoter and 5'UTR of your choice. We will assemble your composite promoter in one of our ready-made pDRIVE plasmids.

Description

Customize Your Promoter

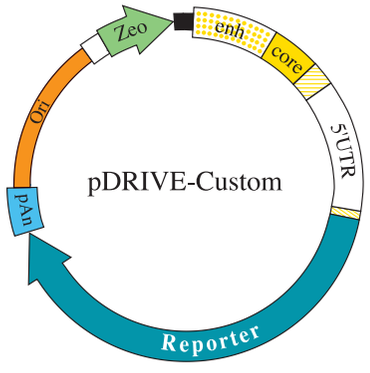
Choose the enhancer, the core promoter and 5'UTR from InvivoGen's lists, and we will generate the composite promoter of your choice.
- **Enhancer:** InvivoGen provides a choice of ubiquitous or specific enhancers. Choose any enhancer from the list below:
- **Core promoter:** You can choose the core promoter from the list of ubiquitous or specific native promoters (see pages 42-43).
- **5'UTR:** The 5' untranslated region can be chosen from the list below:

Pick Your Selection

Custom-made pDRIVE carry the Zeocin™ resistance gene for selection and amplification in *E. coli*. We can replace the bacterial selection cassette with a mammalian selection cassette of your choice derived from a pSELECT plasmid (see page 30).

Choose Your Reporter

- **LacZ** which expression can be determined using chromogenic, luminescent or histochemical detection.
- **Lucia®,** a new secreted synthetic coelenterazine-utilizing luciferase that can be directly measured in the cell culture supernatant using bioluminescent assays, such as QUANTI-Luc™ (see page 23).
- **GFP** which expression can be assessed qualitatively by fluorescence microscopy and quantitatively using a fluorometer or flow cytometry.
- **SEAP** (secreted embryonic alkaline phosphatase) which expression levels can be assayed with luminescent or chromogenic methods, such as QUANTI-Blue™ (see page 20).



Contents and Storage

Each custom-made pDRIVE plasmid is provided as 20 µg lyophilized DNA. Product is shipped at room temperature. Store at -20°C. Plasmids are stable up to one year when properly stored.

PRODUCT	QTY	CAT. CODE
pDRIVE-custom	20 µg	p-custom

Enhancers

Enhancer	Name	Origin	Size	Specificity	References
AFP	Alpha-Fetoprotein	Human	1854 bp	AFP-positive cells	Cao G., 2001, Eur J Cancer. 37(1):140-7
CEA	Carcinoembryonic antigen	Human	1434 bp	CEA-positive cells	Richards CA.,1995, Hum Gene Ther. 6:881-893
CMV	Cytomegalovirus	Viral	405 bp	Ubiquitous	Liu BH., 2004, Gene Ther. 11(1):52-60
P2	Adipocyte P2	Mouse	511 bp	Adipose tissue	Ross SR., 1990, PNAS 1990 Dec;87(24):9590-4
PSA	Prostate specific antigen	Human	1591 bp	PSA-positive cells only	Lee SE., 2000, Anticancer Res. 20(1A):417-22
SV40	Simian virus	Viral	235 bp	Ubiquitous	Moreau P., 1981, Nucleic Acids Res. 9(22):6047-68
Tie2	Angiopoietin receptor	Mouse	282 bp	Vascular ECs	Schlaeger TM., 1997, PNAS. 94(7):3058-63
Tyr	Tyrosinase	Mouse	201 bp	Melanocytes and melanoma	Park BJ., 1999, Hum Gene Ther 10(6):889-98

5' Untranslated Regions

5'UTR	Name	Origin	Size	Activity	References
AdTp	Adenotripartite	Viral	135 bp	Increases mRNA translation	Sheay W., 1993, Biotechniques 15(5):856-62
EF-1α	Elongation factor 1 alpha	Chimpanzee Mouse	988 bp 1014 bp	Increases mRNA translation	Kim SY., 2002, J Biotechnol. 14;93(2):183-7
eIF4g	Eukaryotic initiation factor 4g	Human	314 bp	Contains a putative IRES	Han B., 2002, Mol Cell Biol 22(21):7372-84
HSP70	Heat shock protein 70	Human	195 bp	Increases mRNA translation	Vivinus S., 2001, Eur J Biochem. 268(7):1908-17
NRF	NF-κB repressing factor	Mouse	880 bp	Acts as a potent IRES	Oumard A., 2000, Mol Cell Biol. 20(8):2755-9
HTLV RU5'	Human T-cell leukemia virus	Human	267 bp	Increases mRNA translation	Takebe Y., 1988, Mol. Cell Biol. 8(1): 466-472

E. COLI COMPETENT CELLS

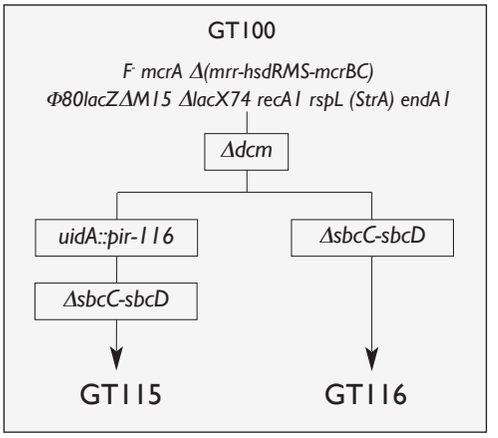
InvivoGen's competent *E. coli* strains have been designed to include genotypes that make them efficient and convenient. These isogenic strains contain genetic modifications which are introduced using an elaborate technique of homologous recombination. This technique allows the generation of targeted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

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 *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 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.

Significant Genetic Markers

Marker	Description	Significance
dcm	Deletion eliminating Dcm methylase activity	Allows for the production of high quality plasmid DNA
endA1	Mutation inactivating endonuclease 1, a potent and non-specific enzyme	Improves the quality of purified plasmid DNA
hsdR	Restriction endonuclease mutation abolishing restriction and modification (r-, m-)	Permits introduction of DNA coming from non-*E. coli* sources such as PCR amplified DNA
lacZΔM15	Defective Φ80 prophage carrying a partial deletion of LacZ that allows α-complementation	Allows for blue/white color selection of clones when supplemented with X-Gal
mcrA, mcrBC	Mutation abolishing degradation of DNA with methylated cytosine	Allows more efficient cloning of 5-methylcytosine containing DNA
mrr	Mutation eliminating restriction of DNA containing 6-methyladenine or 6-methylcytosine	Allows more efficient cloning of DNA containing methyladenine residues
recA	Homologous recombination deficiency	Transformed DNA will not recombine with host DNA ensuring the stability of inserts
uidA::pir-116	*pir* gene integrated in the *uidA* sequence	Allows for high copy number of plasmids containing the R6Kγ origin of replication
sbcC-sbcD	Deletion eliminating the recognition and cleavage of hairpin structures	Increases the stability of hairpin structures and improves the number of recombinant clones

ChemiComp and LyoComp *E. coli*

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.

LyoComp *E. coli* are chemically competent bacteria that have been lyophilized using a proprietary method. LyoComp *E. coli* are easy to handle and more stable than standard competent cells. They are shipped at room temperature eliminating the need of costly dry ice shipping. Their competency is sufficient for cloning and propagation of siRNA-expressing plasmids.

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 pCpG-siRNA plasmids.
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, such as psiRNA plasmids.
Transformation efficiency: 0.1-1 x 10^9 cfu/µg

Contents and Storage

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.) 5 x 0.2 ml (10-20 transf.)	gt115-11 gt115-21
ChemiComp GT116	5 x 0.1 ml (5-10 transf.) 5 x 0.2 ml (10-20 transf.)	gt116-11 gt116-21

LyoComp GT115

(*Δdcm, uidA::pir-116, sbcCD*)

LyoComp GT115 cells are recommended for cloning and propagation of plasmids containing hairpin structures and the R6K gamma origin of replication, such as pCpG-siRNA plasmids.
Transformation efficiency: 1 x 10^6 cfu/µg

LyoComp GT116

(*Δdcm, sbcCD*)

LyoComp GT116 cells are recommended for cloning and propagation of plasmids containing hairpin structures, such as psiRNA plasmids.
Transformation efficiency: 1 x 10^6 cfu/µg

Contents and Storage

LyoComp *E. coli* are provided as 4 or 5 vials, each containing the equivalent of 0.1 or 0.2 ml competent cells for GT115 and 0.5 ml or 1 ml competent cells for GT116. Cells are shipped at room temperature. Upon receipt, store LyoComp *E. coli* at -20°C. Cells are stable for at least 6 months when properly stored.

PRODUCT	QUANTITY	CAT. CODE
LyoComp GT115	5 x 0.1 ml (5-10 transf.) 5 x 0.2 ml (10-20 transf.)	lyo-115-11 lyo-115-21
LyoComp GT116	4 x 0.5 ml (5-10 transf.) 4 x 1 ml (10-20 transf.)	lyo-116-11 lyo-116-21

Recent articles using InvivoGen's competent cells

Competent GT115

Haase R. *et al.*, 2010. pEPito: a significantly improved non-viral episomal expression vector for mammalian cells. BMC Biotechnol. 10:20.

Holz-Schietinger C. & Reich NO., 2010. The inherent processivity of the human de novo methyltransferase 3A (DNMT3A) is enhanced by DNMT3L. J Biol Chem. 285(38):29091-100.

Bartholdson SJ. *et al.*, 2008. Plant host and sugar alcohol induced exopolysaccharide biosynthesis in the Burkholderia cepacia complex. Microbiology. 154(Pt 8):2513-21.

Competent GT116

Kang C. *et al.*, 2009. Silencing epidermal growth factor receptor by RNA interference in glioma. Methods Mol Biol. 542:335-49.

Eckle T. *et al.*, 2008. A2B adenosine receptor dampens hypoxia-induced vascular leak. Blood. 111(4):2024-35.

Ye ZW. *et al.*, 2010. Knockdown of angiotensinogen by shRNA-mediated RNA interference inhibits human visceral preadipocytes differentiation. Int J Obes (Lond). 4(1):157-64.

Fast-Media® - The Hassle-free Way to Prepare *E. coli* Selection 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.

Features and Benefits

No More Time Consuming Medium Preparation

- Rapid preparation: only 5 minutes
- Ready-made: no weighing or mixing of media components
- Pre-sterilized: no autoclaving - just microwave
- Already contains the antibiotic of choice

Fast-Media® is also available with no selective antibiotics.

Ready-to-Use

Fast-Media® is provided in individual sealed pouches. Each pouch contains sufficient reagents to prepare 200 ml of sterile liquid or agar medium in just 5 minutes by using a microwave.

As Easy as 1, 2, 3

1- Empty pouch into a glass bottle
2- Mix pouch contents with 200 ml water
3- Microwave for 3 minutes

Performance and Control

Quality Control

To ensure constant quality and great reproducibility, each new batch of Fast-Media® is rigorously controlled according to approved procedures. Each Fast-Media® lot is tested with widely used *E. coli* K12 strains. Adequate plasmids conferring appropriate resistance to *E. coli* strains are used as positive controls.

Stability and Storage

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

Available with a wide variety of antibiotics

Ampicillin	**Kanamycin**
Blasticidin	**Puromycin**
Hygromycin B	**Zeocin™**

Contents and Storage

Each type of *E. coli* Fast-Media® is 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 12 months at room temperature. After preparation, poured plates or reconstituted media are stable 4 weeks when stored at 4°C.

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.

* InvivoGen has developed a process that guarantees the antibiotic activity after microwave heating.

PRODUCT	ANTIBIOTIC	QUANTITY	CAT. CODE
Preparation of Liquid Medium			
Fast-Media® Base TB	None	30 pouches 500 pouches	fas-l fas-l500
Fast-Media® Amp LB **NEW**	Ampicillin	30 pouches 500 pouches	fas-am-b fas-am-b500
Fast-Media® Amp TB	Ampicillin	30 pouches 500 pouches	fas-am-l fas-am-l500
Fast-Media® Blas TB	Blasticidin S	20 pouches	fas-bl-l
Fast-Media® Hygro TB	Hygromycin B	20 pouches	fas-hg-l
Fast-Media® Kan LB **NEW**	Kanamycin	30 pouches 500 pouches	fas-kn-b fas-kn-b500
Fast-Media® Kan TB	Kanamycin	30 pouches 500 pouches	fas-kn-l fas-kn-l500
Fast-Media® Puro TB	Puromycin	20 pouches	fas-pr-l
Fast-Media® Zeo TB	Zeocin™	20 pouches	fas-zn-l
Preparation of Agar Plates			
Fast-Media® Base Agar	None	30 pouches 500 pouches	fas-s fas-s500
Fast-Media® Amp Agar	Ampicillin	30 pouches 500 pouches	fas-am-s fas-am-s500
Fast-Media® Blas Agar	Blasticidin S	20 pouches	fas-bl-s
Fast-Media® Hygro Agar	Hygromycin B	20 pouches	fas-hg-s
Fast-Media® Kan Agar	Kanamycin	30 pouches 500 pouches	fas-kn-s fas-kn-s500
Fast-Media® Puro Agar	Puromycin	20 pouches	fas-pr-s
Fast-Media® Zeo Agar	Zeocin™	20 pouches	fas-zn-s
Preparation of Agar Plates with X-Gal (+IPTG)			
Fast-Media® Amp Agar X-Gal	Ampicillin	20 pouches	fas-am-x
Fast-Media® Blas Agar X-Gal	Blasticidin S	20 pouches	fas-bl-x
Fast-Media® Hygro Agar X-Gal	Hygromycin B	20 pouches	fas-hg-x
Fast-Media® Kan Agar X-Gal	Kanamycin	20 pouches	fas-kn-x
Fast-Media® Zeo Agar X-Gal	Zeocin™	20 pouches	fas-zn-x
Preparation of Agar Plates with X-Gluc			
Fast-Media® Zeo Agar X-Gluc	Zeocin™	10 pouches	fas-zn-g

Recent articles using InvivoGen's Fast-Media™

Albers CA. *et al.*, 2012. Compound inheritance of a low-frequency regulatory SNP and a rare null mutation in exon-junction complex subunit RBM8A causes TAR syndrome. Nat Genet. 44(4):435-9, S1-2.

Arias CR. *et al.*, 2010. High intragenomic heterogeneity of 16S rRNA genes in a subset of Vibrio vulnificus strains from the western Mediterranean coast. Int Microbiol. 13(4):179-88.

Etheridge SP. *et al.*, 2011. Somatic mosaicism contributes to phenotypic variation in Timothy syndrome. Am J Med Genet A. 155A(10):2578-83.

Stege A. *et al.*, 2010. Overcoming multidrug resistance by RNA interference. Methods Mol Biol. 596:447-65.

Walther W. *et al.*, 2010. Jet-injection of short hairpin RNA-encoding vectors into tumor cells. Methods Mol Biol. 629:123-39.

3 CpG-FREE DNA

CpG-FREE DNA

CpGs and the Immune Response

Bacterial DNA is rich in unmethylated 2'-deoxyribo (cytidine-phosphate-guanosine) (CpG) dinucleotides, in contrast to mammalian DNA, which contains a low frequency of CpG dinucleotides that are mostly methylated. Unmethylated CpGs in specific sequence contexts activate the vertebrate immune system via Toll-Like Receptor (TLR) 9. TLR9 recognizes CpG DNA and initiates a signaling cascade leading to the production of proinflammatory cytokines such as IL-6 and IL-12[1]. Plasmids used for *in vivo* experiments are produced in *E. coli* and therefore their CpGs are unmethylated and induce immune responses through this host defense mechanism. This presents a limitation for the clinical development of DNA vaccines and gene therapy vectors.

CpGs and Gene Silencing

A major limitation of gene delivery vectors for gene therapy applications is the rapid decline of transgene expression *in vivo*. Methylation of CpG dinucleotides within the promoter is a major factor limiting long-lasting gene expression. Indeed, the transcriptional activity of the widely used and CpG-rich cytomegalovirus immediate early gene promoter (CMV) is highly robust but prone to inactivation within a few weeks[2]. Replacement of the CMV promoter with a cellular promoter combined with the use of a CpG-reduced backbone was shown by our team (figures 1 & 2) and other labs to increase the duration of expression and lower the inflammatory response[3, 4, 5]. It has been shown that CpGs in plasmid DNA (pDNA) enhance the clearance of PEG-coated pDNA-lipoplexes, a phenomenon that could be reduced by the use of CpG-free pDNA allowing repeated dosing[6].

Construction of CpG-free Plasmids

InvivoGen has developed a family of plasmids that are completely devoid of CpG dinucleotides, named pCpGfree. pCpGfree plasmids contain elements that either naturally lack CpG dinucleotides, or were modified to remove all CpGs, or entirely synthesized such as genes encoding selectable markers or reporters. These plasmids yield high levels of transgene expression both *in vitro* and *in vivo*, and in contrast to CMV-based plasmids allow sustained expression *in vivo* (see ref. 7 and figure 1).

Applications of CpG-free Plasmids

A major application of CpG-free plasmids is the treatment through gene therapy of inherited diseases caused by a single gene defect, such as cystic fibrosis and hemophilia, . Hyde *et al.* have reported promising results for the treatment of cystic fibrosis using a pCpGfree-derived plasmid[8]. They show that a CpG-free pDNA can achieve sustained lung transgene expression in contrast to a pDNA containing even a single CpG. These results have led to an ongoing clinical trial initiated by the UK CF Gene Therapy Consortium. pCpGfree plamids represent valuable tools to study the effects of CpGs on gene expression using cell lines expressing TLR9[9], as well as their effects on the innate and acquired immune systems. Furthermore, pCpGfree plasmids are also useful when analyzing promoter methylation[10].

Plasmid	Application	Mode of delivery	Duration of expression	Ref.
CpG-free plasmid	Gene therapy in chronic lung	Aerosol delivery	56 days	8
pCpG-free plasmid	Gene therapy in chronic lung	Aerosol delivery	70 days	4
CpG-free plasmid	Gene therapy in liver	Hydrodynamic delivery	80 days	3
pCpGfree-MCS	Treatment of atopic dermatitis	Intravenous injection	80 days	7
pCpGfree-vitro	Lumbar gene delivery	Intrathecal injection	116 days	11

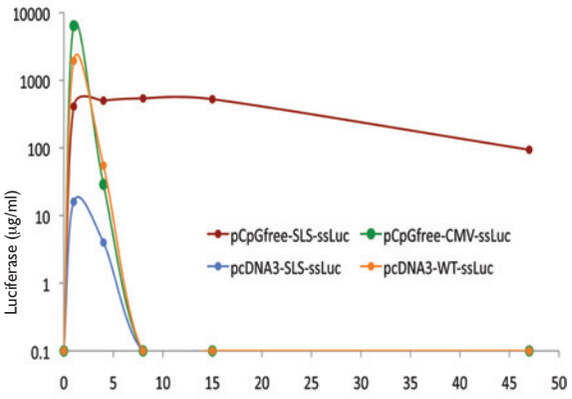


Figure 1. Time course of luciferase expression. Plasmids (30 µg) expressing a CpG-free luciferase (ssLuc) gene and containing different amounts of CpGs in their backbone were hydrodynamically injected in mice. Luciferase expression was evaluated at different time points. Expression of ssLuc from pcDNA3-CMV (CpG-rich plasmid with CMV promoter) peaked at day 1 after injection but had drastically fallen at day 4 to reach background levels by day 8. In contrast, ssLuc expression from pCpGfree-SLS (CpG-free plasmid with a CpG-free engineered albumin promoter) was high and persisted to day 47. Rapid reduction of luciferase expression was also observed with pCpGfree-CMV (CpG-free plasmid with CMV promoter) and pcDNA3-SLS (CpG-rich plasmid with CpG-free promoter).

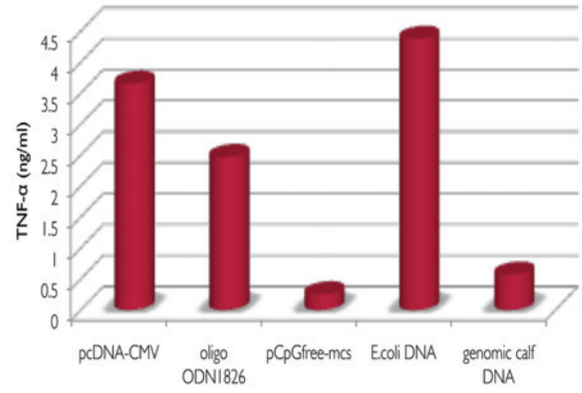


Figure 2. DNA-induced immune response. Mice were injected i.p. with 500 µg CpG-rich or CpG-free DNA and the levels of serum TNF-α assessed 4h post-injection. As expected CpG-free DNA (pCpGfree-mcs and genomic calf DNA) induced negligeable amounts of TNF-α whereas CpG-rich DNA (pDNA-CMV, CpG ODN1826 and *E. coli* DNA) induced significant amounts of TNF-α. All DNAs injected were endotoxin-free.

1. Bauer S. *et al.*, 2001. Human TLR9 confers responsiveness to bacterial DNA via species-specific CpG motif recognition. PNAS. 98(16):9237-42. **2. Scharfmann R. *et al.*, 1991.** Long-term *in vivo* expression of retrovirus-mediated gene transfer in mouse fibroblast implants. PNAS. 88(11):4626-30. **3. Magnusson T. *et al.*, 2011.** Sustained, high transgene expression in liver with plasmid vectors using optimized promoter-enhancer combinations. J Gene Med. 13:382-91. **4. Davies LA. *et al.*, 2012.** The use of CpG-free plasmids to mediate persistent gene expression following repeated aerosol delivery of pDNA/PEI complexes. Biomaterials 33: 5618e5627. **5. Clim A. *et al.*, 2012.** *In vivo* studies on non-viral transdifferentiation of liver cells towards pancreatic beta cells. J Endocrinol. [Epub ahead of print]. **6. Tagami T. *et al.*, 2010.** CpG motifs in pDNA-sequences increase anti-PEG IgM production induced by PEG-coated pDNA-lipoplexes. J Control Release. 142(2):160-6. **7. Hattori K. *et al.*, 2010.** Sustained exogenous expression of therapeutic levels of IFN-{gamma} ameliorates atopic dermatitis in NC/Nga mice via Th1 polarization. J Immunol. 184(5):2729-35. **8. Hyde S. *et al.*, 2008.** CpG-free plasmids confer reduced inflammation and sustained pulmonary gene expression. Nat Biotechnol. 26(5):549-51. **9. Yasuda K. *et al.*, 2009.** Requirement for DNA CpG content in TLR9-dependent dendritic cell activation induced by DNA-containing immune complexes. J Immunol. 183(5):3109-17. **10. Klug M & Rehli M., 2006.** Functional analysis of promoter CpG methylation using a CpG-free luciferase reporter vector. Epigenetics. 1(3):127-30. **11. Hughes T. *et al.*, 2009.** Intrathecal injection of naked plasmid DNA provides long-term expression of secreted proteins. Mol Ther. 17(1):88-94.

pCpGfree - CpG-free Plasmids

Description

CpG-free Plasmid Backbone

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 were 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[1].
- **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 typically AT-rich that are able to form barriers between independently regulated domains[2]. pCpG plasmids contain two MARs, from the 5' region of the human IFN-β gene or β-globin gene that were chosen because they are naturally CpG-free. The MARs are placed between the bacterial and mammalian transcription units.
- **Selectable marker:** The Zeocin™ resistance gene is a small gene (<400 bp) that contains numerous CpG dinucleotides. A synthetic new allele was created that contains no CpGs.

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

Provided with the *E. coli* GT115 Strain

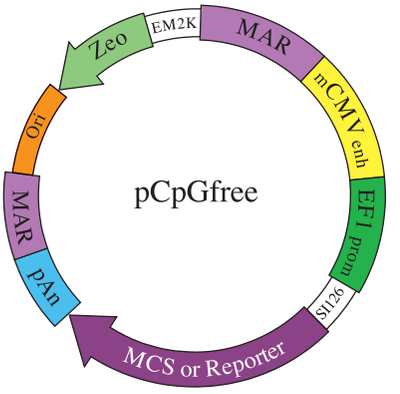
Due to the presence of the R6K gamma origin of replication, pCpGfree plasmids can only be amplified in an *E. coli* mutant strain expressing a *pir* mutant gene. They will not replicate in standard *E. coli* strains. Therefore, pCpGfree plasmids are provided with the *E. coli* GT115 strain, a *pir* mutant also deficient in *Dcm* methylation (see page 46).

1. Wu F. *et al.*, **1995.** A DNA segment conferring stable maintenance on R6K gamma-origin core replicons. J Bacteriol. 177(22):6338-45. **2.** Bode J. *et al.*, **1996.** Scaffold/matrix-attached regions: topological switches with multiple regulatory functions. Crit Rev Eukaryot Gene Expr. 6(2-3):115-38.

Contents and Storage

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	QTY	CAT. CODE
pCpGfree-mcs	20 µg	pcpgf-mcs
pCpGfree-LacZ	20 µg	pcpgf-lacz
pCpGfree-Lucia **NEW**	20 µg	pcpgf-lucia
pCpGfree-mSEAP	20 µg	pcpgf-mseap
pCpGrich-mcs	20 µg	pcpgr-mcs



pCpGfree-mcs

pCpGfree-mcs contains a multiple cloning site (MCS) featuring several commonly used restriction sites, for convenient cloning of a CpG-free gene, such as the genes provided in the pSELECT plasmid (see page 32), or any open reading frame or cDNA.

MCS 5'- Bsr GI, Bgl II, Acc65 I, Eco O109I, Bsp 120I, Nco I, Nhe -3'

pCpGfree-reporter

pCpGfree reporter family comprises three plasmids expressing CpG-free alleles of three different reporter genes (see pages 17-23):

- **pCpGfree-LacZ,** *E. coli* β-galactosidase (LacZ) gene
- **pCpGfree-mSEAP,** mouse secreted embryonic alkaline phosphatase (mSEAP) gene,
- **NEW! pCpGfree-Lucia,** novel synthetic secreted luciferase (Lucia®)

pCpGrich-mcs

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.

Recent articles using pCpGfree plasmids

Ando M. *et al.*, 2012. Constant and steady transgene expression of interferon-γ by optimization of plasmid construct for safe and effective interferon-γ gene therapy. J Gene Med. 14(4):288-95.

Cim A. *et al.*, 2012. In vivo studies on non-viral transdifferentiation of liver cells towards pancreatic beta cells. J Endocrinol. [Epub ahead of print]

Kato M. *et al.*, 2012. Collagenase-1 injection improved tumor distribution and gene expression of cationic lipoplex. Int J Pharm. 423(2):428-34.

Klausner EA. *et al.*, 2012. Corneal gene delivery: chitosan oligomer as a carrier of CpG rich, CpG free or S/MAR plasmid DNA. J Gene Med. 14(2):100-8.

Pringle IA. *et al.*, 2012. CpG-free plasmid expression cassettes for cystic fibrosis gene therapy. Biomaterials. [Epub ahead of print]

Uccellini MB. *et al.*, 2012. Selective binding of anti-DNA antibodies to native dsDNA fragments of differing sequence. Immunol Lett. ;143(1):85-91.

pCpGfree-basic & pCpGfree-promoter

Promoter CpG Methylation Study

Methylation of CpG dinucleotides within the promoter region of genes is often associated with transcriptional silencing. This epigenetic event plays an important role in the regulation of gene activity in normal and cancer cells. InvivoGen provides pCpGfree-basic and pCpGfree-promoter, two reporter plasmids completely devoid of CpG dinucleotides, that allow to study the effect of promoter CpG methylation in transfection assays. The lack of CpGs within the plasmid backbone limits *in vitro* CpG methylation to the CpG dinucleotides present in the inserted promoter fragment. Thus, the pCpGfree-basic and pCpGfree-promoter plasmids represent useful tools to analyze the effect of DNA methylation on CpG-containing promoters.

Description

pCpGfree-basic and pCpGfree-promoter comprise four plasmids that derive from the pCpGfree-mSEAP or pCpGfree-Lucia plasmid by deletion of the enhancer/promoter region or the enhancer sequence alone, respectively.

- **pCpGfree-basic (mSEAP) & pCpGfree-promoter (mSEAP)** express the murine secreted embryonic alkaline phosphatase (mSEAP)
- **NEW! pCpGfree-basic-Lucia & pCpGfree-promoter-Lucia** express Lucia®, a novel secreted luciferase (see page 17) reporter gene.

pCpGfree-basic

The pCpGfree-basic plasmids lack the entire promoter region (i. e. murine CMV enhancer and human EF-1α promoter). They contain a multiple cloning site upstream of the mSEAP or Lucia® reporter genes. Expression of mSEAP or Lucia® in cells transfected with this plasmid depends on the insertion of a functional promoter or enhancer/promoter cassette upstream from the reporter gene. Thus, pCpGfree-basic plasmids allow to study the effect of CpG methylation on a promoter, alone or combined with enhancer elements.

pCpGfree-promoter

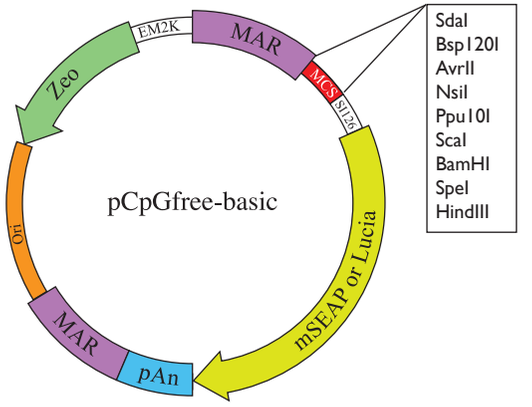
The pCpGfree-promoter plasmid contains the human EF-1α promoter and a multiple cloning site in place of the murine CMV enhancer. It is specifically designed to analyze the effect of methylation on CpG residues present in enhancer elements.

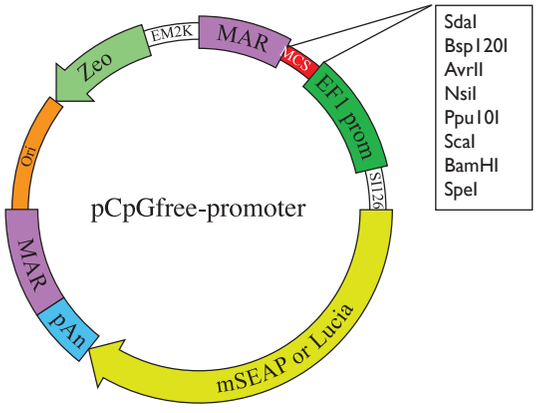
Contents and Storage

pCpGfree-basic and pCpGfree-promoter 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.

Procedure

1- Insert promoter and/or enhancer fragment into pCpGfree-basic or pCpGfree-promoter.
2- Treat recombinant plasmid with CpG methylase (Sss I) or site specific-methylase (Hha I, Hpa II).
3- Transiently transfect cells with methylase-treated or untreated plasmid.
4- Analyze promoter CpG methylation by determining reporter expression using the appropriate detection reagent.





PRODUCT	QTY	CAT. CODE
pCpGfree-basic (mSEAP)	20 µg	pcpgf-bas
pCpGfree-basic-Lucia NEW	20 µg	pcpgf-baslc
pCpGfree-promoter (mSEAP)	20 µg	pcpgf-prom
pCpGfree-promoter-Lucia NEW	20 µg	pcpgf-promlc

pCpGfree-vitro - Selectable CpG-free Plasmids

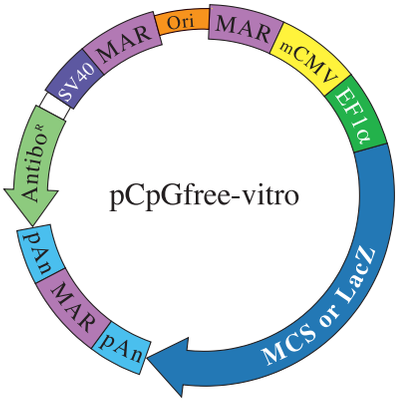
Description

CpG-free Plasmid Backbone

pCpGfree-vitro is a family of expression vectors completely devoid of CpG dinucleotides that are selectable in mammalian cells. Similarly to the other pCpGfree plasmids, all the elements required for replication and selection of the plasmids in bacteria, and gene expression in mammalian cells have been modified to remove all CpG dinucleotides.

A Choice of Different Backbones

To better suit your needs, the pCpGfree-vitro plasmid comes with a choice of selection: blasticidin, hygromycin or kanamycin/G418.
Each pCpGfree-vitro is available with a CpG-free allele of the LacZ gene or a multiple cloning site (MCS). This MCS contains several commonly used restriction sites, for convenient cloning of a CpG-free gene, such as the genes provided in the pSELECT-zeo plasmid (see page 56) or any open reading frame or cDNA.



MCS 5'- BsrGI, ScaI, BglII, ApaLI, Bsp120I, NcoI, NheI, MscI -3'

Applications

Study the immunostimulatory effect of CpG motifs

pCpGfree-vitro plasmids represent innovative tools to study the effects of CpG dinucleotides in a number of applications. DNA vaccination exploits the immunostimulatory character of certain CpG motifs to prime and boost the immune response. However, these immunostimulatory CpG motifs are antagonized by CpG dinucleotides in certain distinct base contexts, termed neutralizing CpG motifs. Both types of CpG motifs are usually present in plasmidic DNA, and therefore may lead to an unfavorable immune response. pCpGfree-vitro is the ideal tool to overcome this problem, and may be used to study the effects of these two types of CpG motifs by adding them in different configurations to the pCpGfree-vitro backbone.

Study CpG methylation

CpG dinucleotides are key elements in a number of cellular functions associated with chromatin. Several large multisubunit complexes, consisting of methyl-CpG binding (MBD) proteins and histone deacetylases, have been implicated in the regulation of chromatin dynamics. These complexes are recruited to methylated CpG dinucleotides by DNA methyl transferases (DNMTs) and induce chromatin remodeling. However the specific roles of these complexes are still to be explored. Due to the absence of CpG dinucleotides within its backbone, pCpGfree-vitro is not the target of DNMTs and thus MBD proteins. Therefore, it provides a useful model to study the other proteins involved in these complexes, in particular the histone deacetylases. It can also be used to analyze the effects of CpG methylation on the regulation and duration of gene expression.

Contents and Storage

Each pCpGfree-vitro plasmid is 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.

Selections Available in pCpGfree-vitro Plasmids

Blasticidin
Hygromycin
Neomycin (G418)

PRODUCT	QUANTITY	CAT. CODE
pCpGfree-vitroBmcs	20 µg	pcpgvtb-mcsg2
pCpGfree-vitroBLacZ	20 µg	pcpgvtb-lz
pCpGfree-vitroHmcs	20 µg	pcpgvth-mcsg2
pCpGfree-vitroHLacZ	20 µg	pcpgvth-lz
pCpGfree-vitroNmcs	20 µg	pcpgvtn-mcsg2
pCpGfree-vitroNLacZ	20 µg	pcpgvtn-lz

Related Products

Blasticidin, page 13
G418 Sulfate, page 13
Hygromycin B, page 14
LyoComp GT115, page 47

pCpGfree-siRNA & pCpGfree-siRNADUO

Description

pCpGfree-siRNA and pCpGfree-siRNADUO are CpG-free plasmids that allow the expression of one or two shRNAs, respectively. The absence of CpG dinucleotides in their backbone makes these plasmids less immunogenic and insensitive to CpG methylation thus ideal for long lasting expression of shRNA(s) *in vivo*.

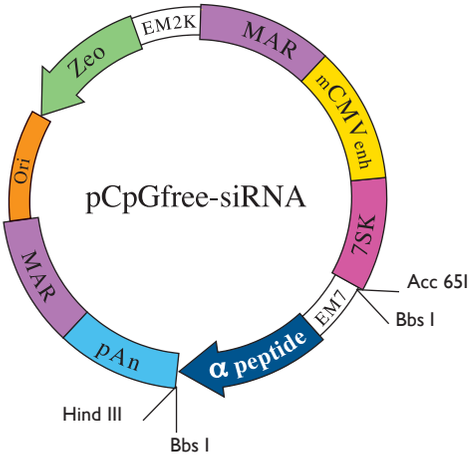
pCpGfree-siRNA

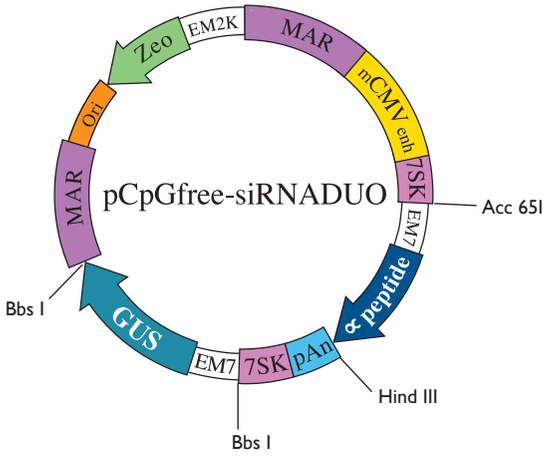
The pCpGfree-siRNA plasmid combines the backbone of the pCpGfree plasmid with the shRNA expression cassette of the psiRNA plasmid. pCpGfree-siRNA features the human 7SK promoter, in which all CpG dinucleotides have been removed. The activity of this RNA Pol III promoter is augmented by the addition of the enhancer of the murine CMV immediate-early promoter, a Pol II enhancer. The enhancer has been modified to remove all CpGs.
The pCpGfree-siRNA plasmid uses Bbs I, an unusual restriction enzyme that generates asymmetric cohesive overhangs that are not compatible with each other to eliminate the risk of self-ligation of the vector.
A second cloning strategy can be chosen by using Acc 65I and Hind III restriction sites.
The pCpGfree-siRNA plasmid exploits the white/blue selection system to facilitate the screening of recombinant clones. It features an EM7-LacZ α-peptide cassette between the cloning sites to allow the easy discrimination between blue parental and white recombinant clones.

pCpGfree-siRNADUO

The pCpGfree-siRNADUO plasmid contains two shRNA cassette driven by a CpG-free version of the human 7SK promoter. The murine CMV enhancer has been added to increase the activity of both 7SK promoters. The first cassette features Acc65 I / Hind III cloning sites and a LacZ α-peptide bacterial expression cassette for white/blue selection in *E. coli*.
The second cassette contains Bbs I / Bbs I cloning sites and a GUS bacterial expression cassette, another system that allows white/blue selection in *E. coli*.

pCpGfree-siRNA and pCpGfree-siRNADUO are provided with LyoComp GT115, a lyophilized competent *E. coli* mutant strain permissive with the white and blue color selection from the LacZ and GUS systems.





Contents and Storage

The pCpGfree-siRNA and pCpGfree-siRNADUO plasmids are provided as 50 µg of lyophilized DNA in a kit that includes the following components:
- 20 µg control plasmid
- 1 vial of LyoComp GT115
- 10 µg of each sequencing primer
- 4 pouches of Fast-Media® Zeo X-Gal or,
- 2 pouches of Fast-Media® Zeo X-Gal and 2 pouches of Fast-Media® Zeo X-Gluc

Products are shipped at room temperature. Store Fast-Media® pouches at room temperature. Store all other components at -20°C.

PRODUCT	QUANTITY	CAT. CODE
pCpGfree-siRNA	1 kit	kcpgf-sirna
pCpGfree-siRNADUO	1 kit	kcpgf-sirna2

Related Products

LyoComp GT115, page 47
Zeocin™, page 15
Fast-Media® Zeo-X-Gal, page 49
Fast-Media® Zeo-X-Gluc, page 49

CpG-Free Genes - Synthetic CpG-free Genes

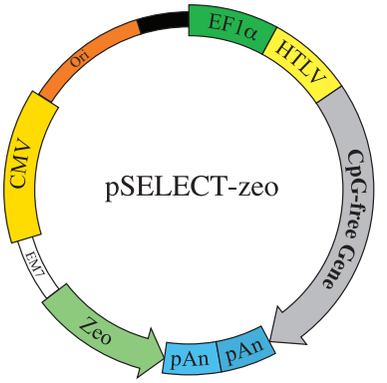
Many non-mammalian genes are widely used as reporter or suicide genes in molecular and cellular studies. However these genes are recognized as foreign DNA by the vertebrate host leading to a progressive decline of their expression. To circumvent this limitation, InvivoGen has synthesized new alleles of these genes completely devoid of CpG dinucleotides. These synthetic CpG-free genes display higher activity and lower immunogenicity than their wild-type counterparts.

Description

The DNA sequence of the CpG-free genes was modified by optimizing the codon usage, eliminating the CpG nucleotides and avoiding secondary DNA structures without changing the amino acid sequence of the wild type proteins.

CpG-free genes were chemically synthesized by assembling large oligonucleotides. Sequence integrity was verified by double-stranded sequencing.

CpG-free genes are provided in the pSELECT-zeo plasmid (see page 30). pSELECT-zeo is a mammalian expression plasmid selectable in *E. coli* and mammalian cells with Zeocin™. Each CpG-free gene is flanked by a unique restriction site at the 5' and 3' end to facilitate its subcloning into another vector.



Note: The pSELECT-zeo plasmid backbone contains CpG dinucleotides.

Contents and Storage

Each pSELECT-zeo-<CpGfree gene> 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 up to one year when properly stored. Each plasmid is supplied with 4 pouches of *E. coli* Fast-Media® Zeo (2 TB and 2 Agar, see pages 48-49).

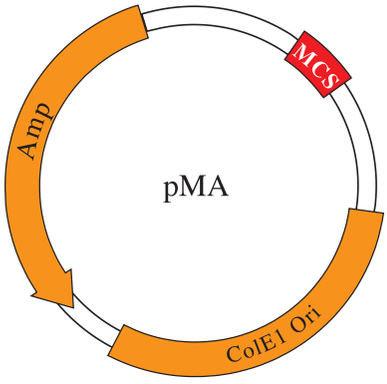
GENE	DESCRIPTION	CpGs IN NATIVE GENE	QUANTITY	CAT. CODE
Suicide Genes				
Fcy::fur*	*S. cerevisiae* cytosine deaminase-uracil phosphoribosyl transferase fusion	33	20 µg	psetz-fcyfur
HSV1-tk	Herpes simplex virus 1 (HSV1) thymidine kinase	121	20 µg	psetz-hsv1tk
HSV1-tk::Sh*	HSV1 thymidine kinase-Zeocin resistance fusion	372	20 µg	psetz-hsv1tksh
Reporter Genes				
GFP::Sh*	Green fluorescent protein-Zeocin resistance fusion	110	20 µg	psetz-zgfpsh
GFP::Bsr*	Green fluorescent protein-Blasticidin resistance fusion	110	20 µg	psetz-zgfpbsr
LacZ	β-Galactosidase	289	20 µg	psetz-lacz
LacZnls	β-Galactosidase with SV40 nuclear localization signal	289	20 µg	psetz-lacznls
LacZ::Sh*	β-Galactosidase-Zeocin resistance fusion	341	20 µg	psetz-laczsh
Lucia® **NEW**	Engineered secreted luciferase	-	20 µg	psetz-lucia
Luc::Sh*	Firefly luciferase-Zeocin resistance fusion	151	20 µg	psetz-lucsh
SEAP	Mouse secreted embryonic alkaline phosphatase	65	20 µg	psetz-mseap

* Fusion gene

Custom-Made CpG-Free Genes

InvivoGen is an expert in developing CpG-Free genes and now offers a CpG-Free gene custom service. Send us the sequence of your gene of interest, and our specialists will design, synthesize and clone into an expression vector a CpG-Free allele of this gene. We guarantee that the sequence of the synthetic gene will 100% match the designed sequence.

- **Free Sequence Design**
- **Short Turnaround Time**
- **Competitive Price**
- **Confidentiality Guaranteed**



Description

Sequence Design

Our experts will design the sequence of a CpG-free allele of your gene of interest using a proprietary software that will include the following:
- elimination of all CpG dinucleotides
- humanization of the sequence
- elimination of secondary structures such as hairpins and long direct repeated sequences in order to increase the stability and expression of the mRNA
- elimination/reduction of *dam* and *dcm* methylations to diminish *E. coli* signature

Cloning in an Expression Vector

The Custom-made CpG-free Gene is cloned into the pMA plasmid. We can clone it in the vector of your choice for an additional charge.

Sequencing

The Custom-made CpG-free Gene is 100% sequence verified, both strands are sequenced.

Processing

Send us the nucleic (Genbank accession number) or proteic sequence of your gene of interest. The synthesis of the CpG-free allele will start after you confirm its sequence.
The turnaround time is 2-3 weeks for sequences shorter than 1 kb.
We can process sequences up to 5 kb.

Contents and Storage

Custom-made CpG-free genes are provided as 20 µg of lyophilized DNA. Products are shipped at room temperature. Store at -20°C.

PRODUCT	QTY	CAT. CODE
Custom-made CpG-free Gene	20 µg	p-custom

Contact us for more information.
info@invivogen.com

4 RNA INTERFERENCE

RNA INTERFERENCE

RNA interference (RNAi) is one of the most exciting discoveries of the past decade in functional genomics. RNAi is rapidly becoming an important method for analyzing gene functions in eukaryotes and holds promise for the development of therapeutic gene silencing.

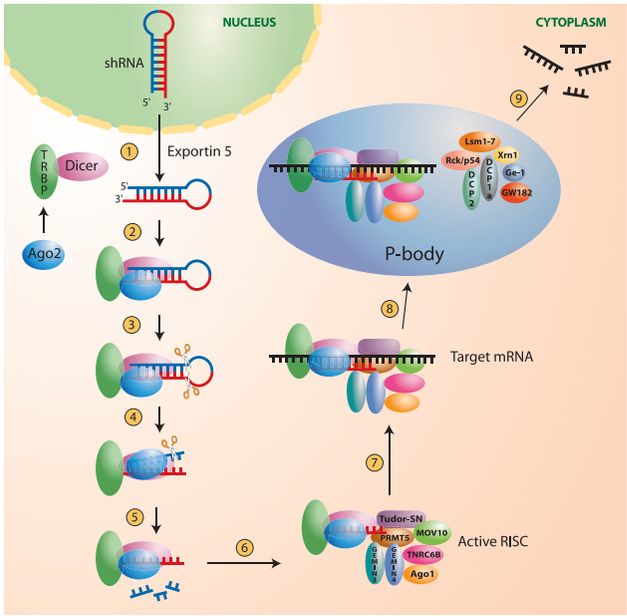
RNA interference (RNAi) is a post-transcriptional process triggered by the introduction of double-stranded RNA (dsRNA) which leads to gene silencing in a sequence-specific manner. The first evidence that dsRNA could achieve efficient gene silencing through RNAi came from studies on the nematode *Caenorhabditis elegans*. Further analyses in the fruit fly *Drosophila melanogaster* have contributed greatly toward understanding the biochemical nature of the RNAi pathway[1]. Long dsRNAs are cleaved by the RNase III family member, Dicer, into 19-23 nucleotides (nt) fragments with 5' phosphorylated ends and 2-nt unpaired and unphosphorylated 3' ends. These small dsRNAs are called small interfering RNAs (siRNAs). Each siRNA duplex is formed by a guide strand and a passenger strand. The endonuclease Argonaute 2 (Ago 2) catalyzes the unwinding of the siRNA duplex. Once unwound, the guide strand is incorporated into the RNA Interference Specificity Complex (RISC), while the passenger strand is released. RISC uses the guide strand to find the mRNA that has a complementary sequence leading to the endonucleolytic cleavage of the target mRNA[2] (see figure and legend).

Even though dsRNA were shown to induce gene-specific interference in early mouse embryos[3], preliminary attempts to use dsRNA in mammalian systems were not conclusive. These experiments employed long dsRNAs which instead of triggering RNAi generated an overall decrease in mRNA eventually leading to apoptosis, a response mediated by the interferon-activated dsRNA-dependent protein kinase[4]. Tuschl and colleagues[1] have demonstrated that this non-specific response can be bypassed by using chemically synthesized 19 to 23 nt dsRNAs[5-8]. Transfection of these siRNAs resulted in strong and sequence-specific suppression of gene expression in different mammalian cell lines. This discovery has led to the widespread use of this technology to study mammalian gene function including clinically relevant genes, alluding to the potential therapeutic applications of RNAi-based technologies[9].

Chemically synthesized siRNAs are expensive and they induce only transient gene silencing due to their short life length. Another limiting step for efficient gene silencing is cell transfectability. To overcome these limitations, InvivoGen has designed an efficient, highly transfectable and simple-to-use plasmid, called psiRNA™, that allows the production of siRNAs within the cells (see page 60). psiRNA is an RNA polymerase III-based plasmid that produces short hairpin RNAs (shRNAs). psiRNA is used to insert a DNA fragment of approximately 50 mer designed in such a way that after transcription from the human 7SK RNA polymerase III promoter it will generate shRNAs. shRNAs are more stable than synthetic siRNAs and since they are continuously expressed within the cells, this method permits long-lasting silencing of your gene of interest.

One critical step for efficient gene silencing using RNAi is the design of siRNAs/shRNAs that greatly reduce the expression of the target transcripts without affecting unintended targets. InvivoGen has developed a design algorithm, named siRNA Wizard, that reliably identifies siRNA/shRNA sequences for any given gene. The sequence selection is based on several criteria, such as thermodynamic stability, base composition and secondary structures (see page 60). The siRNA wizard algorithm is experimentally validated. It is used to generate ready-made psiRNAs, an expanding collection of plasmids that express shRNAs that induce silencing of their target transcript expression of >70% (see page 66). The silencing efficiency of these ready-made psiRNAs is tested using the psiTEST™ system, a rapid and simple method to screen for functional siRNA or shRNA sequences (see page 64).

RNAi-mediated gene silencing in mammals using shRNAs



① Plasmid-expressed short hairpin RNA (shRNA) requires the activity of endogenous Exportin 5 for nuclear export[10]. ② Ago2 (Argonaute 2) is recruited by TRBP[11], that forms a dimer with Dicer[12], and then receives the shRNA[13-15]. ③ The shRNA is cleaved in one step by Dicer generating a 19-23 nt duplex siRNA with 2 nt 3' overhangs. ④ After identification of the "guide strand" in the siRNA duplex, the "passenger strand" is cleaved by Ago2[13]. ⑤ The "passenger strand" is released. ⑥ The "guide strand" is integrated in the active RNA Interference Specificity Complex (RISC) that contains different argonautes and argonaute-associated proteins[16]. ⑦ The siRNA guides RISC to the target mRNA. ⑧ RISC delivers the mRNA to cytoplasmic foci named processing bodies (P-bodies or GW-bodies) wherein mRNA decay factors are concentrated[17, 18]. ⑨ The target mRNA is cleaved by Ago2 and degraded.

1. Elbashir S.M. *et al.*, 2001. RNA interference is mediated by 21- and 22-nucleotide RNAs. Genes Dev, 15(2):188-200. **2. Fuchs U *et al.*, 2004.** Silencing of disease-related genes by small interfering RNAs. Curr. Mol. Med 4:507-517. **3. Wianny F. and M. Zernicka-Goetz, 2000.** Specific interference with gene function by double-stranded RNA in early mouse development. Nat Cell Biol, 2(2):70-5. **4. Gil J. and M. Esteban, 2000.** Induction of apoptosis by the dsRNA-dependent protein kinase (PKR): mechanism of action. Apoptosis, 5(2):107-14. **5. Elbashir SM. *et al.*, 2001.** Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nature, 411(6836):494-8. **6. Brummelkamp TR. *et al.*, 2002.** A system for stable expression of short interfering RNAs in mammalian cells. Science, 296(5567):550-3. **7. Lee NS. *et al.*, 2002.** Expression of small interfering RNAs targeted against HIV-1 rev transcripts in human cells. Nat Biotechnol, 20(5): 500-5. **8. Scherr M. *et al.*, 2003.** Gene silencing mediated by small interfering RNAs in mammalian cells. Curr Med Chem. 10(3):245-56. **9. Ryther RCC *et al.*, 2005.** SiRNA therapeutics : big potential from small RNAs. Gene Ther 12(1): 5-11. **10. Yi R. *et al.*, 2005.** Overexpression of exportin 5 enhances RNA interference mediated by short hairpin RNAs and microRNAs. RNA. 11(2):220-6. **11. Chendrimada TP. *et al.*, 2005.** TRBP recruits the Dicer complex to Ago2 for microRNA processing and gene silencing. Nature. 436(7051):740-4. **12. Haase AD. *et al.*, 2005.** TRBP, a regulator of cellular PKR and HIV-1 virus expression, interacts with Dicer and functions in RNA silencing. EMBO Rep. 6(10):961-7. **13. Matranga C. *et al.*, 2005.** Passenger-strand cleavage facilitates assembly of siRNA into Ago2-containing RNAi enzyme complexes. Cell. 123(4):607-2. **14. Gregory RI. *et al.*, 2005.** Human RISC couples microRNA biogenesis and posttranscriptional gene silencing. Cell. 123(4):631-40. **15. Rivas FV. *et al.*, 2005.** Purified Argonaute2 and an siRNA form recombinant human RISC. Nat Struct Mol Biol. 12(4):340-9. **16. Meister G. *et al.*, 2005.** Identification of novel argonaute-associated proteins. Curr Biol. 15(23):2149-55. **17. Liu J. *et al.*, 2005.** A role for the P-body component GW182 in microRNA function. Nat Cell Biol. 7(12):1161-6. **18. Jakymiw A. *et al.*, 2005.** Disruption of GW bodies impairs mammalian RNA interference. Nat Cell Biol. 7(12):1167-74.

psiRNA™ System

InvivoGen provides a plasmid-based system developed to knockdown efficiently the expression of a wide variety of mammalian genes. This system represents a simple and affordable method to generate short hairpin RNAs (shRNAs) by eliminating the need to synthesize RNA oligonucleotides. The psiRNA System is designed to assist you in all the steps necessary to obtain efficient silencing of a gene of interest from the selection of an effective siRNA/shRNA to its prevalidation.

➢ Selection of Candidate siRNA/shRNA and Design of Hairpin Insert:

siRNA Wizard Software - **www.sirnawizard.com**

The design of siRNAs and short hairpin RNAs (shRNAs) remains an empirical process since the molecular mechanisms underlying RNAi are not yet sufficiently understood to allow for their rational design. However, based on the research from various laboratories including our own, InvivoGen has been able to develop siRNA Wizard, a **free software accessible online** from our homepage, that will help you do the following:

▶ Select Candidate siRNA/shRNAs

The siRNA Wizard algorithm allows to select effective and specific siRNAs/shRNAs against your gene of interest based on thermodynamic and sequence-related criteria. Two search options are available:

- Standard Search

"Standard Search" uses default criteria described in the "siRNA/shRNA Design Guidelines" paragraph to select several candidate siRNAs/shRNAs against your gene of interest.

- Advanced Search

"Advanced Search" lets you manually set the selection criteria.

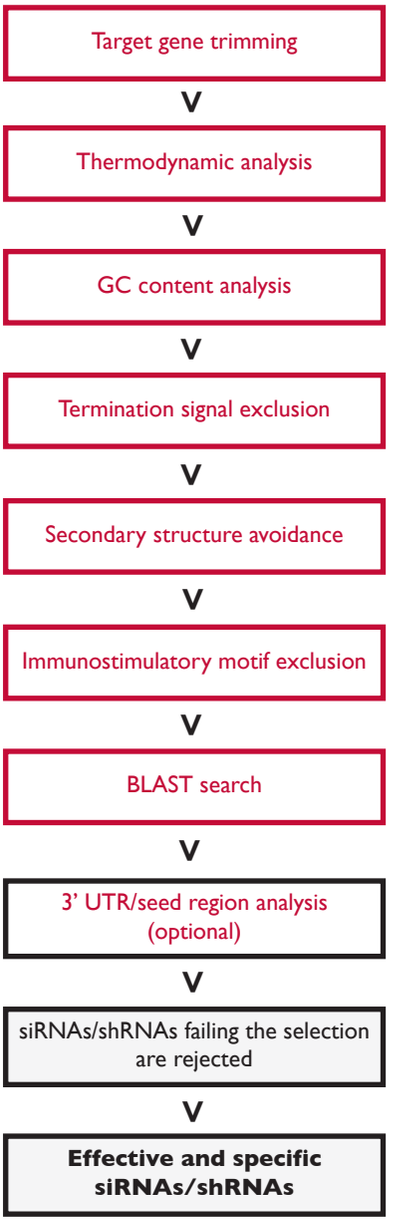
▶ Design Hairpin Insert

Using your selected siRNA/shRNA sequence, this tool will design two complementary oligonucleotides necessary to create the hairpin insert for psiRNA cloning vectors and let you choose the sequence of the loop.

▶ Generate siRNA/shRNA Scramble Sequence

This tool will return a scramble sequence with no match with any mRNA of the selected species database. This scramble sequence that serves as a negative control contains the same nucleotide composition as the selected siRNA/shRNA sequence.

"Standard Search" Selection Criteria



➢ Cloning of siRNA/shRNA Insert: psiRNA-h7SK Plasmids

InvivoGen provides psiRNA-h7SK, a family of cloning vectors featuring the human 7SK RNA polymerase III promoter and a choice of selection markers (see page 62). psiRNA-h7SK plasmids exploit the white/blue selection system to facilitate the screening of recombinant bacterial clones. Furthermore, they feature a GFP::Zeo fusion gene that allows to evaluate transfection efficiency and normalize silencing experiments.

psiRNA-h7SK plasmids are provided in a kit that contains, in addition to the cloning vector, two control vectors, and a set of tools designed to facilitate the cloning of shRNAs in the plasmid. The psiRNA-h7SK tools are the following:

- *E. coli* GT116 & GT115
- Sequencing Primers
- Fast-Media® X-Gal & X-Gluc

E. coli GT116 & GT115

InvivoGen has engineered two specific *E. coli* strains, named GT116 and GT115, to facilitate the cloning of shRNAs into psiRNA plasmids. Hairpin structures are known to be unstable in *E. coli* due to their elimination by a protein complex called SbcCD that recognizes and cleaves hairpins. To increase their stability in *E. coli*, we developed GT116 and GT115 by deleting the *sbcC* and *sbcD* genes. Both strains are more compatible with hairpin structures than commonly used *E. coli* strains increasing the probability of obtaining the correct clone in a minimum number of steps. GT115 is further mutated in the uidA gene rendering this strain deficient for β-glucuronidase (see page 46).
Both strains are provided as lyophilized chemically competent cells, named LyoComp GT116 and LyoComp GT115 respectively, that can also be purchased separately (see page 47).

GT116 Genotype: *F¯ mcrA Δ(mrr-hsdRMS-mcrBC) Φ80lacZΔM15 ΔlacX74 recA1 rspL (StrA) endA1 Δdcm ΔsbcC-sbcD*
GT115 Genotype: *F¯ mcrA Δ(mrr-hsdRMS-mcrBC) Φ80lacZΔM15 ΔlacX74 recA1 rspL (StrA) endA1 Δdcm uidA(ΔMluI)::pir-116 ΔsbcC-sbcD*

Sequencing Primers

Several sequencing primer pairs have been designed, one for each psiRNA plasmid backbone, that allow full reading of the insert sequence by using conventional sequencing methods.
- OL559-OL408 pair, for all psiRNA-h7SK plasmids
- OL178-OL408 and OL906-OL176 pairs for the α-peptide cassette and the GUS cassette of the psiRNA-DUO plasmid, respectively

Fast-Media® X-Gal & X-Gluc

Fast-Media® X-Gal and Fast-Media® X-Gluc are ready-to-use microwaveable LB-based medium powder for *E. coli* selection. They already contain the selective antibiotic and X-Gal (LacZ) or X-Gluc (Gus) at the appropriate concentration. These media allow to prepare high quality white/blue selection medium in less than 5 minutes.

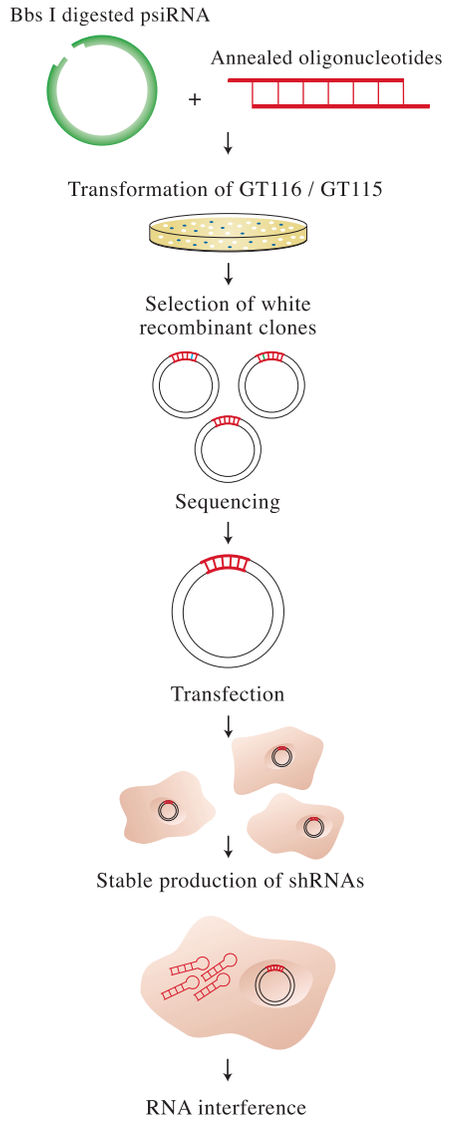
Fast-Media® X-Gal is available with four different selections: blasticidin, hygromycin, kanamycin and Zeocin™. Fast-Media® X-Gluc is available with Zeocin™.

Fast-Media® can be purchased separately (see pages 48-49).

➢ Prevalidation of siRNA/shRNA: psiTEST System

The psiTEST System was developed to provide a rapid, simple, and convenient method to screen for functional siRNA and shRNA sequences (see page 64). The silencing efficiency of a given siRNA or shRNA is evaluated by monitoring the reduction of expression of a chimeric gene consisting of a secreted alkaline phosphatase (SEAP) reporter gene transcriptionally fused to the target gene. Transfection of effective siRNAs or shRNAs triggers the RNAi process resulting in the degradation of the chimeric mRNA and therefore the absence of SEAP activity.

Strategy for psiRNA-mediated RNA interference



psiRNA-h7SK - 7SK-based expression of shRNAs

Features and Benefits

RNA Polymerase III Based Plasmid

psiRNA-h7SK is a family of expression vectors designed to generate shRNA from the human 7SK RNA polymerase III (Pol III) promoter.
7SK is an abundant and evolutionarily conserved small nuclear RNA transcribed by RNA polymerase III[1]. The human 7SK promoter is ideal for the production of shRNAs as it can generate high amounts of shRNAs[1,2]. In a series of experiments aimed to compare the strength of the human 7SK, H1 and U6 promoters, we found that the best silencing efficiencies of various target genes was consistently obtained with the 7SK promoter. psiRNA-h7SK has been successfully used to achieve gene silencing *in vitro* and *in vivo*[3-5]. Furthermore, the 7SK promoter can be used in combination with other RNA pol III promoters for multiple shRNA expression strategies[6].

Cloning Options

psiRNA-h7SK plasmids offer two cloning strategies for the cloning of an shRNA insert, either Acc 65I / Hind III or Bbs I / Bbs I. The two Bbs I sites are different although recognized by the same restriction enzyme thus preventing self-ligation of the plasmid.

White and Blue Selection

psiRNA-h7SK plasmids exploit the white/blue selection system to facilitate the screening of recombinant clones. The cloning sites flank a bacterial LacZ α-peptide expression cassette allowing the discrimination between blue parental clones and white recombinant clones. Although over 90% of the white clones have integrated a fragment, it is necessary to sequence the insert to verify the integrity of the sequence since only one base difference can lead to an inactive shRNA.

Variety of Selectable Markers

psiRNA-h7SK plasmids are available with several selectable markers that function in *E. coli* and mammalian cells. They are driven by a strong mammalian promoter in tandem with a constitutive bacterial promoter.

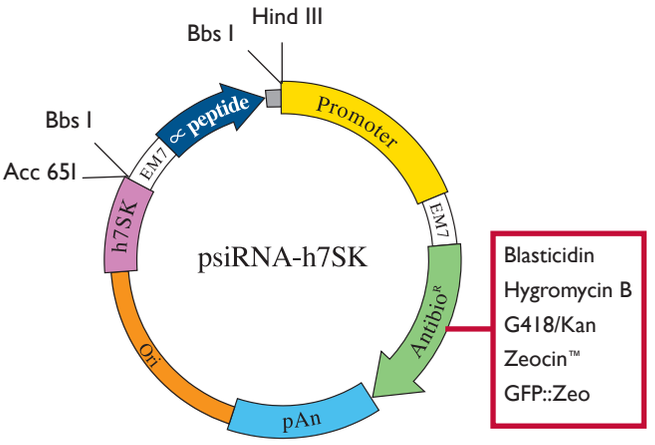
Normalize Your Silencing Experiments with GFP::Zeo

psiRNA-h7SKGFPzeo features a GFP::zeo fusion gene that combines the GFP reporter gene and the Zeocin™ resistance gene. This fusion gene allows the evaluation of transfection efficiency by determining the percentage of transfected cells by assaying the reporter activity. Therefore, psiRNA-h7SKGFPzeo use allows to normalize your silencing experiments.

Easily Convertible into an Adenovector

The shRNA expression cassette is flanked by Spe I at the 5' end and Hind III at the 3' end. This cassette can be easily excised from any psiRNA-h7SK plasmid using these two restriction enzymes and subcloned into one of the shuttle plasmids commercially available digested with Nhe I (compatible with Spe I) and Hind III.

1. Czauderna F. *et al.*, 2003. Inducible shRNA expression for application in a prostate cancer mouse model. Nucleic Acids Res. 31(21):e127. **2. Koper-Emde D. *et al.*,2004.** RNA interference by small hairpin RNAs synthesised under control of the human 7S K RNA promoter. Biol Chem. 385(9):791-4. **3. Triantafilou K. *et al.*, 2005.** Human cardiac inflammatory responses triggered by Coxsackie B viruses are mainly Toll-like receptor (TLR) 8-dependent. Cell Microbiol. 7(8):1117-26. **4. Tajeddine N. *et al.*, 2005.** Tumor-associated antigen preferentially expressed antigen of melanoma (PRAME) induces caspase-independent cell death in vitro and reduces tumorigenicity in vivo. Cancer Res.65(16):7348-55. **5. Mazzanti CM. *et al.*, 2004.** Early genetic mechanisms underlying the inhibitory effects of endostatin and fumagillin on human endothelial cells. Genome Res. 14(8):1585-93. **6. ter Brake O. *et al.*, 2008.** Lentiviral Vector Design for Multiple shRNA Expression and Durable HIV-1 Inhibition. Mol Ther. 16(3):557-64.



Contents and Storage

psiRNA-h7SK kits contain the following components:
- 20 µg of the psiRNA-h7SK plasmid of your choice (see list)
- 20 µg of the corresponding control plasmid 1 (psiRNA-h7SKgfp for all selectable markers and psiRNA-h7SKlacZ for GFP::zeo)
- 20 µg of the corresponding control plasmid 2 (psiRNA-h7SKluc for all selectable markers)
- 1 vial of LyoComp GT116
- 2 x 10 µg of the corresponding sequencing primer pair
- 4 pouches of the appropriate Fast-Media® X-Gal

Products are shipped at room temperature. Store Fast-Media® at room temperature. Store all other components at -20°C.

PRODUCT	QTY	CAT. CODE
psiRNA-h7SKblasti	1 kit	ksirna3-b21
psiRNA-h7SKhygro	1 kit	ksirna3-h21
psiRNA-h7SKneo	1 kit	ksirna3-n21
psiRNA-h7SKzeo	1 kit	ksirna3-z21
psiRNA-h7SKGFPzeo	1 kit	ksirna4-gz21

Recent articles using psiRNA plasmids

Courtial N. *et al.*, 2012. Tal1 regulates osteoclast differentiation through suppression of the master regulator of cell fusion DC-STAMP. FASEB J. 26(2):523-32.

Keestra AM. *et al.*, 2010. Chicken TLR21 is an innate CpG DNA receptor distinct from mammalian TLR9. J Immunol. ;185(1):460-7.

Siewe BT. *et al.*, 2011. In vitro requirement for periostin in B lymphopoiesis. Blood. 117(14):3770-9.

Ho JJ. et al., 2012. Functional importance of Dicer in the adaptive cellular response to hypoxia. J Biol Chem. [Epub ahead of print]

Takezaki T. *et al.*, 2011. Essential role of the Hedgehog signaling pathway in human glioma-initiating cells. Cancer Sci. 102(7):1306-12.

Derbigny WA. *et al.*, 2010. The Chlamydia muridarum-induced IFN-β response is TLR3-dependent in murine oviduct epithelial cells. J Immunol. 185(11):6689-97.

psiRNA-DUO - Expression of Two shRNAs

Description

psiRNA-DUO generates two shRNAs from the same plasmid for the silencing of either a single target gene (with/without polymorphisms) or two different target genes. psiRNA-DUO contains special features designed to make the cloning of the shRNA inserts and selection of recombinant clones simple and rapid.

The major features of the psiRNA-DUO plasmid are its two RNA Pol III cassettes and the GFP-Zeo fusion gene.

7SK RNAi Cassettes

The first cassette contains the following elements:
- Human 7SK promoter (see page 62)
- Acc65 I / Hind III cloning sites
- LacZ α-peptide expression cassette for white and blue selection

The second cassette contains the following elements:
- Human 7SK promoter
- Bbs I / Bbs I cloning sites
- GUS expression cassette for white and blue selection

β-Glucuronidase (GUS, *uidA*) is an *E. coli* enzyme capable of hydrolyzing the chromogenic substrate X-Gluc to generate a blue pigment. Therefore, similarly to LacZ, the expression of GUS in permissive *E. coli* allows the discrimination between blue parental clones and white recombinant clones.
psiRNA-DUO is provided with the *E. coli* GT115, a *lacZ uidA* mutant strain, enabling the white and blue color selection from the LacZ and GUS systems.

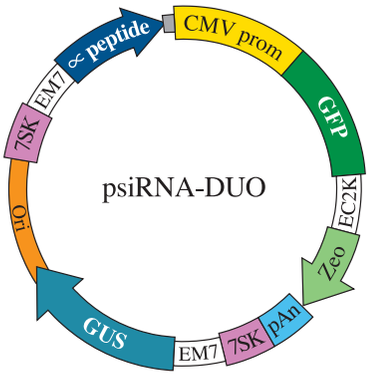
GFP-Zeo Fusion Gene

The GFP-Zeo gene is a transcriptional fusion between the Green Fluorescent Protein reporter gene and the Zeocin™ resistance (Zeo^R) gene. The two moieties are separated by a bacterial promoter (EC2K) located within an intron. In *E. coli*, Zeo^R is expressed from the EC2K promoter that is spliced out in mammalian cells. A composite CMV promoter drives the expression of the GFP-Zeo fusion gene, allowing the monitoring of transfection efficiency and the selection of stable clones.

Contents and Storage

psiRNA-DUO is provided as a kit composed of following:
- 20 µg of psiRNA-DUO-GFPzeo
- 20 µg of psiRNA-LucLac-GFPzeo (control plasmid)
- 1 vial of LyoComp GT115
- 2 sets of sequencing primers (2 x 10 µg each)
- 2 pouches of Fast-Media® Zeo X-Gal
- 2 pouches of Fast-Media® Zeo X-Gluc

For more information on LyoComp GT115, sequencing primers and Fast-Media® X-Gal and X-Gluc, see page 45.
Products are shipped at room temperature. Store Fast-Media® at room temperature. Store all other components at -20°C.



Procedures

Two Cloning Step Procedure

1- Clone first shRNA insert in first 7SK RNAi cassette opened with Acc65 I and Hind III.
2- Select white recombinant clones on X-Gal plates prepared with Fast-Media® Zeo X-Gal.
3- Clone second shRNA insert in second 7SK RNAi cassette opened with Bbs I.
4- Select white recombinant clones on X-Gluc plates prepared with Fast-Media® Zeo X-Gluc.

One Cloning Step Procedure

1- Digest psiRNA-DUO with Acc65 I, Hind III and Bbs I.
2- Ligate both shRNA inserts with the two psiRNA-DUO fragments (Hind III/Bbs I and Bbs I/Acc65 I).
3- Select sequentially white recombinant clones on X-Gal plates and X-Gluc plates.

PRODUCT	QTY	CAT. CODE
psiRNA-DUO	1 kit	ksirna4-gz3

Related Products

Zeocin™, page 15
LacZ staining kits, page 18
ChemiComp GT116, page 47
Fast-Media® Zeo X-Gal, page 49
Fast-Media® Zeo X-Gal, page 49
LyoComp GT116, page 47

psiTEST™ System - Prevalidation of siRNAs and shRNAs

The psiTEST system enables you to screen for effective siRNAs and shRNAs without using time-consuming and costly reagents that are specific to your target gene, such as antibodies. The psiTEST system can be used to evaluate RNAi on virtually any gene of any species for which the sequence is known. The silencing efficiency of a given siRNA or shRNA is evaluated by monitoring the reduction of expression of a chimeric gene consisting of a secreted alkaline phosphatase (SEAP) reporter gene transcriptionally fused to the target gene. Transfection of effective siRNAs or shRNAs triggers the RNAi process resulting in the degradation of the chimeric mRNA and therefore the absence of SEAP activity.

Description

The psiTEST system is based on a transcriptional fusion between a reporter gene and your target gene. The reporter gene is an optimized alkaline phosphatase gene engineered to be secreted (SEAP). The efficacy of an siRNA or shRNA to induce RNAi on your target gene is determined by measuring the expression levels of the SEAP reporter gene. Since SEAP is a secreted protein, the expression level can be evaluated from supernatants therefore allowing to perform kinetics of the silencing process.

Features and Benefits

The psiTEST system contains two major components, the psiTEST plasmid and QUANTI-Blue™.

psiTEST plasmid

The psiTEST plasmid features the SEAP gene fused at the 3' end after its Stop codon to a multiple cloning site (MCS). This MCS contains many common restriction sites compatible with many other restriction sites. psiTEST also features a GFP::zeo fusion gene: the zeo moiety allows to amplify the plasmid in *E. coli* and select stable mammalian clones while the GFP moiety enables the monitoring of transfection efficiency.
psiTEST can be used to clone fragments of genes or entire genes, the optimum size being 1.5 kb, although genes up to 3.5 kb can be targeted.

QUANTI-Blue™

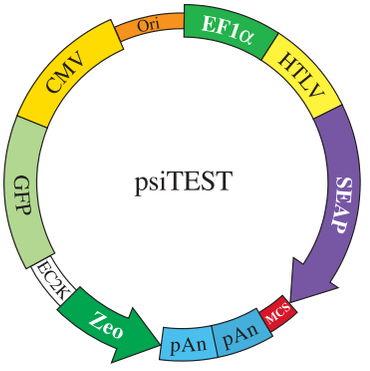
QUANTI-Blue™ is a SEAP detection medium that turns purple/blue in the presence of phosphatase activity (see pages 20-21). Functional siRNAs or shRNAs will induce the degradation of the SEAP mRNA resulting in the reduction or absence of SEAP activity.
Silencing efficiencies of the siRNAs or shRNAs can be observed visually and unlike fluorescent reporters can also be easily quantified using a microplate reader or spectrophotometer.

Contents and Storage

The psiTEST system is provided with several control plasmids: psiTEST-hp53, psiRNA-hp53 and psiRNA-Luc. psiTEST-hp53 contains a fusion between the SEAP and human p53 genes. psiRNA-hp53 produces an shRNA that functionally silences the human p53 resulting in the absence of phosphatase activity. psiRNA-Luc expresses an shRNA insert that targets luciferase GL3 and therefore will not affect the expression of the phosphatase reporter gene.

The psiTEST system contains the following components:
- 20 µg of psiTEST and 20 µg of psiTEST-hp53 (control plasmid)
- 20 µg of psiRNA-hp53 and 20 µg of psiRNA-Luc
- 2 pouches of QUANTI-Blue™ (100 ml each)
- 4 pouches of Fast-Media® Zeo (2 TB, 2 Agar)

Products are shipped at room temperature. Store Fast-Media® at room temperature. Store all other components at -20°C.



Procedure

1- Clone your target gene (or a fragment) into the psiTEST plasmid.
2- Cotransfect mammalian cells with recombinant psiTEST™ and siRNA or shRNA-producing plasmid, such as psiRNA™.
3- Incubate at 37°C with 5% CO_2 for 72 hours.
4- Collect supernatant and add to a QUANTI-Blue™-containing well.
5- Incubate at 37°C for 30 min to 3 hours.
6- Determine the functionality of your siRNA or shRNA by observing the coloration of QUANTI-Blue visually and/or with a spectrophotometer at 620-655 nm.

PRODUCT	QTY	CAT. CODE
psiTEST™	1 kit	ksitest
QUANTI-Blue™	5 pouches	rep-qb1

Related Products

Zeocin™, page 15
ChemiComp GT116, page 47
Fast-Media® Zeo, page 49
LyoComp GT116, page 47

IFNr qRT-Primers - Detection of the IFN Response

Double strand RNA (dsRNA) is a molecular pattern associated with viral infection. It is recognized by the innate immune system leading to the production of type I interferons and the subsequent induction of a variety of antiviral genes. Recent reports indicate that certain siRNAs and shRNAs can activate the IFN pathway, potentially leading to severe side effects. To determine whether a given siRNA or shRNA is immunostimulatory, InvivoGen has developed a set of primers that detect the expression of human genes involved in the IFN response by real-time quantitative PCR using the SYBR® Green detection.

- ▶ The IFNr qRT-Primers provide highly specific and sensitive results in real-time quantitative PCR.
- ▶ Each IFNr qRT-Primer Pair is carefully designed and tested.
- ▶ The size of the amplified fragments varies from 80 to 280 bp.
- ▶ The IFNr qRT-Primers allow to perform 100 x 50 µl reactions (in a 96-well plate) or 250 x 20 µl reactions (in a 384-well plate).

Description

The IFNr qRT-Primers are a set of primer pairs designed to measure the mRNA expression of 5 genes induced by IFNα:
- IFNβ
- OAS1
- MX1 (also known as MxA)
- G1P2 (also known as ISG15)
- IFIT1 (also known as CDKL2 or P56),

and the housekeeping gene GAPDH as a control.

The IFNr qRT-Primers allow one-step or two-step qRT-PCR in combination with SYBR® Green I dye.

Contents and Storage

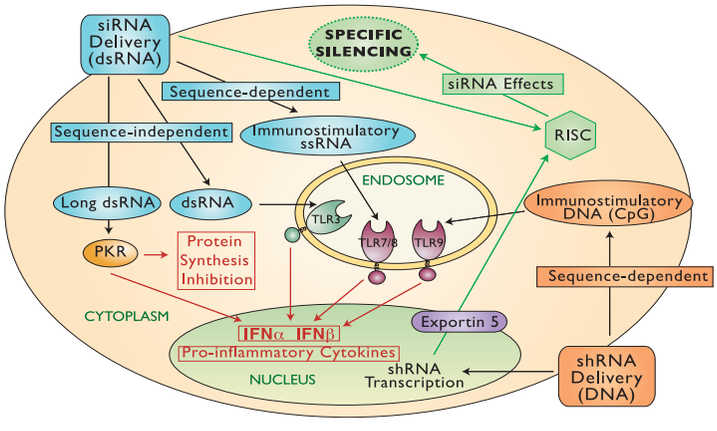
The IFNr qRT-Primers contain 10 µM of each primer. The 5' sense primer and the 3' antisense primer are provided in separate vials. Products are lyophilized and shipped at room temperature.

PRODUCT	QTY	CAT. CODE
IFNr qRT-Primers	1 kit	rts-hifnr

Interferon Response: An Off-Target Effect

The RNAi reagents, siRNA and shRNA, can be potent inducers of interferons (IFNs) and inflammatory cytokines both *in vivo* and *in vitro*[1-3] due to their recognition by cellular receptors of foreign RNA and DNA. Much of the IFN response is caused by the activation of the dsRNA-dependent protein kinase R (PKR), that recognizes long double-stranded (ds)RNA, leading to a global inhibition of protein synthesis. siRNAs that are shorter than 30 bp can evade PKR activation, but are recognized by other receptors, such as the Toll-like receptors (TLRs). TLR3, which ligand is dsRNA, and TLR7 and TLR8, that sense single-stranded RNA, have been implicated in the recognition of siRNAs[1-4]. Recognition by TLR7 and TLR8 appears to sequence-dependent as several immunostimulatory sequences have been identified. These sequences are characterized by a high content of guanosine and uridine residues[1, 5]. They have been integrated in InvivoGen's siRNA Wizard algorithm and are systematically excluded to limit the IFN response.

The IFN response induced by shRNAs results mainly from the DNA vector that contains non-methylated CpG sequences that are recognized by TLR9. InvivoGen has designed a CpG-free vector, pCpGfree-siRNA, to overcome this problem (see page 55).



1. Hornung V. *et al.*, 2005. Sequence-specific potent induction of IFN-alpha by short interfering RNA in plasmacytoid dendritic cells through TLR7. Nat Med. 11(3):263-70. **2. Sioud M., 2005.** Induction of inflammatory cytokines and interferon responses by double-stranded and single-stranded siRNAs is sequence-dependent and requires endosomal localization. J Mol Biol. 348(5):1079-90. **3. Agrawal S. & Kandimalla ER. 2004.** Antisense and siRNA as agonists of Toll-like receptors. Nat. Biotechnol. 22: 1533-1537. **4. Kariko K. *et al.*, 2004.** Exogenous siRNA mediates sequence-independent gene suppression by signaling through toll-like receptor 3. Cells Tissues Organs. 177(3):132-8. **5. Judge AD. *et al.*, 2005.** Sequence-dependent stimulation of the mammalian innate immune response by synthetic siRNA. Nat Biotechnol. 23(4):457-62.

Ready-Made psiRNA™ - Plasmids Expressing Functional shRNAs

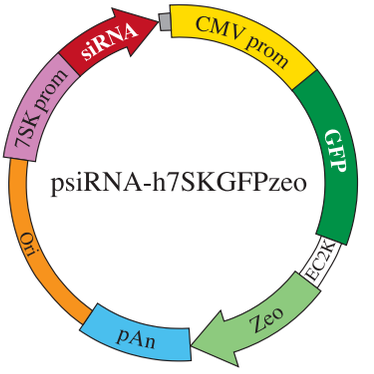
Ready-made psiRNA is a new family of plasmids expressing a growing list of shRNAs which functionality has been described in the literature or validated in house. Ready-made psiRNA plasmids eliminate the need to design and clone several siRNA sequences before identifying an effective one. They express shRNAs that silence the expression of a target gene by >70%.

Features and Benefits

Ready-made psiRNAs are psiRNA-h7SKGFPzeo-derived plasmids. They feature the human 7SK RNA Pol III promoter that generates high amounts of short hairpin RNAs. They also feature the GFP::Zeo fusion gene which confers both reporter and antibiotic resistance activities making these plasmids very useful for the following applications:

- Monitor transfection efficiency
- Standardize gene silencing efficiency
- Select clones that stably express a validated siRNA

The silencing efficiency of each Ready-made psiRNA plasmid has been tested using the psiTEST system (see page 64). The genes or fragments of the genes targeted by the Ready-made psiRNA have been fused to the secreted embryonic alkaline phosphatase (SEAP) reporter gene within the psiTEST plasmid. Silencing efficiencies have been confirmed by the absence of SEAP activity after cotransfecting HEK293 cells with each recombinant psiTEST and corresponding Ready-made psiRNA.



Contents and Storage

Ready-made psiRNA plasmids are available alone or in a kit.
Ready-made psiRNA plasmids are provided as 20 µg of lyophilized DNA.
Ready-made psiRNA kits contain the following components:
- 20 µg of a Ready-made psiRNA plasmid
- 20 µg of a control psiRNA plasmid (psiRNA-Luc)
- 1 vial of LyoComp GT116
- 4 pouches of Fast-Media® Zeo

Products are shipped at room temperature. Store at -20°C.

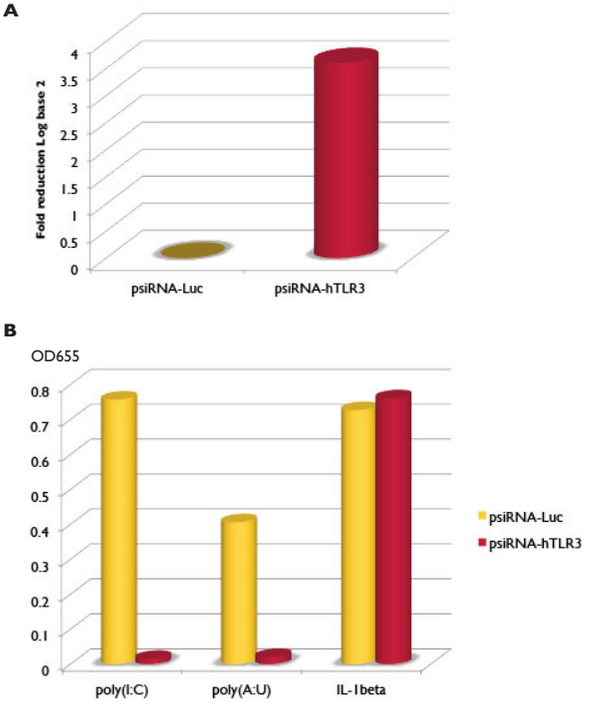
PRODUCT	QTY	CAT. CODE*
Ready-made psiRNA	20 µg	psirna42<gene>
Ready-made psiRNA Kit	1 kit	ksirna42<gene>

* See Table

Related Products

Zeocin™, page 15
LyoComp GT116, page 47
Fast-Media® Zeo, page 49

Example of silencing using Ready-made psiRNA-hTLR3



HEK293-SEAP cells, which express endogenous levels of TLR3 and stably express an NF-κB-inducible SEAP construct, were stably transfected with psiRNA-hTLR3 or psiRNA-Luc (negative control). (A) The fold reduction of TLR3 gene expression was determined by quantitative RT-PCR analysis. (B) The response of the transfected cells to the TLR3 agonists, poly(I:C) (3 µg/ml) and poly(A:U) (3 µg/ml), was assessed following 24h stimulation by determining NF-κB-induced SEAP levels using QUANTI-Blue™, a SEAP detection reagent.

GENE NAME/ALIASES	SPECIES	CAT. CODE (Plasmid)**
A20 / TNFAIP3	Human, mouse	psirna42-(h/m)a20
ASC / CARD5	Human, mouse	psirna42-(h/m)asc
AIM2 / IFI210	Human, mouse	psirna42-(h/m)aim2
ATF3	Human	psirna42-hatf3
ATG5	Human, mouse	psirna42-(h/m)atg5
BAF47 / SMARCB1	Human	psirna42-hbaf47
BPI	Human	psirna42-hbpi
BCL2	Human	psirna42-hbcl2
BCL3	Human, mouse	psirna42-(h/m)bcl3
BCL10 / CLAP	Human, mouse	psirna42-(h/m)bcl10
Beclin-1	Human, mouse	psirna42-(h/m)beclin
CARD8 / Cardinal	Human	psirna42-hcard8
CARD9	Human, mouse	psirna42-(h/m)card9
Caspase 1 / ICE	Human, mouse	psirna42-(h/m)casp1
Caspase 8 / FLICE	Human, mouse	psirna42-(h/m)casp8
CD14	Human, mouse	psirna42-(h/m)cd14
CD18 NEW	Human	psirna42-hcd18
CD36	Human, mouse	psirna42-(h/m)cd36
CD44	Human, mouse	psirna42-(h/m)cd44
CLEC9A	Human, mouse	psirna42-(h/m)clec9a
CXCL16	Human, mouse	psirna42-(h/m)cxcl16
CXCR4 / CD184	Human	psirna42-hcxcr4
CYLD	Human	psirna42-hcyld
DAI / ZBP1	Human, mouse	psirna42-(h/m)dai
DAK	Human, mouse	psirna42-(h/m)dak
DC-SIGN / CD209	Human, mouse	psirna42-(h/m)dcsign
DDX3 / DDX3X	Human, mouse	psirna42-(h/m)ddx3x
DDX41 NEW	Human, mouse	psirna42-(h/m)ddx41
Dectin-1	Human, mouse	psirna42-(h/m)dectin1
DNMT1	Human	psirna42-hdnmt1
DNMT2	Human	psirna42-hdnmt2
DNMT3A	Human	psirna42-hdnmt3a
DNMT3B	Human	psirna42-hdnmt3b
DNMT3L	Human	psirna42-hdnmt3l
DUBA / OTUD5	Mouse	psirna42-mduba
FADD / MORT1	Human, mouse	psirna42-(h/m)fadd
FLII / Fliih	Human	psirna42-hflii
GM-CSF	Human, mouse	psirna42-(h/m)gmcsf
HDAC1	Human	psirna42-hhdac1
HDAC2	Human	psirna42-hhdac2
HDAC3	Human	psirna42-hhdac3
HDAC6	Human	psirna42-hhdac6
HDAC8	Human	psirna42-hhdac8
HPRT	Human	psirna42-hhprt
IFI16 / PYHIN2	Human, mouse	psirna42-(h/m)ifi16
IFIT1 NEW	Human, mouse	psirna42-(h/m)ifit1
IFNAR1	Human, mouse	psirna42-(h/m)ifnar1
IFNAR2	Human, mouse	psirna42-(h/m)ifnar2
IKKε / IKBKE / IKK-i	Human, mouse	psirna42-(h/m)ikke
IL1R1 / IL1RA	Human	psirna42-hil1r1
IPAF / CARD12	Human	psirna42-hipaf

GENE NAME/ALIASES	SPECIES	CAT. CODE (plasmid)**
IPS-1 / MAVS / VISA	Human, mouse	psirna42-(h/m)ips1
IRAK-1	Human, mouse	psirna42-(h/m)irak1
IRAK-4	Human, mouse	psirna42-(h/m)irak4
IRAK-M	Human	psirna42-hirakm
IRF1	Human, mouse	psirna42-(h/m)irf1
IRF3	Human, mouse	psirna42-(h/m)irf3
IRF5	Human, mouse	psirna42-(h/m)irf5
IRF7	Human, mouse	psirna42-(h/m)irf7
IRF9	Human, mouse	psirna42-(h/m)irf9
JAK1 / JAK1A	Human, mouse	psirna42-(h/m)jak1
KAISO / ZBTB33	Human	psirna42-hkaiso
KRAS	Human, mouse	psirna42-(h/m)kras
Ku70 / XRCC6 NEW	Human, mouse	psirna42-(h/m)ku70
LacZ	*E. coli*	psirna42-lacz
Lamin A/C	Human	psirna42-hlamin
LC3B NEW	Human	psirna42-hlc3b
LGP2 / DHX58	Human, mouse	psirna42-(h/m)lgp2
LRRFIP1 NEW	Human, mouse	psirna42-(h/m)lrrfip1
LRRFIP2	Human	psirna42-hlrrfip2
Luc GL3	Firefly	psirna42-lucgl3
MBL2	Human	psirna42-hmbl2
MBD1 / PCM1	Human	psirna42-hmbd1
MD2	Human, mouse	psirna42-(h/m)md2
MDA-5 / IFIH1	Human, mouse	psirna42-(h/m)mda5
MeCP2	Human	psirna42-hmecp2
MEKK3 / MAP3K	Human, mouse	psirna42-(h/m)mekk3
Mincle / CLEC4E	Human, mouse	psirna42-(h/m)mincle
MNDA / PYHIN3	Human	psirna42-hmnda
MSK1	Human, mouse	psirna42-(h/m)msk1
MULAN / MUL1	Human, mouse	psirna42-(h/m)mul1
MyD88	Human, mouse	psirna42-(h/m)myd88
Naip5 / BIRC1E	Mouse	psirna42-mnaip5
NALP1 / CARD7	Human	psirna42-hnalp1
NALP2 / PAN1	Human	psirna42-hnalp2
NALP3 / NLRP3	Human, mouse	psirna42-(h/m)nalp3
NALP11 / NLRP11	Human	psirna42-hnalp11
NALP12 / Monarch1	Human	psirna42-hnalp12
NAP1 / AZI2	Human, mouse	psirna42-(h/m)nap1
NLRC5 NEW	Human	psirna42-hnlrc5
NOD1 / CARD4	Human, mouse	psirna42-(h/m)nod1
NOD2 / CARD15	Human, mouse	psirna42-(h/m)nod2
NOD9 / NLRX1	Human, mouse	psirna42-(h/m)nod9
P2X7 / P2RX7 NEW	Human, mouse	psirna42-(h/m)p2x7
p53	Human, mouse	psirna42-(h/m)p53
PACT / PRKRA	Human, mouse	psirna42-(h/m)pact
Pannexin 1 / PANX1	Human, mouse	psirna42-(h/m)panx1
Pellino 1 / PELI1	Human, mouse	psirna42-(h/m)pellino1
Pellino 2 / PELI2	Human, mouse	psirna42-(h/m)pellino2
Pellino 3 / PELI3	Human	psirna42-hpellino3
PIN1 / DOB	Human, mouse	psirna42-(h/m)pin1
PKD1 / PRKD1	Human	psirna42-hpkd1

** For the catalog code of the kit, replace psirna42 by ksirna42

GENE NAME/ALIASES	SPECIES	CAT. CODE (plasmid)**
PKR / EIFAK2	Human, mouse	psirna42-(h/m)pkr
RAC1	Human, mouse	psirna42-(h/m)rac1
RAGE / AGER	Human, mouse	psirna42-(h/m)ager
RIG-I / DDX58	Human, mouse	psirna42-(h/m)rigi
RIP1 / RIPK1	Human, mouse	psirna42-(h/m)rip1
RIP2 / RIPK2	Human, mouse	psirna42-(h/m)rip2
RNF125 / TRAC-1	Human, mouse	psirna42-(h/m)rnf125
RP105 / CD180	Human	psirna42-hrp105
S100A8	Mouse	psirna42-ms100a8
S100A9	Mouse	psirna42-ms100a9
SARM1	Human	psirna42-hsarm1
SHIP	Human	psirna42-hship
SIGIRR / TIR8	Human	psirna42-hsigirr
SIGNR1	Mouse	psirna42-msignr1
SIGNR2	Mouse	psirna42-msignr2
SIGNR3	Mouse	psirna42-msignr3
SIKE	Human, mouse	psirna42-(h/m)sike
Smad3	Human	psirna42-hsmad3
Smad4	Human	psirna42-hsmad4
SOCS-1	Human, mouse	psirna42-(h/m)socs1
SOCS-3	Human	psirna42-hsocs3
ST2 / IL1RL1 / T2	Human	psirna42-hst2
STAT1 / STAT91	Human, mouse	psirna42-(h/m)stat1
STAT2 / STAT113	Human, mouse	psirna42-(h/m)stat2
STAT3 / APRF	Human	psirna42-hstat3
STING	Human, mouse	psirna42-(h/m)sting
SUGT1 / SGT1	Human	psirna42-hsugt1
SUV39H1 / KMTIA	Human	psirna42-hsuv39h1
TAK1 / MAP3K7	Human, mouse	psirna42-(h/m)tak1
TANK	Human, mouse	psirna42-(h/m)tank
TBK1	Human, mouse	psirna42-(h/m)tbk1

GENE NAME/ALIASES	SPECIES	CAT. CODE (plasmid)**
TBKBP1 / SINTBAD	Human	psirna42-htbkbp1
TICAM1 / TRIF	Human, mouse	psirna42-(h/m)ticam1
TICAM2 / TRAM	Human, mouse	psirna42-(h/m)ticam2
TIFA	Human, mouse	psirna42-(h/m)tifa
TIRAP / MAL	Human, mouse	psirna42-(h/m)tirap
TLR1 / CD281	Human, mouse	psirna42-(h/m)tlr1
TLR2 / CD282	Human, mouse	psirna42-(h/m)tlr2
TLR3 / CD283	Human, mouse	psirna42-(h/m)tlr3
TLR4 / CD284	Human, mouse	psirna42-(h/m)tlr4
TLR5	Human, mouse	psirna42-(h/m)tlr5
TLR6 / CD286	Human, mouse	psirna42-(h/m)tlr6
TLR7	Human, mouse	psirna42-(h/m)tlr7
TLR8 / CD288	Human, mouse	psirna42-(h/m)tlr8
TLR9 / CD289	Human, mouse	psirna42-(h/m)tlr9
TLR10 / CD290	Human	psirna42-htlr10
TNFR1 / TNFRSF1A	Human	psirna42-htnfr1
TNFR2 / TNFRSF1B	Human, mouse	psirna42-(h/m)tnfr2
Tollip / IL-1RAcP1P	Human	psirna42-htollip
TRADD	Human, mouse	psirna42-(h/m)tradd
TRAF1	Human	psirna42-htraf1
TRAF3 / CAP-1	Human, mouse	psirna42-(h/m)traf3
TRAF4	Human, mouse	psirna42-(h/m)traf4
TRAF6 / RNF85	Human, mouse	psirna42-(h/m)traf6
TRAFD1 / FLN29	Human	psirna42-htrafd1
TREX1 **NEW**	Human, mouse	psirna42-(h/m)trex1
Triad3A	Human	psirna42-htriad3a
TRIM56 **NEW**	Human, mouse	psirna42-(h/m)trim56
Tyk2 / JTK1	Human, mouse	psirna42-(h/m)tyk2
UBP43	Human, mouse	psirna42-(h/m)ubp43
UNC93B1	Human, mouse	psirna42-(h/m)unc93
VEGF	Human, mouse	psirna42-(h/m)vegf

** For the catalog code of the kit, replace psirna42 by ksirna42

Recent articles using Ready-made psiRNA plasmids

psiRNA-hBcl2 - Mena S. *et al.*, 2012. Glutathione and Bcl-2 targeting facilitates elimination by chemoradiotherapy of human A375 melanoma xenografts overexpressing bcl-xl, bcl-2, and mcl-1. J Transl Med. 10:8.

psiRNA-hBeclin-1 - Kuwahara Y. *et al.*, 2011. Enhancement of autophagy is a potential modality for tumors refractory to radiotherapy. Cell Death Dis. 2:e177.

psiRNA-LacZ & psiRNA-LucGL3 - Ho JJ. *et al.*, 2012. Functional importance of Dicer in the adaptive cellular response to hypoxia. J Biol Chem. [Epub ahead of print]

psiRNA-hLaminA/C - De Vos WH. *et al.*, 2010. Increased plasticity of the nuclear envelope and hypermobility of telomeres due to the loss of A-type lamins. Biochim Biophys Acta. 1800(4):448-58.

psiRNA-hMDA-5 - Peltier DC. *et al.*, 2010. Human neuronal cells possess functional cytoplasmic and TLR-mediated innate immune pathways influenced by phosphatidylinositol-3 kinase signaling. J Immunol. 184(12):7010-21.

psiRNA-hp53 & psiRNA-mp53 - Hara MR. *et al.*, 2011. A stress response pathway regulates DNA damage through β2-adrenoreceptors and β-arrestin-1. Nature. 477(7364):349-53.

psiRNA-hRIG-I - Morosky SA. *et al.*, 2011. Retinoic acid-induced gene-I (RIG-I) associates with nucleotide-binding oligomerization domain-2 (NOD2) to negatively regulate inflammatory signaling. J. Biol. Chem. 286(32):28574-83.

psiRNA-TICAM2 - Ohnishi H. *et al.*, 2012. TRAM Is Involved in IL-18 Signaling and Functions as a Sorting Adaptor for MyD88. PLoS One. 7(6):e38423.

psiRNA-hTLR2 & psiRNA-hTLR4 - Lee HM. *et al.*, 2011. Autophagy negatively regulates keratinocyte inflammatory responses via scaffolding protein p62/SQSTM1. J Immunol. 186(2):1248-58.

psiRNA-mTLR3 - Derbigny WA. *et al.*, 2010. The Chlamydia muridarum-induced IFN-β response is TLR3-dependent in murine oviduct epithelial cells. J Immunol. 185(11):6689-97.

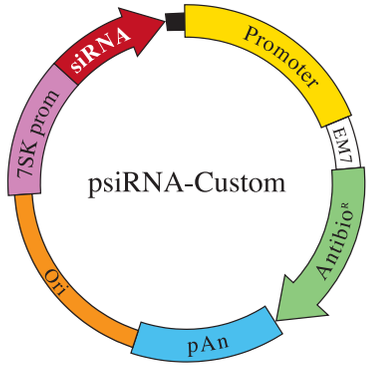
psiRNA-hTLR5 - Arikawa K. & Nishikawa Y., 2010. Interleukin-8 induction due to diffusely adherent Escherichia coli possessing Afa/Dr genes depends on flagella and epithelial Toll-like receptor 5. Microbiol Immunol. 54(9):491-501.

psiRNA-hTLR9 - Wu JY. & Kuo CC., 2012. Pivotal Role of ADP-ribosylation Factor 6 in Toll-like Receptor 9-mediated Immune Signaling. J Biol Chem. 287(6):4323-34.

Custom-Made psiRNA™

InvivoGen provides a complete siRNA service to help you speed-up your gene silencing experiments. Just give us the accession number of your gene of interest and we will take care of all the steps, from helping you choose the target sequence on your gene to sequencing the resulting siRNA insert. InvivoGen provides a choice of plasmidic vectors to better suit your needs.

- **Full Custom Service**
- **Your siRNA vector Ready-to-Use**
- **Cost-effective**
- **Rapid Processing**



Features and Benefits

Choose your siRNA sequence

You can either send us your siRNA sequence or, using the siRNA Wizard software, InvivoGen will select the best candidate siRNA sequence for your target gene.

Choose your psiRNA Plasmid

- **psiRNA-h7SK** (see page 62)
- **psiRNA-DUO** (see page 63)

Choose your selectable marker

psiRNA plasmids are available with various selectable markers that confer antibiotic resistance in both *E. coli* and mammalian cells:
- **Selectable marker:** blasticidin^R, hygromycin B^R, Kanamycin^R / G418^R, or Zeocin^R
- **Reporter and selectable marker:** GFP::Zeo, a transcriptional fusion between the Green Fluorescent Protein reporter gene and the Zeocin™ resistance gene.

Description

Construction of custom-made psiRNA plasmids includes the following:
- Selection of the siRNA sequence for the target gene
- Synthesis of the siRNA insert oligonucleotides
- Cloning of the annealed oligonucleotides in psiRNA
- Sequencing of the siRNA insert

Contents and Storage

Custom-made psiRNA are provided as 20 µg of lyophilized DNA. They can also be provided as a kit that contains in addition
- 20 µg of psiRNA-EGFP or psiRNA-LucGL3 as a control
- 1 vial of LyoComp GT116
- 4 pouches of the corresponding Fast-Media®

Products are shipped at room temperature. Store at -20°C.

Although the siRNA Wizard software can facilitate the selection of functional shRNAs, there is no guarantee that the resulting shRNA will be effective enough for your experiment. Thus it is usually recommended to select 3-5 candidate shRNAs. Furthermore, most journal article reviewers require that gene silencing results be confirmed with a second effective siRNA to a target before an article is accepted for publication.

Orders of multiple psiRNA plasmids targeting the same gene will be eligible for a volume discount.

PRODUCT	QTY	CAT. CODE
Custom-made psiRNA	20 µg	p-custom
Custom-made psiRNA Kit	1 kit	k-custom

5 INHIBITORS

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.

- **Inhibitors of innate immunity signaling**
- **Inhibitors of NF-κB and MAPK activation**
- **Inhibitors of mTOR and calcineurin signaling**
- **Inhibitors of JAK/STAT activation**
- **Inhibitors of DNA synthesis and chromatin remodeling**
- **Inhibitors of Hsp90**

PRODUCT	DESCRIPTION	TARGET	WORKING CONCENTRATION	QTY	CATALOG CODE	INFO
17-AAG	GA analogue	Hsp90	1 nM - 10 µM	5 mg 25 mg	ant-agl-5 ant-agl-25	p 81
17-AEP-GA	GA analogue	Hsp90	1 nM - 10 µM	1 mg 5 mg	ant-egl-1 ant-egl-5	p 81
17-DMAG	GA analogue	Hsp90	1 nM - 10 µM	5 mg 25 mg	ant-dgl-5 ant-dgl-25	p 81
17-DMAP-GA	GA analogue	Hsp90	1 nM - 10 µM	1 mg 5 mg	ant-mgl-1 ant-mgl-5	p 81
17-GMB-APA-GA	GA analogue for conjugation to MAb	Hsp90	1 nM - 10 µM	1 mg	gmbapa-ga	p 82
17-NHS-ALA-GA	GA analogue	Hsp90	1 nM - 10 µM	1 mg	ant-nhgl-1	p 82
2-Aminopurine	PKR inhibitor	Innate immunity	1 - 10 mM	250 mg	tlrl-apr	p 73
3-Methyladenine	PI3K inhibitor	mTOR / Calcineurin	5 mM	50 mg	tlrl-3ma	p 77
5-Aza-cytidine	DNA methyltransferase inhibitor	DNA / Chromatin	2 µM	100 mg	inh-aza	p 79
5-Aza-2'-deoxycytidine	DNA methyltransferase inhibitor	DNA / Chromatin	0.1 - 10 µM	10 mg 50 mg	met-adc-1 met-adc-5	p 79
5-Fluorocytosine	Thymidine synthesis inhibitor	DNA / Chromatin	200 - 500 µg/ml	250 mg	sud-5fc	p 79
5-Fluorouracil	Thymidine synthesis inhibitor	DNA / Chromatin	1 - 300 µg/ml	250 mg	sud-5fu	p 79
AG490	JAK family tyrosine kinase inhibitor	JAK / STAT	1 -100 µM	10 mg	tlrl-ag4	p 78
Bafilomycin A1	V-ATPase inhibitor	Innate immunity	0.1 - 1 µM	10 µg	tlrl-baf	p 73
Bay11-7082	IκB-α inhibitor	NF-κB / MAPK	1 - 10 µM	10 mg	tlrl-b82	p 75
Biotin-GA	Biotin-labeled GA analogue	Hsp90	1 nM - 10 µM	1 mg 5 mg	ant-bgl-1 ant-bgl-5	p 82
Bix-01294	G9a histone methyltransferase inhibitor	DNA / Chromatin	1 µM	2 mg	inh-bix	p 79
BX795	TBK1/IKKε and PDK1 inhibitor	Innate immunity	10 nM - 1 µM	5 mg	tlrl-bx7	p 73
Celastrol	NF-κB inhibitor	NF-κB / MAPK	2.5 - 10 µM	1 mg	ant-cls	p 75
Chloroquine	Endosomal acidification inhibitor	Innate immunity	10 µM	250 mg	tlrl-chq	p 73
CI-994 **NEW**	HDAC inhibitor	DNA / Chromatin	1 - 10 µg/ml	10 mg	inh-ci99	p 79
CLI-095	TLR4 signaling inhibitor	Innate immunity	50 nM - 1 µM	1 mg	tlrl-cli95	p 73
CP-690550 **NEW**	JAK3 inhibitor	JAK / STAT	50 nM - 1 µM	5 mg	tlrl-cp69	p. 78
Cyclosporin A	Calcineurin inhibitor	mTOR / Calcineurin	50 nM - 1.5 µM	100 mg	tlrl-cyca	p 77
Dexamethasone	NF-κB and MAPK inhibitor	NF-κB / MAPK	100 nM	100 mg	tlrl-dex	p 75

PRODUCT	DESCRIPTION	TARGET	WORKING CONCENTRATION	QTY	CATALOG CODE	INFO
Everolimus NEW	mTOR inhibitor	mTOR / Calcineurin	1 - 300 nM	5 mg	tlrl-eve	p. 77
FK506 (Tacrolimus) NEW	Calcineurin inhibitor	mTOR / Calcineurin	1- 100 nM	10 mg	tlrl-fk5	p. 77
Ganciclovir	Thymidine synthesis inhibitor	DNA / Chromatin	1 - 100 µg/ml	250 mg	sud-gcv	p. 80
Gefitinib (Iressa)	RIP2 tyrosine kinase inhibitor	Innate immunity	10 µM	10 mg	tlrl-gef	p. 73
Geldanamycin (GA)	Hsp90 inhibitor	Hsp90	1 nM - 10 µM	5 mg 25 mg	ant-gl-5 ant-gl-25	p. 81
Glybenclamide (glyburide)	ATP-dependent K+ channel inhibitor	Innate immunity	25 µg/ml	1 g	tlrl-gly	p. 73
H-89	PKA inhibitor	Innate immunity	5 - 50 µM	5 mg	tlrl-h89	p. 73
Leptomycin B	Nuclear export inhibitor	DNA / Chromatin	50 - 200 nM	5 µg	tlrl-lep	p. 80
LY294002	PI3K inhibitor	mTOR / Calcineurin	50 - 100 µM	5 mg	tlrl-ly29	p. 77
MG-132	26S proteasome inhibitor	NF-κB / MAPK	10 µM	5 mg	tlrl-mg132	p. 75
Metformin NEW	AMPK activator / mTOR inhibitor	mTOR / Calcineurin	2 - 10 mM	1 g	tlrl-metf	p. 77
OxPAPC	TLR2 and TLR4 inhibitor	Innate immunity	30 µg/ml	1 mg	tlrl-oxp1	p. 74
PD98059	MAP kinase kinase inhibitor	NF-κB / MAPK	50 - 100 µM	10 mg	tlrl-pd98	p. 75
PD0325901	MEK inhibitor	NF-κB / MAPK	0.5 µM	2 mg	inh-pd32	p.75
Pepinh-MYD	MyD88 inhibitory peptide	Innate immunity	5 - 100 µM	2 mg	tlrl-pimyd	p. 74
Pepinh-TRIF	TRIF inhibitory peptide	Innate immunity	5 - 100 µM	2 mg	tlrl-pitrif	p. 74
Perifosine NEW	Akt inhibitor	mTOR / Calcineurin	5 - 50 µM	5 mg	tlrl-peri	p. 77
Piceatannol	Syk inhibitor	Innate immunity	1 - 25 µM	5 mg	tlrl-pct	p. 74
Polymyxin B	LPS-induced TLR4 activation inhibitor	Innate immunity	10 µg/ml	100 mg	tlrl-pmb	p. 74
Rapamycin (Sirolimus)	mTOR inhibitor	mTOR / Calcineurin	10-100 nM	5 mg	tlrl-rap	p. 77
Resveratrol	NF-κB and AP-1 inhibitor	NF-κB / MAPK	10 - 100 µM	100 mg	tlrl-resv	p. 75
Ruxolitinib NEW	Pan JAK inhibitor	JAK / STAT	100 - 1000 nM	5 mg	tlrl-rux	p. 78
SAHA NEW	Pan HDAC inhibitor	DNA / Chromatin	0.1 - 10 µM	25 mg	inh-saha	p. 80
SB202190	p38 MAP kinase inhibitor	NF-κB / MAPK	1 - 20 µM	5 mg	tlrl-sb90	p. 75
SB203580	p38 MAP kinase inhibitor	NF-κB / MAPK	1 - 20 µM	5 mg	tlrl-sb20	p. 76
SB431542	TGF-β receptor inhibitor	NF-κB / MAPK	2 µM	5 mg	inh-sb43	p. 76
SP600125	JNK inhibitor	NF-κB / MAPK	10 - 500 µM	10 mg	tlrl-sp60	p. 76
Tamoxifen	Estrogen receptor antagonist	mTOR / Calcineurin	10 - 100 µM	200 mg	tlrl-txf	p. 77
Trichostatin A	Histone deacetylase inhibitor	DNA / Chromatin	150 - 300 nM	1 mg 5 mg	met-tsa-1 met-tsa-5	p. 80
Triptolide	NF-κB activation inhibitor	NF-κB / MAPK	10 - 100 nM	1 mg	ant-tpl	p. 76
U0126	MEK1-MEK2 inhibitor	NF-κB / MAPK	10 - 50 µM	5 mg	tlrl-u0126	p. 76
Valproic Acid	Histone deacetylase inhibitor	DNA / Chromatin	2 mM	5 g	inh-vpa	p. 80
Wortmannin	PI3K inhibitor	mTOR / Calcineurin	0.1 - 2.5 µM	5 mg	tlrl-wtm	p. 78
Z-VAD-FMK	Pan-caspase Inhibitor	Innate immunity	10 µg/ml (20 µM)	1 mg	tlrl-vad	p. 74

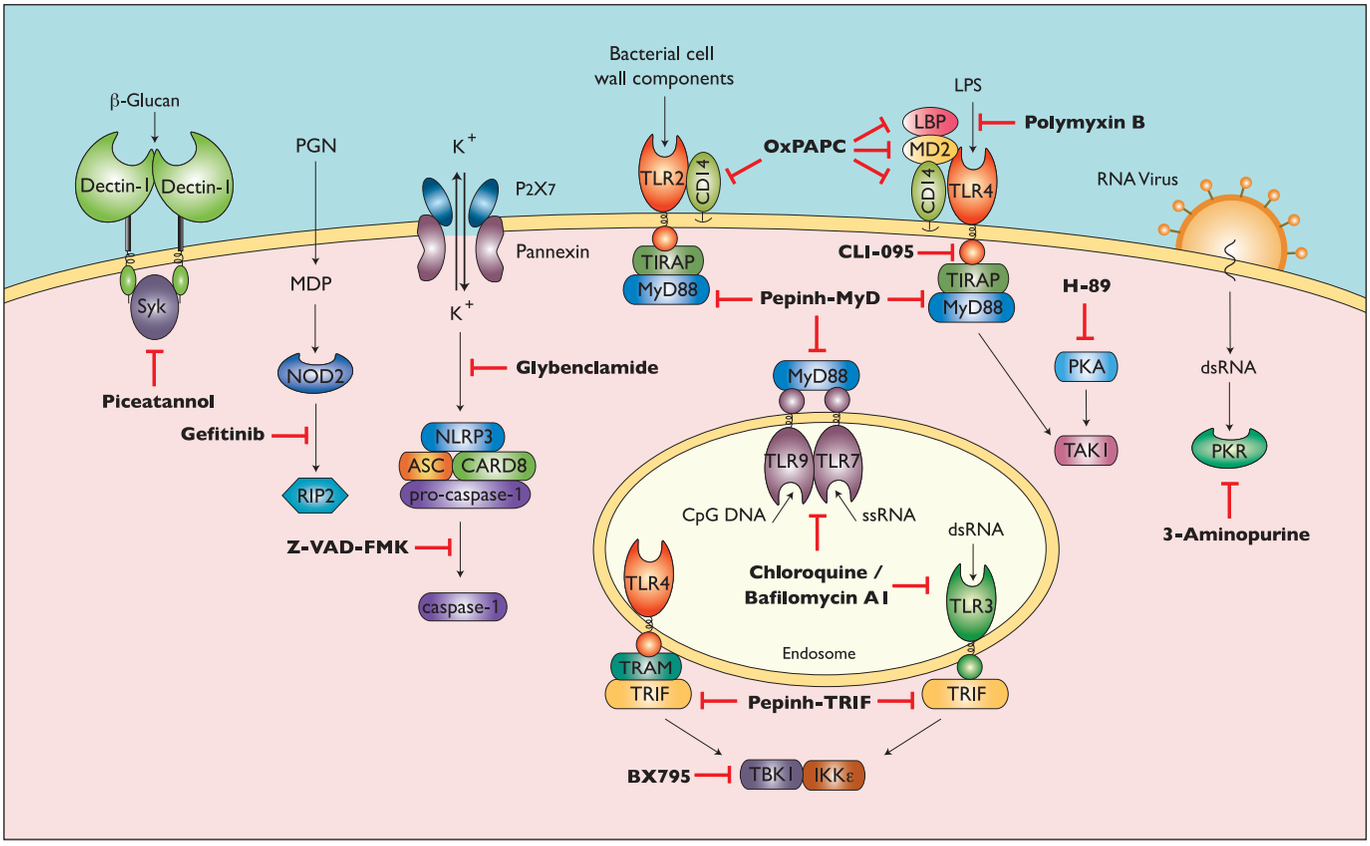
Contents and Storage

Products are provided as solids and shipped at room temperature. Store at room temperature, 4°C or -20°C according to the product label. Geldanamycin and analogues, and triptolide should be kept in the dark. Products are stable at least 6 months.

Inhibitors and activators of Autophagy

Many of the inhibitors provided by InvivoGen can act either as inhibitors or activators of autophagy by targeting diverse molecules that regulate this process at different levels.

See illustration page 83



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].

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[4,5]. Furthermore, by blocking endo-lysosomal compartment acidification, bafilomycin interferes with intracellular TLR signaling[6].

BX795 - TBK/IKKε Inhibitor

BX795, an aminopyrimidine compound, was developed as an inhibitor of 3-phosphoinositide-dependent kinase 1 (PDK1)[7]. 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[8]. 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[9,10]. 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[11,12].

CLI-095 - TLR4 Signaling Inhibitor

CLI095, also known as TAK-242, is a novel cyclohexene derivative that specifically suppresses TLR4 signaling, inhibiting the production of NO and pro-inflammatory cytokines[13]. 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[14].

Gefitinib - RIP2 Tyrosine Kinase inhibitor

Gefitinib (also known as Iressa) is a selective inhibitor of epidermal growth factor, a growth factor that plays a pivotal role in the control of cell growth, apoptosis, and angiogenesis. Recent studies demonstrated that Gefitinib can inhibit 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[15]. Gefitinib is used in the treatment of advanced non-small cell lung cancer[16].

Glybenclamide (glyburide) - Potassium channel inhibitor

Glybenclamide, also known as glyburide, blocks the maturation of caspase-1 and pro-IL-1β by inhibiting K+ efflux[17]. Glybenclamide was shown to potently block the activation of the NLRP3 inflammasome induced by PAMPs, DAMPs and crystalline substances[18]. 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)[19]. 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[20].

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[21,22].

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)[23] preceeded by a protein transduction sequence (RQIKIWFQNRRMKWKK) derived from antennapedia which enables the peptide to translocate through the cell membrane[24]. 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)[25] linked to the cell-penetrating segment of the antennapedia homeodomain (RQIKIWFQNRRMKWKK)[24]. 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[26].

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[27,28]. The neutralizing effect of PMB on LPS is dose-dependent and specific for LPS[29]. PMB is widely used to eliminate the effects of endotoxin contamination, both *in vitro* and *in vivo*.

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[30]. 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[31].

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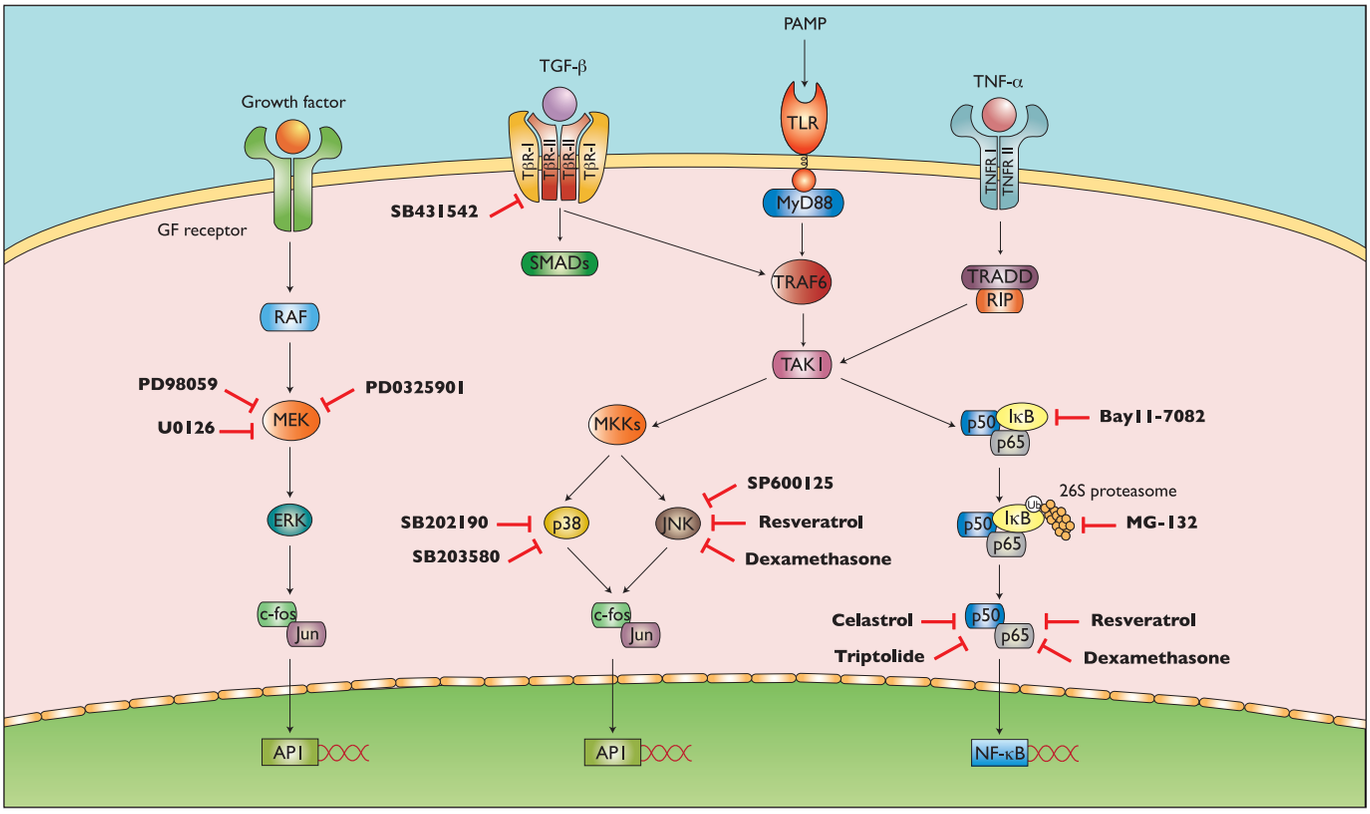
Inhibitors of NF-κB and MAPK Activation



Bay 11-7082 - IκB-α Inhibitor

Bay 11-7082 is an irreversible inhibitor of TNF-α-induced IκB-α phosphorylation resulting in the inactivation of NF-κB[1]. TNF-α-dependent effects of NF-κB are important for TLR expression and cytokine production[2]. Recently, Bay-11-7082 was identified as a potent inhibitor of the NLRP3 inflammasome independent of its inhibitory effect on NF-κB. Bay 11-7082 is believed to act by suppressing the ATPase activity of NLRP3[3].

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[4,5].

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[6]. Dexamethasone has been shown to repress a large set of pro-inflammatory genes by blocking NF-κB and MAPK activation during TLR engagement depending on the TLR ligand and whether the adapters MyD88 and TRIF are activated individually or coincidently[7].

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[8].

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)[9]. 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[10]. 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. Treatment with PD0325901 has been shown to enhance reprogramming efficiencies of induced pluripotent stem cells[11].

Resveratrol - NF-κB & MAPK Inhibitor

Resveratrol (3,4',5-trihydroxy-trans-stilbene) is a polyphenol found in plants known to possess anti-inflammatory and chemopreventive properties. Resveratrol has been shown to inhibit the expression of proinflammatory markers, including inducible nitric oxide synthase and cyclooxygenase-2 in macrophages and cancer cells[12], block TRIF-dependent signaling pathway of TLR3 and TLR4[13] and modulate bacterial phagocytosis of macrophages[14]. Resveratrol may exert these diverse effects by suppressing NF-κB and/or AP-1 activation in a cell or tissue-specific manner[15].

SB202190 - p38/RK MAP Kinase Inhibitor

SB202190, a close relative of SB203580, is widely used to assess the physiological roles of p38α and p38β MAPKs. Recent studies have identified other protein kinases, including GAK, CK1 and RIP2, that are potently inhibited by SB202190 (as well as SB203580)[16]. Further, SB202190 was shown to induce autophagic vacuoles through cross-inhibition of the PI3K/mTOR pathway[17].

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[18]. SB203580 inhibits also the phosphorylation and activation of protein kinase B (PKB, also known as Akt)[19].

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[20]. TGF-β is involved in many cellular processes including cell growth, cell differentiation, apoptosis, and specification of developmental fate. TGF-β signals through the Smad proteins, but it can also activate MAPKs through TAK1 and PI3K in a Smad-independent manner[21]. Inhibition of TGF-β signaling is known to induce the de-repression of epithelial fate and was shown to benefit the reprogramming process. Indeed, treatment of OSKM-transduced human primary fibroblasts with a combination of SB431542 and PD0325901, a MEK inhibitor, was found to improve reprogramming efficiency of human cells[22].

SP600125 - JNK Inhibitor

SP600125 is a potent, cell-permeable, selective and reversible inhibitor of c-Jun N-terminal kinase (JNK)[23]. 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[24].

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[25].

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BAY11-7082, PD98059, SB203580 & SP600125 - Watanabe T. *et al.*, 2012. Involvement of activation of toll-like receptors and nucleotide-binding oligomerization domain-like receptors in enhanced IgG4 responses in autoimmune pancreatitis. Arthritis Rheum. 64(3):914-24.

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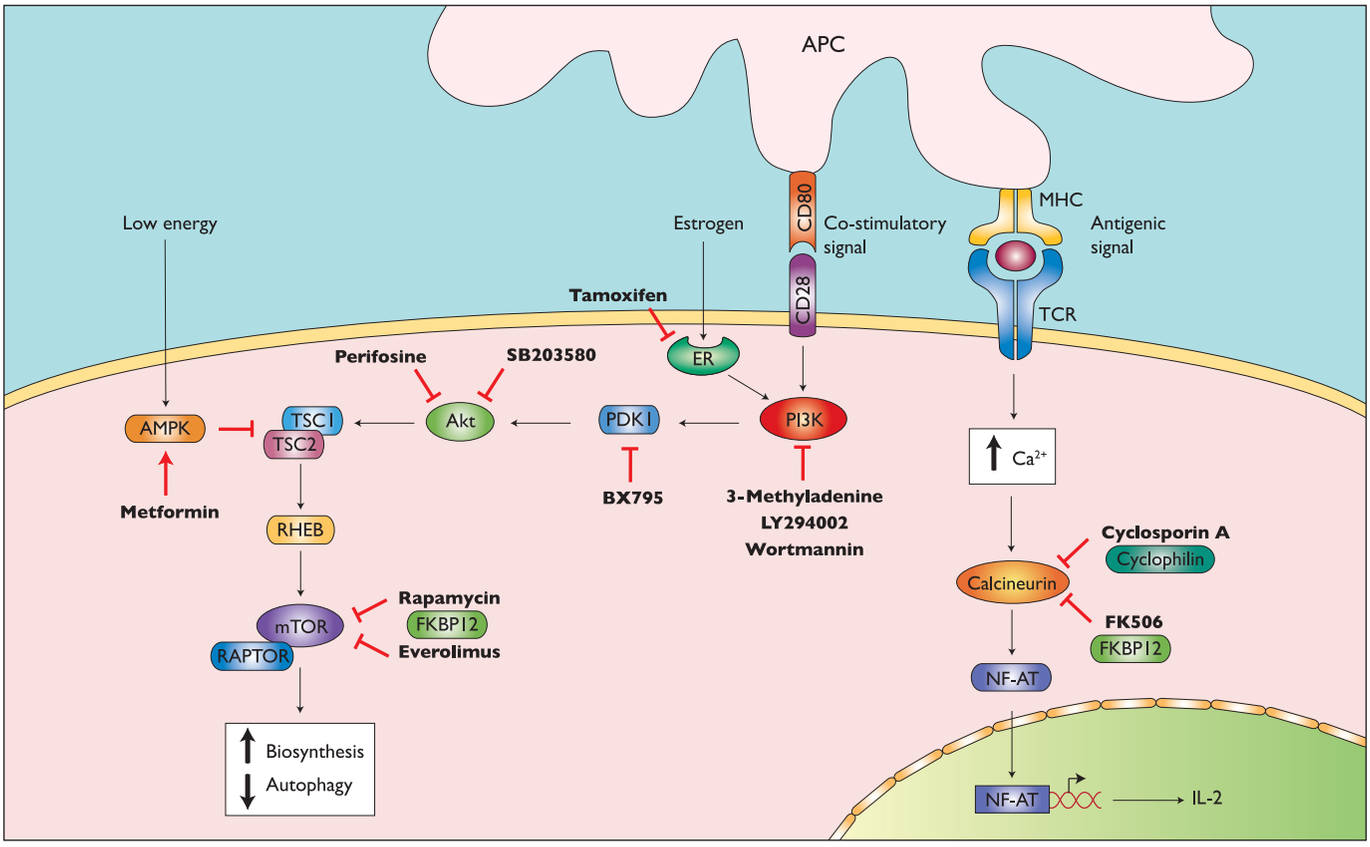
Inhibitors of mTOR and Calcineurin Signaling



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].

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 (NF-AT), thus preventing the transcription of T cell effector cytokines[4]. 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[5].

Everolimus - mTOR inhibitor **NEW**

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[6]. It has been demonstrated that in cancer models Everolimus promotes autophagy through the inhibition of mTOR[7].

FK506 - Calcineurin inhibitor **NEW**

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[8].

LY294002 - PI3K Inhibitor

LY294002 is a potent, cell permeable inhibitor of PI3K that acts on the ATP binding site of the enzyme[9]. LY294002 is often used to study the role of PI3K in apoptosis[4] and autophagy[10].

Metformin - AMPK activator **NEW**

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[11]. Experimental data indicate that metformin blocks lymphoma cell growth through the inhibition of the mTOR pathway and the induction of autophagy[12].

Perifosine - Akt inhibitor **NEW**

Perifosine is an alkylphospholipid exhibiting antitumor activity. It induces cell cycle arrest and apoptosis through the inhibition of the serine-threonine protein kinase Akt[13]. In addition, perifosine inhibits mTOR signaling through a different mechanism than classical mTOR inhibitors such as rapamycin[14].

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[15].

Tamoxifen - Estrogen Receptor Antagonist / Autophagy Inducer

Tamoxifen is an antagonist of the estrogen receptor known to induce autophagy and cell death[16]. Tamoxifen stimulates autophagy by increasing the intracellular level of ceramide and abolishing the inhibitory effect of the class-I PI3K pathway on autophagy[17].

Wortmannin - PI3K Inhibitor

Wortmannin is a cell-permeable, fungal metabolite that acts as a potent, selective and irreversible inhibitor of PI3K[18]. Wortmannin has been used to determine the involvement of PI3K in many cellular processes, such as apoptosis[4], autophagy[2] and TLR signaling[19].

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Recent articles using InvivoGen's Inhibitors

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Inhibitors of JAK/STAT Activation

AG490 - JAK2 Inhibitor

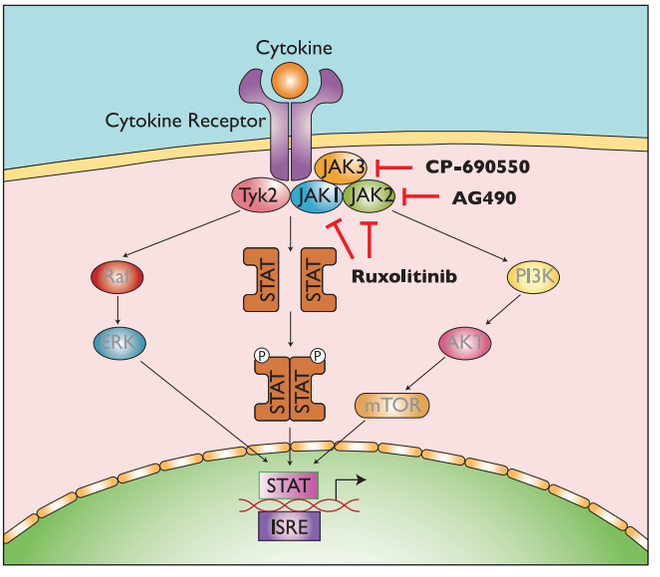
AG490 is a specific and potent inhibitor of the Janus kinase 2 protein (JAK2)[1]. JAK2 regulates the phosphorylation of JNK, primarily through PI3K. It has been established that JAK2 plays an important role in TLR-mediated biological responses, blocking TLR4-mediated responses to LPS[2] and TLR5-mediated responses to flagellin[3].

CP-690550 - JAK3 Inhibitor **NEW**

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[4]. In experimental models, treatment with CP-690550 decreases IL-6 production, a critical cytokine that drives inflammation[5]. Recently, it has been shown that CP-690550 also inhibits TNF-induced chemokine expression[6].

Ruxolitinib - Pan JAK Inhibitor **NEW**

Ruxolitinib (INCB018424) is a small-molecule ATP mimetic that potently inhibits JAK1 and JAK2 in the blood[7]. Ruxolitinib is clinically used for the treatment of myelofibrosis, a bone marrow disorder[8], and is being investigated for the treatment of certain cancers and autoimmune diseases, such as psoriasis.



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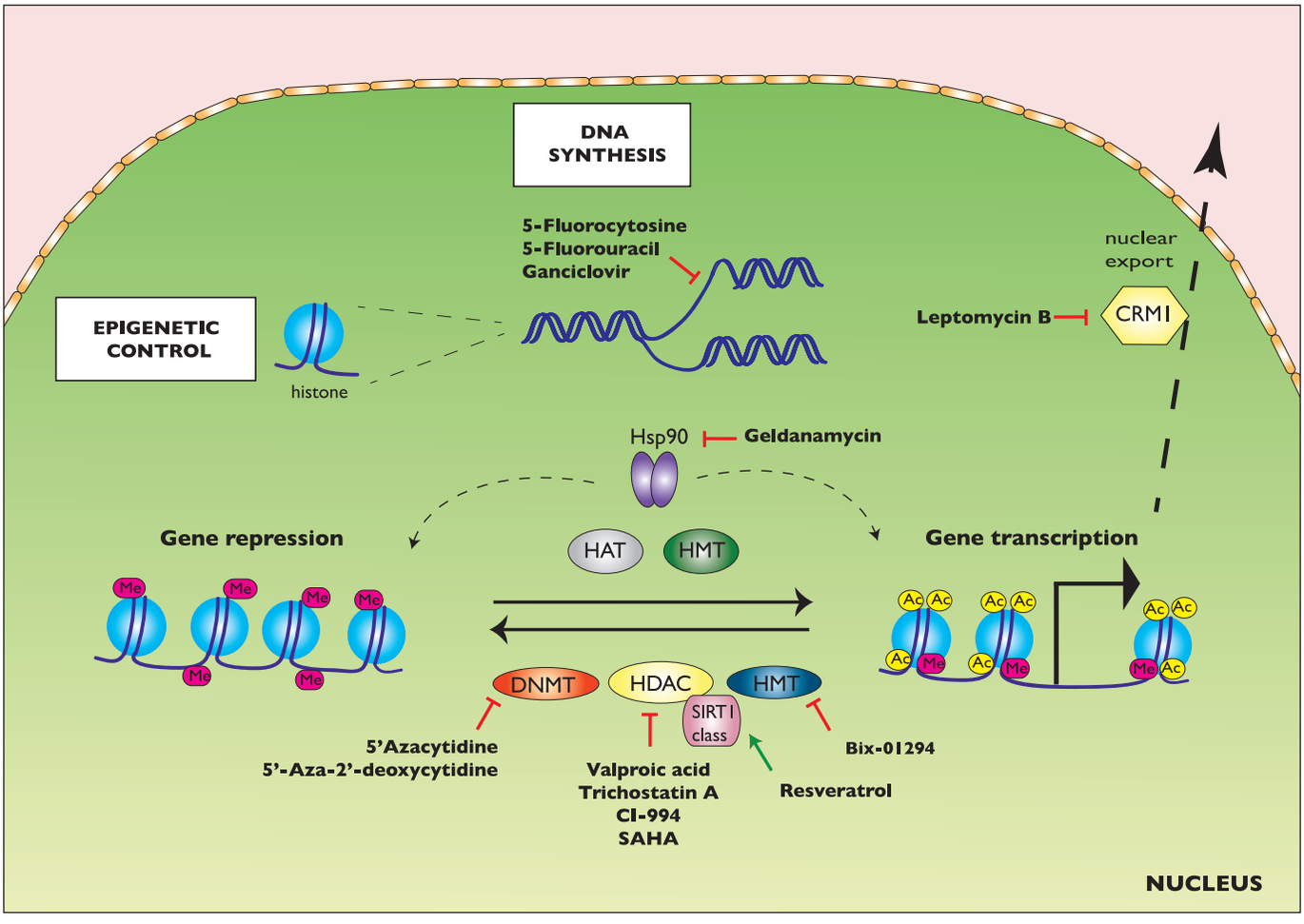

5-Aza-cytidine - DNA Methyltransferase Inhibitor

5-aza-cytidine (AZA) is a potent inhibitor of DNA methyltransferase 1 (DNMT1), approved by the FDA for the treatment of myelodysplastic syndrome[1]. As an analog of cytidine, AZA can be incorporated into RNA and DNA chains. The incorporation into RNA leads to the disassembly of polyribosomes, defective methylation and inhibition of the protein production. Its incorporation into DNA leads to a covalent binding with DNA methyltransferases, which prevents DNA synthesis. As a demethylating agent, AZA is used to enhance the efficiency of induced pluripotent stem (iPS) cells generation[2,3].

5-Aza-2'-deoxycytidine - DNA Methyltransferase Inhibitor

5-Aza-2'-deoxycytidine (5-AzadCyD, 5-Aza-CdR, decitabine), the deoxy derivative of AZA, is also 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. 5-AzadCyD was demonstrated to be a potent antineoplastic agent against leukemia and tumors in animal models[4,5].

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[6].

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 and its metabolites possess a number of different mechanisms of action. 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.

Bix-01294 - G9a Histone Methyltransferase Inhibitor

Bix-01294 is an inhibitor of the G9a histone methyltransferase, a key regulator of DNA methylation and transcriptional silencing in pluripotent cells[7]. Bix-01294 is believed to facilitate the reactivation of pluripotency genes and induce passive demethylation, thus promoting reprogramming. Indeed, Bix-01294 was found to improve reprogramming efficiencies of Oct4-Klf4-(OK)-infected neural progenitor cells by approximately 8 fold[8].

CI-994 - Histone deacetylase Inhibitor **NEW**

CI-994 (N-acetyldinaline, Tacedinaline, PD-123654) is a histone deacetylase (HDAC) inhibitor of the benzamide class[9]. CI-994 was demonstrated to inhibit HDAC-1 and HDAC-2 in a concentration-dependent fashion with no effect on the activity of the prototypical histone acetyltransferase GCN5. CI-994 has been investigated in combination with gemcitabine against solid tumors and shown to be effective against acute myeloid leukemia *in vitro* and *in vivo*[10,11].

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*)[6]. 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[12].

Leptomycin B - Nuclear export inhibitor

Leptomycin B is an inhibitor of nuclear export used to study nucleo-cytoplasmic translocation. It has been demonstrated that Leptomycin B can result in the accumulation of proteins that shuttle the cytosol and nucleus, such as IRAK-1 and NLRC5[13]. The cellular target of leptomycin B has been idenetified as CRM1 (exportin 1), an evolutionarily conserved receptor for the nuclear export signal of proteins. Recently, leptomycin B has been used to trap the transcription factor Foxo1 in the nucleus[14].

SAHA - Pan-Histone deacetylase (HDAC) Inhibitor **NEW**

SAHA (Vorinostat), suberoylanilide hydroxamine, also known as Vorinostat, is a pan-HDAC inhibitor. SAHA 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 a anti-cancer drug, and has has antifibrotic and anti-inflammatory potential[15,16]. 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 U.S. Food and Drug Administration (FDA) for the treatment of advanced cutaneous T-cell-lymphoma[17].

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[18]. Furthermore, TSA is used to improve the genomic reprogramming of embryos generated by somatic cell nuclear transfer[19].

Valproic acid - Histone deacetylase Inhibitor

Valproic acid (VPA) is a histone deacetylase inhibitor with potent antitumor activity[20]. VPA treatment promotes histone acetylation allowing the chromatin to adopt a relaxed structure facilitating the binding of ectopically expressed transcription factors or downstream secondary factors. VPA was found to significantly enhance reprogramming efficiencies of OSKM-, OSK- and OS-infected fibroblasts, eliminating the need for the oncogenes *c-Myc* or *Klf4*[2, 21]. VPA is believed to induce global transcriptional changes, in particular upregulating ES cell-specific genes while downregulating MEF-specific genes.

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Trichostatin A

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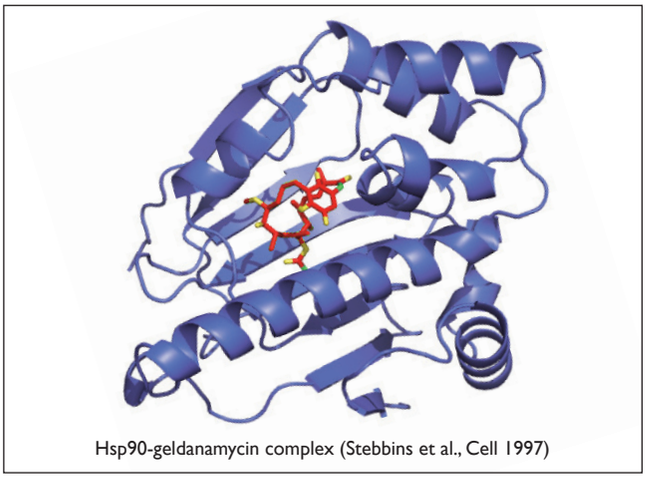
Inhibitors of Heat Shock Protein 90

Heat shock protein 90 (Hsp90) is a ubiquitous molecular chaperone critical for the folding, assembly and activity of multiple mutated and overexpressed signaling proteins that promote the growth and/or survival of tumor cells. Hsp90 client proteins include mutated p53, Raf-1, Akt, ErbB2 and hypoxia-inducible factor 1a (HIF-1a). Geldanamycin (GA), a benzoquinone ansamycin antibiotic, selectively inhibits Hsp90 leading to the degradation of its client proteins. GA inhibits the proliferation of cancer cells and shows anti-cancer activity in experimental animals. However due to poor aqueous solubility and liver toxicity, GA has not moved forward in clinical trials. To overcome these undesirable properties, numerous GA analogs have been synthesized which differ only in their 17-substituent.

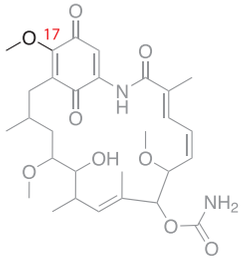


Hsp90-geldanamycin complex (Stebbins et al., Cell 1997)

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].

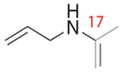
Geldanamycin
$C_{29}H_{40}N_2O_9$
MW: 560
Purity: 99%



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-AAG
$C_{31}H_{43}N_3O_8$
MW: 586
Purity: 99%

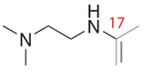


17-DMAG - Water-soluble GA Analog

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.

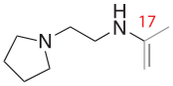
17-DMAG
$C_{32}H_{48}N_4O_8$, HCl
MW: 617
Purity: 99%



17-AEP-GA - Water-soluble GA Analog

17-[2-(Pyrrolidin-1-yl)ethyl]amino-17-demethoxygeldanamycin (17-AEP-GA) is a new geldanamycin (GA) analogue with an alkylamino group in place of the methoxy moiety at C17. 17-AEP-GA is less cytotoxic than GA and remains biologically active[7]. 17-AEP-GA was shown to induce similar tumor cell growth inhibition than 17-AAG and, unlike 17-AAG which is soluble in DMSO, to be water soluble.

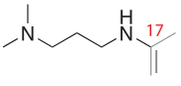
17-AEP-GA
$C_{34}H_{50}N_4O_8$
MW: 643
Purity: 99%



17-DMAP-GA - Water-soluble GA Analog

17-(Dimethylaminopropylamino)-17-demethoxygeldanamycin (17-DMAP-GA) belongs to a new set of geldanamycin analogs that have been synthesized based on binding affinity to Hsp90 and water solubility. 17-DMAP-GA was shown to greatly inhibit the growth of cancer cells (IC50 below 100 nM)[7]. Its binding affinity to Hsp90 was not significantly affected while its water solubility was highly improved compared to 17-AAG.

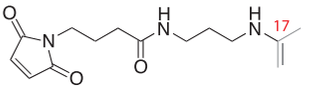
17-DMAP-GA
$C_{33}H_{50}N_4O_8$
MW: 631
Purity: 99%



17-GMB-APA-GA - GA Analog for Conjugation to a Monoclonal Antibody

17-GMB-APA-GA is a maleimido derivative of geldanamycin that enables the conjugation of GA to a monoclonal antibody (mAb) such as Herceptin, the first mAb approved for therapy of solid tumors. This geldanamycin immunoconjugate induces less systemic toxicity than GA by being selectively delivered into malignant cells, a property conferred by the mAb that acts as the targeting vehicle. NCI has reported that Herceptin-GA conjugates deliver a more potent selective cytotoxic impact to Her2-overexpressing tumors than Herceptin alone[8]. To prepare such conjugates, GA is modified to introduce a latent primary amine[9]. After deprotection, this primary amine provides a site for introduction of a maleimide group that enables linkage to proteins.

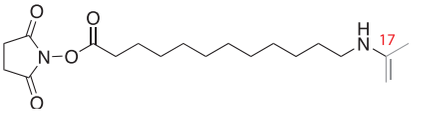
17-GMB-APA-GA
$C_{39}H_{53}N_5O_{11}$
MW: 768
Purity: 99%



17-NHS-ALA-GA - GA Analog for Coupling of NH2-Containing Molecules

17-NHS-ALA-GA is an NHS (N-hydroxysuccinimide) activated geldanamycin analog designed for easy coupling of NH2-containing molecules. NHS coupling forms a chemically stable amide bond with ligands containing primary amino groups such as small proteins and peptides. Thus, 17-NHS-ALA-GA can be used for conjugation of GA to a monoclonal antibody, similarly to 17-GMB-APA-GA. It can also be used to perform affinity chromatography to purify GA binding proteins such as Hsp90.

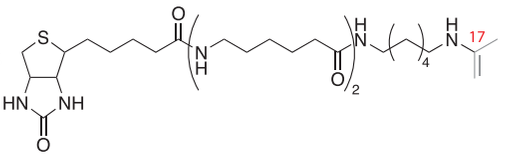
17-NHS-ALA-GA
$C_{44}H_{84}N_4O_{12}$
MW: 841
Purity: 99%



Biotin-GA - Labeled GA Analog

Biotin-GA was generated by biotinylation of geldanamycin at the C17 position. This labeled GA can be used to identify novel Hsp90 inhibitors through time-resolved fluorescence resonance energy transfer-based HTS that measures the binding of biotin-GA to the N-terminal ATP-binding domain of Hsp90[10].

Biotin-GA
$C_{56}H_{88}N_8O_{12}S$
MW: 1097
Purity: 99%



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. Tian ZQ. *et al.*, 2004.** Synthesis and biological activities of novel 17-aminogeldanamycin derivatives. Bioorg Med Chem. 12(20):5317-29. **8. Mandler R. *et al.*, 2004.** Herceptin-geldanamycin immunoconjugates: pharmacokinetics, biodistribution, and enhanced antitumor activity. Cancer Res. 64(4):1460-7. **9. Mandler R. *et al.*, 2002.** Modifications in synthesis strategy improve the yield and efficacy of geldanamycin-herceptin immunoconjugates. Bioconjug Chem. 13(4):786-91. **10. Zhou V. *et al.*, 2004.** A time-resolved fluorescence resonance energy transfer-based HTS assay and a surface plasmon resonance-based binding assay for heat shock protein 90 inhibitors. Anal Biochem. 331(2):349-57. **11. Kelland LR *et al.*, 1999.** DT-Diaphorase expression and tumor cell sensitivity to 17-allylamino, 17-demethoxygeldanamycin, an inhibitor of heat shock protein 90. J Natl Cancer Inst. 91(22):1940-9. **12. Guo W *et al.*, 2005.** Formation of 17-allylamino-demethoxygeldanamycin (17-AAG) hydroquinone by NAD(P)H:quinone oxidoreductase 1: role of 17-AAG hydroquinone in heat shock protein 90 inhibition. Cancer Res. 65(21):10006-15. **13. Guo W *et al.*, 2006.** The bioreduction of a series of benzoquinone ansamycins by NAD(P)H:quinone oxidoreductase 1 to more potent heat shock protein 90 inhibitors, the hydroquinone ansamycins. Mol Pharmacol. 70(4):1194-203.

Recent articles using InvivoGen's Inhibitors

Geldanamycin

Mandal AK. *et al.*, 2011. Role of molecular chaperones in biogenesis of the protein kinome. Methods Mol Biol. 787:75-81.

Blum G. *et al.*, 2012. In vitro and in vivo role of heat shock protein 90 in Amphotericin B resistance of Aspergillus terreus. Clin Microbiol Infect. [Epub ahead of print]

Koo BH & Apte SS., 2010. Cell-surface processing of the metalloprotease pro-ADAMTS9 is influenced by the chaperone GRP94/gp96. J Biol Chem. ;285(1):197-205.

Zhu XJ. *et al.*, 2010. The L279P mutation of nuclear distribution gene C (NudC) influences its chaperone activity and lissencephaly protein 1 (LIS1) stability. J Biol Chem. 285(39):29903-10.

17-AAG

Karkoulis PK. *et al.*, 2010. 17-Allylamino-17-demethoxygeldanamycin induces downregulation of critical Hsp90 protein clients and results in cell cycle arrest and apoptosis of human urinary bladder cancer cells. BMC Cancer. 10:481.

Laederich MB. *et al.*, 2011. Fibroblast growth factor receptor 3 (FGFR3) is a strong heat shock protein 90 (Hsp90) client: implications for therapeutic manipulation. J Biol Chem. 286(22):19597-604.

17-DMAG

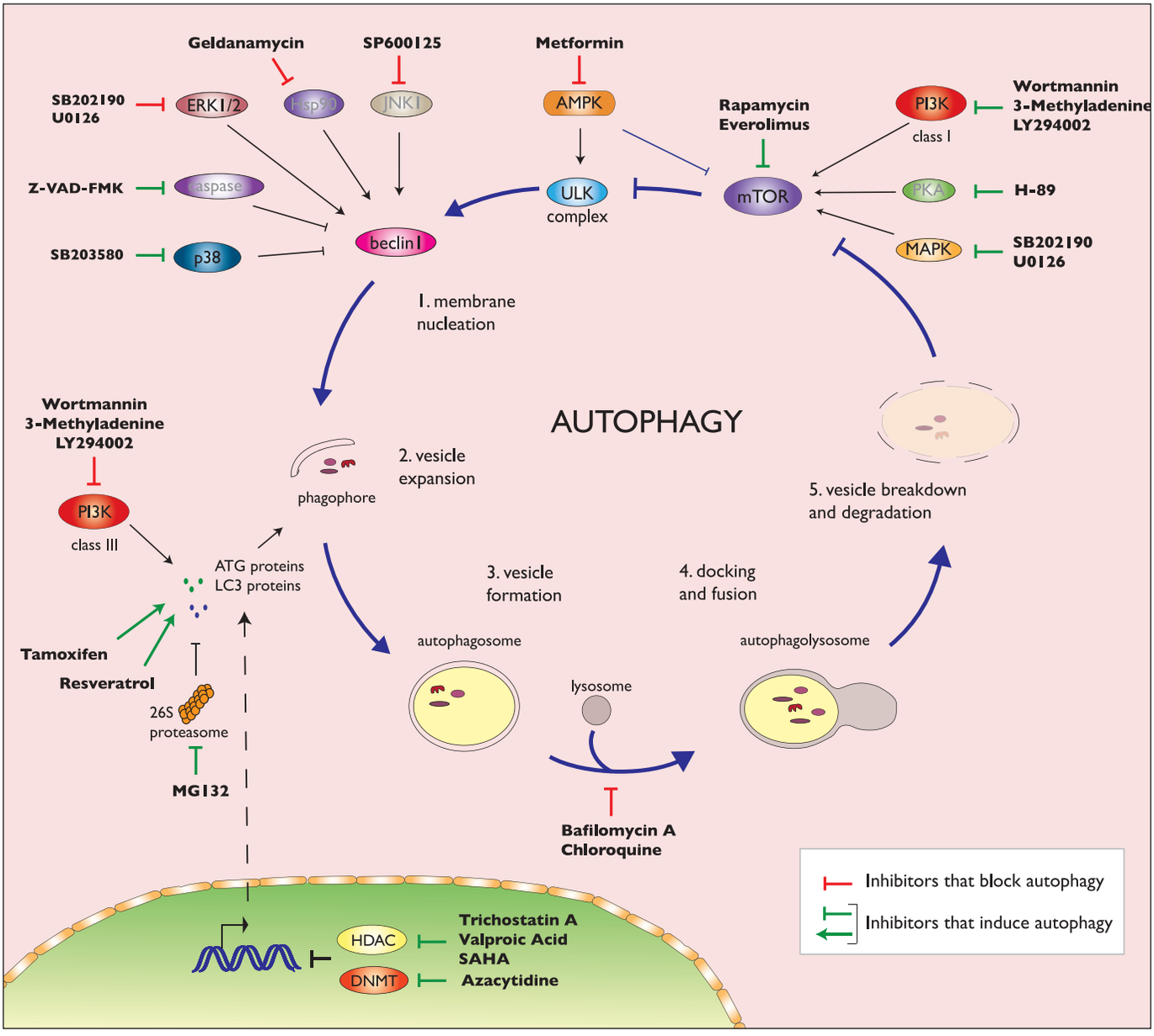
Hackl C. *et al.*, 2010. Activating transcription factor-3 (ATF3) functions as a tumor suppressor in colon cancer and is up-regulated upon heat-shock protein 90 (Hsp90) inhibition. BMC Cancer. 10:668.

Kiang JG. *et al.*, 2011. 17-DMAG diminishes hemorrhage-induced small intestine injury by elevating Bcl-2 protein and inhibiting iNOS pathway, TNF-α increase, and caspase-3 activation. Cell Biosci. 1(1):21.

Kasperkiewicz M. *et al.*, 2011. Heat-shock protein 90 inhibition in autoimmunity to type VII collagen: evidence that nonmalignant plasma cells are not therapeutic targets. Blood. 117(23):6135-42.

Inhibitors and Activators of Autophagy

Autophagy is a process to eliminate the cell's own cytoplasmic material. Several signaling pathways sense the status of different types of stress and regulate autophagy for cell survival and homeostasis. A number of inhibitors of various kinases and signaling molecules impact autophagy at different levels of the process, as depicted in the illustration below, and can be used to assess or manipulate autophagy in cells.



Inhibitors that block Autophagy

The primary step in inducing autophagy involves membrane nucleation, controlled by ULK complex and Beclin1. Inhibitors of positive regulators of of the ULK complex and Beclin1 have been demonstrated to block autophagy. These include inhibitors to the kinases, AMPK, JNK1 and ERK1/2. Inhibitors of Hsp90 has been shown to destablize Beclin1 and block autophagy. The induction of ATG protein and LC3 proteins is required for vesicle expansion and formation. Inhibitors of the class III PI3kinases can block autophagy. In a later step of the autophagic process, inhibitors that inhibit lysosome acidification, Bafilomycin A and Chloroquine, essentially block the formation of autophagosome and autophagic degradation.

Inhibitors that induce autophagy

mTOR is a major negative regulatory axis of autophagy and is influenced by several nutrient signaling pathways. Direct inhibitors of mTOR and those of pathways activating mTOR, subsequently induce autophagy by inhibiting mTOR. In addition, Beclin1 is negatively regulated by caspases and p38, the inhibitors of which act to promote Beclin1 action to induce the initial stages of autophagy. Furthermore, inhibitors of the 26S proteasome and the epigenetic regulators, HDACs and DNMTs, result in the increase of ATG and LC3 proteins levels essential to the process of autophagy.

6 ANTIBODIES & VACCINATION

ANTIBODY ISOTYPES

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.

Characteristics of Immunoglobulin Isotypes

Human Isotype	IgG	IgA	IgM	IgD	IgE
Sub-isotype	γ1 / γ2 / γ3 / γ4	α1 / α2			
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)	+++/±/++/+	+	-	-	-

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.

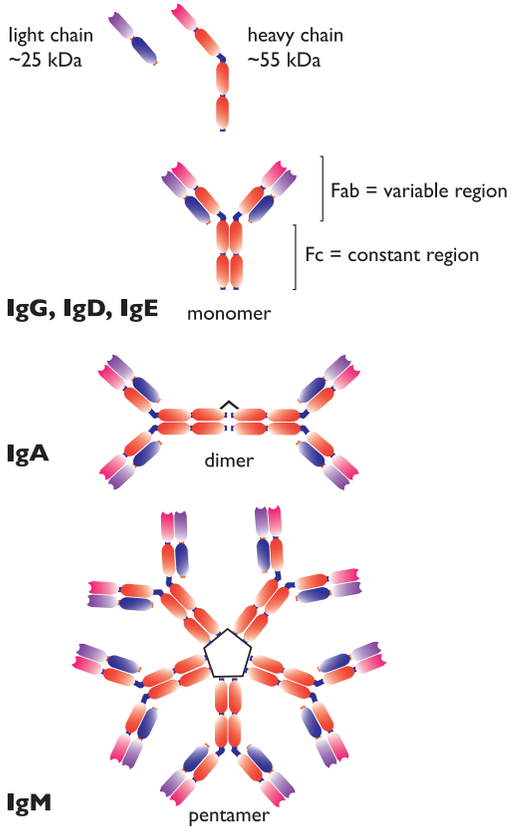
IgD is monovalent and found on the surface of B-lymphocytes and together with monomeric IgM, serves as antigen receptor for the activation of B cells.

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.

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).

Immunoglobulins (Ig) isotypes



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.

pFUSE-CHIg and pFUSE2-CLIg - Antibody Generation

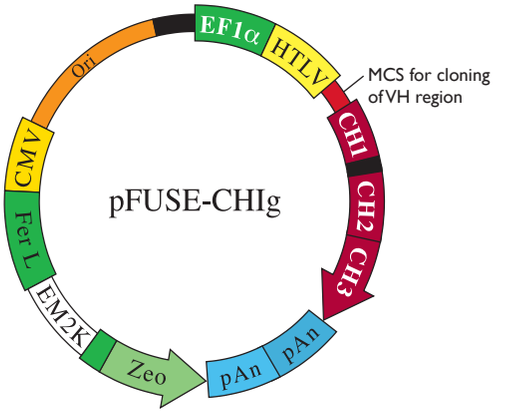
pFUSE-CHIg and pFUSE2-CLIg plasmids are designed to change a monoclonal antibody from one immunoglobulin G subclass to another therefore enabling the generation of antibodies with the same antigen affinity but with different effector functions, such as increased or reduced ADCC and CDC. They can also 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.

- Isotype switch to generate antibodies with different effector functions
- Generation of entire IgG antibodies, chimeric, humanized or fully human
- **NEW !** IgA, IgD, IgE and IgM classes available

Description

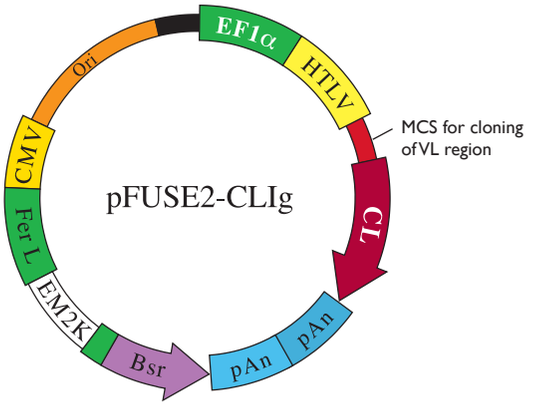
pFUSE-CHIg and pFUSE2-CLIg plasmids express the constant regions of heavy (CH) and the light (CL) chains, respectively. They contain a multiple cloning site (MCS) upstream of these constant regions to enable the cloning of the variable (VH and VL) regions of a given antibody, Fab or ScFv. Co-transfection 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 85).

pFUSE-CHIg and pFUSE2-CLIg plasmids feature the same backbone made up of two expression cassettes: the first drives the expression of the CH or CL region, and the second encodes the resistance gene.



pFUSE-CHIg - Heavy chain constant region
pFUSE-CHIg plasmids express a large choice of isotypes of the heavy chain constant region:
- Human IgA1, IgA2, IgD, IgE, IgG1, IgG2, IgG3, IgG4 and IgM
- Murine IgA, IgD, IgE, IgG1, IgG2A, IgG2B, IgG3 and IgM
- Rabbit IgG

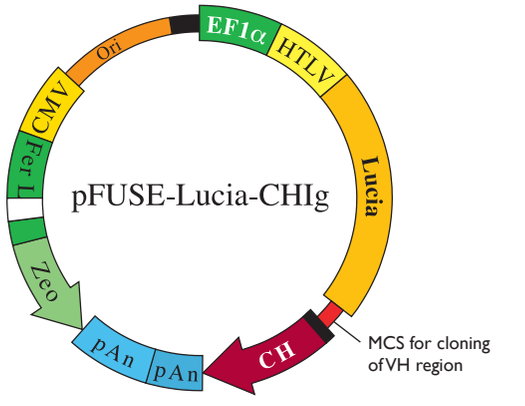
pFUSEss-CHIg plasmids derive from the corresponding pFUSE-CHIg plasmids by addition of the IL-2 signal sequence (IL2ss) upstream of the MCS. It allows the secretion of the recombinant heavy chains generated using Fab or scFv fragments selected from phage display libraries that lack a signal sequence.



pFUSE2-CLIg - Light chain constant region
pFUSE2-CLIg plasmids express different isotypes of the light chain constant region:
- Human kappa, lambda 2
- Mouse kappa, lambda 1, lambda 2
- Rabbit kappa

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.

NEW! pFUSE-Lucia-CHIg
Lucia® is a new secreted luciferase reporter protein (see page 17) with high protein carrier ability. pFUSE-Lucia-CHIg plasmids contain the Lucia® 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® in the cell supernatants reflects the presence of full-length antibodies.



All pFUSE plasmids are selectable in *E. coli* and mammalian cells with the same antibiotic. pFUSE2-CLIg plasmids are selectable with blasticidin. pFUSE-CHIg and pFUSE-Lucia-CHIg plasmids are selectable with Zeocin™.

Principle

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.

3- Antibody production

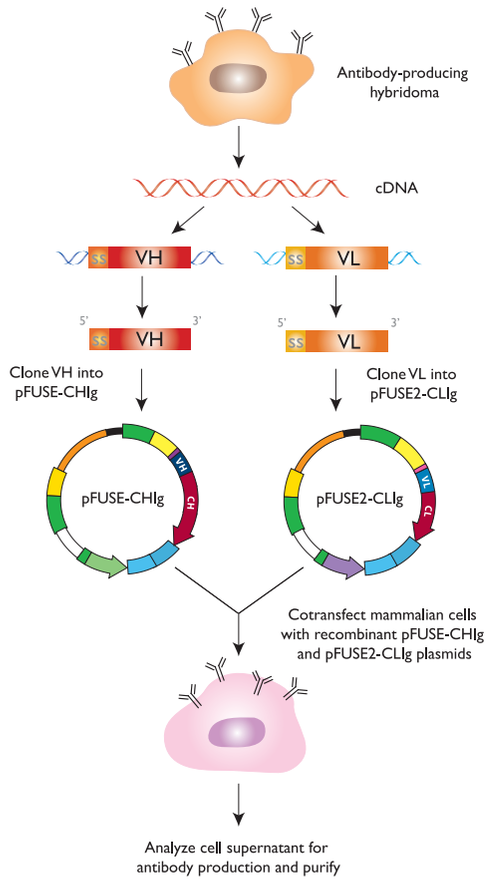
Antibody production depends greatly on the ratio of heavy chain (HC) to light chain (LC) expression. Typically, a pFUSE-CHIg (HC):pFUSE2-CLIg (LC) ratio of 2:3 is used to cotransfect mammalian cells such as HEK293 or CHO cells. Production of antibodies in cell supernatants can be analyzed using different 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 (see table page 85).

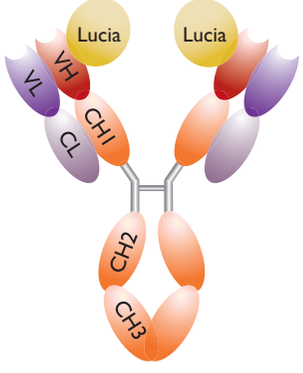
See page 88 for catalog codes

Antibody generation using pFUSE-CHIg/pFUSE2-CLIg

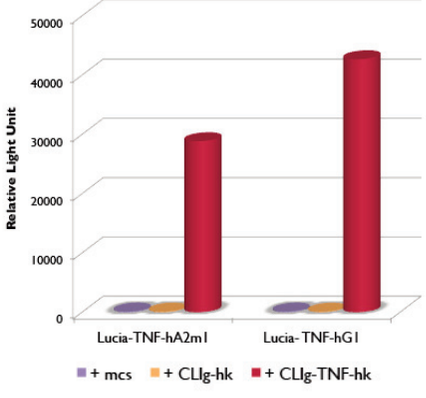


Generation and validation of Lucia®-tagged anti-hTNF-α antibody

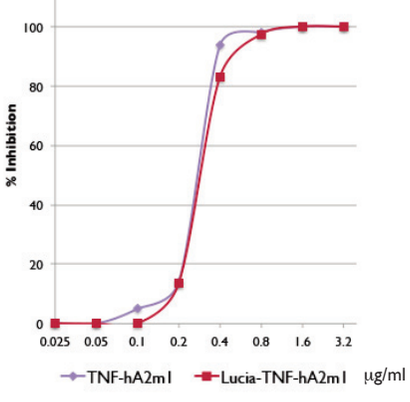
A. Lucia-tagged antibody



B. Luciferase activity



C. Neutralizing TNF-α activity



(A) Schematic representation of a Lucia®-tagged antibody. (B) Luciferase activity of Lucia-tagged anti-hTNF-α antibodies. CHO cells were stably co-transfected with a heavy chain expressing plasmid, pFUSE-Lucia-TNF-CHIg-hA2m1 (TNF-CHIg-hA2m1) or pFUSE-Lucia-TNF-CHIg-hG1 (TNF-CHIg-hG1) and a light chain expressing plasmid, pFUSE2-TNF-CLIg-hk (TNF-CLIg-hk) to generate Lucia-tagged anti-hTNF-α antibodies, Lucia-anti-hTNF-α-hIgA2m1and Lucia-anti-hTNF-α-hIgG1, of human IgAm2 and human IgG1 isotypes, respectively. pFUSE2-CLIg-hk (CLIg-hk), which expresses no VL, and pSELECT-blasti-mcs (mcs) were used as negative controls. Supernatants were collected and the luciferase levels determined using QUANTI-Luc™ (see page 23). Only the cells co-producing a Lucia-anti-hTNF-α heavy-chain and an anti-hTNF-α light chain displayed luciferase activity. **(C) Neutralizing activity of anti-hTNF-α antibodies.** The activity of anti-hTNF-α-hIgA2m1 and Lucia-anti-hTNF-α-hIgA2m1 antibodies, purified from the supernatants of CHO transfected cells, was determined by performing an TNF-α neutralizing assay. Both antibodies display similar neutralizing activities, thus fusion of the Lucia® tag at the N-terminus of the heavy chain does not alter the functionality of the antibody.

PRODUCT	ISOTYPE	QUANTITY	CAT. CODE (No IL2ss)	CAT. CODE (With IL2ss)
pFUSE2-CLIg				
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-rk1	Rabbit kappa 1	20 µg	pfuse2-rclk1	pfuse2ss-rclk1
pFUSE2-CLIg-rk2	Rabbit kappa 2	20 µg	pfuse2-rclk2	pfuse2ss-rclk2
pFUSE-CHIg				
pFUSE-CHIg-hA1 **NEW**	Human IgA1	20 µg	pfuse-hcha1	pfusess-hcha1
pFUSE-CHIg-hA2m1 **NEW**	Human IgA2 (allele m1)	20 µg	pfuse-hcha2m1	pfusess-hcha2m1
pFUSE-CHIg-hD **NEW**	Human IgD (allele 2)	20 µg	pfuse-hchd	pfusess-hchd
pFUSE-CHIg-hE **NEW**	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 **NEW**	Human IgM (allele 3)	20 µg	pfuse-hchm	pfusess-hchm
pFUSE-CHIg-mA **NEW**	Mouse IgA	20 µg	pfuse-mcha	pfusess-mcha
pFUSE-CHIg-mD **NEW**	Mouse IgD	20 µg	pfuse-mchd	pfusess-mchd
pFUSE-CHIg-mE **NEW**	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 **NEW**	Mouse IgM (allele 1)	20 µg	pfuse-mchm	pfusess-mchm
pFUSE-CHIg-rG	Rabbit IgG	20 µg	pfuse-rchg	pfusess-rchg
pFUSE-Lucia-CHIg				
pFUSE-Lucia-CHIg-hG1 **NEW**	Human IgG1	20 µg	pfuselc-hchg1	-
pFUSE-Lucia-CHIg-hG2 **NEW**	Human IgG2	20 µg	pfuselc-hchg2	-
pFUSE-Lucia-CHIg-hG3 **NEW**	Human IgG3	20 µg	pfuselc-hchg3	-
pFUSE-Lucia-CHIg-hG4 **NEW**	Human IgG4	20 µg	pfuselc-hchg4	-
pFUSE-Lucia-CHIg-mG1 **NEW**	Mouse IgG1	20 µg	pfuselc-mchg1	-
pFUSE-Lucia-CHIg-mG2a **NEW**	Mouse IgG2a	20 µg	pfuselc-mchg2a	-
pFUSE-Lucia-CHIg-mG2b **NEW**	Mouse IgG2b	20 µg	pfuselc-mchg2b	-
pFUSE-Lucia-CHIg-mG3 **NEW**	Mouse IgG3	20 µg	pfuselc-mchg3	-

Contents and Storage

pFUSE2-CLIg, pFUSE-CHIg and pFUSE-Lucia-CHIg plasmids are provided as 20 µg of lyophilized DNA. Product is shipped at room temperature and should be stored at -20°C. Each plasmid is provided with 4 pouches of *E. coli* Fast-Media® Blas or 95 Fast-Media® Zeo (2 TB and 2 Agar, see pages 48-49).

Related Products

Blasticidin, page 13
Zeocin™, page 15
Peptide M, page 92
Fast-Media® Blas, page 49
Fast-Media® Zeo, page 49
Protein L, page 92

Antibody Isotype Collections

Immunoglobulins (Ig) are divided in isotypes: nine in humans (IgG1, IgG2, IgG3, IgG4, IgM, IgA1, IgA2, IgD, IgE) and eight in mice (IgG1, IgG2a, IgG2b, IgG3, IgM, IgA, IgD, IgE). Each isotype displays distinct structural and effector properties. These properties are key features in choosing the backbone for a therapeutic antibody. To help you decide which Ig isotype is the most suitable for your application, InvivoGen provides two well-known monoclonal antibodies, anti-hCD20 (rituximab) and anti-hTNF-α (adalimumab), available in the most common human and murine isotypes. These antibody isotype collections will help you study the effector functions between different isotypes or between the same isotype of two different species.

Description

The anti-hCD20 and anti-hTNF-α isotype collections consist of monoclonal antibodies comprising the same variable region, that targets the human CD20 antigen or human TNF-α cytokine respectively, and the constant region of different isotypes.

Variable Region

• **The Anti-hCD20 isotype collection** features the variable region of rituximab. Rituximab is a mouse/human chimeric 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. Rituximab has been approved by the FDA for the treatment of various lymphoid malignancies, including B-cell non-Hodgkin's lymphoma and B-cell chronic lymphocytic leukemia.

• **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. Adalimumab has been approved by the FDA for the treatment of various inflammatory diseases, such as rheumatoid arthritis and Crohn's disease.

Constant Region

The constant region consists of the human or murine kappa light chain and the heavy chain of different isotypes. Eight human and three murine isotypes are available:

- human isotypes: IgG1, IgG2, IgG3, IgG4, IgM, IgA1, IgA2, IgE
- murine isotypes: IgG1, IgG2a, IgA

The anti-hCD20 and anti-hTNF-α isotype collections have been generated by recombinant DNA technology. They have been produced in CHO cells and purified by different types of affinity chormatography: protein G for IgG isotypes, protein L for IgE and IgM, and peptide M for IgA isotypes.

PRODUCT	ISOTYPE	QTY	CAT. CODE
Anti-hCD20 isotype collection			
Anti-hCD20-hIgG1	Human IgG1	100 µg	hcd20-mab1
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-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
Anti-[anti-hTNF-α] NEW	Mouse IgG1	100 µg	mab-idtnf

NEW! Anti-[anti-hTNF-α] antibody

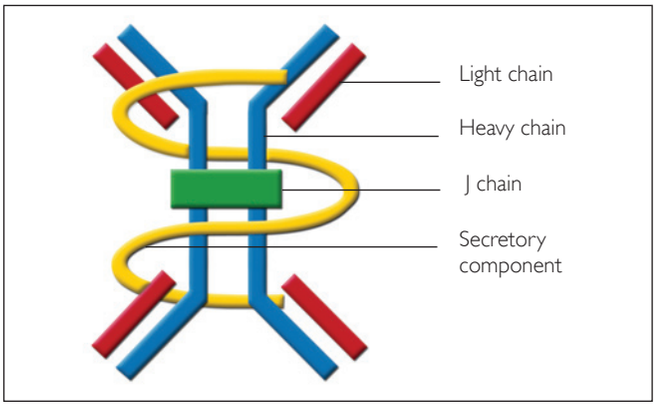
Anti-[anti-hTNF-α] is a mouse monoclonal antibody against all the antibodies of the anti-hTNF-α isotype collection. It was generated through immunization of mice with anti-hTNF-α-mIgG2a (cat code htnfa-mab10) and screened for its ability to neutralize all the anti-hTNF-α antibodies of the collection. Anti-[anti-hTNF-α] can be used as a positive control for the detection of antibodies against anti-hTNF-α antibodies.

Contents and Storage

Antibodies of the anti-hCD20 and anti-hTNF-α isotype collection and the anti-[anti-hTNF-α] antibody are provided lyophilized from a 0.2 µm filtered buffered solution with stabilizers. Product should be reconstituted in sterile water. Lyophilized antibodies are stable greater than six months when stored at -20°C. Reconstituted antibodies are stable 1 month when stored at 4°C and 6 months when aliquoted and stored at -20°C.

IMMUNOGLOBULIN A

The mucosal surfaces represent the largest area of exposure of the body to external pathogens. Immunoglobulin A (IgA), in its secretory form, is the main effector of the mucosal immune system and provides an important first line of defense against most pathogens that invade the body at a mucosal surface[1]. Secretory IgA (SIgA) represents the most abundant immunoglobulin of body secretions such as saliva, tears, colostrum and gastrointestinal secretions. The molecular stability and effector immune functions make SIgA particularly well suited to provide mucosal protection against pathogens.



SIgA is produced by plasma cells predominantly as polymeric IgA (pIgA) consisting of two or more monomers linked by the J (joining) chain. pIgA is actively transported by the epithelial polymeric Ig receptor (pIgR) and released into mucosal secretions with a bound secretory component (the extracellular domain of the pIgR) that protects the molecule from proteolytic enzymes. IgA mediates a variety of protective functions[2, 3]. Luminal SIgA is believed to interfere with pathogen adherence to mucosal epithelial cells, a process called immune exclusion. In addition, IgA appears to have two other defense functions: intracellular neutralization, and virus excretion. In response to continuous stimulation by microbes, the IgA repertoire is spontaneously diversified by somatic hypermutation (SHM). IgA that has undergone defective SHM can neither regulate intestinal microbial homeostasis nor effectively protect the host from pathogens emphasizing the importance of IgA in maintaining intestinal homeostasis and efficient mucosal defense[4]. IgA is also found as a monomer in the serum where it may function as a second line of defence by eliminating pathogens that have breached the mucosal surface. Serum IgA interacts with an Fc receptor called FcαR1 triggering antibody-dependent-cell-mediated cytotoxicity (ADCC).

Due to their specific effector functions, IgA present an interesting therapeutic potential for mucosal protection against virus and bacteria. Indeed, monoclonal IgA antibodies have been shown to be efficient in protecting against infection by various bacteria[5] and viruses, including HIV-1[6-7].

Despite this great potential and in contrast to monoclonal antibodies (MAbs) of the IgG isotype, their development as research tools or human therapeutics has been scarce. This is mostly due to the difficulties encountered in producing and purifying biologically active IgA. IgA MAbs can hardly be obtained through the classical hybridoma technique that involves the fusion between murine splenocytes and myeloma cells[8]. Studies of IgA would be much facilitated by the availability of a simple method to isolate and detect IgA.

As a specialist of the Toll-like receptors (TLRs) and innate immunity, InvivoGen believes that IgA is a new hot topic in this field and therefore is initiating a vast IgA program. In this regard, we are using two innovative methods to generate IgA MAbs. The first relies on the use of a transgenic mouse, named Cα, obtained through insertion of the human α1 gene in place of the switch sequence Sμ[8], that allows the isolation of primarily chimeric IgA MAbs via the classical hybridoma technique. The second combines hybridoma and recombinant DNA technologies and involves an IgG-IgA class-switch. In both methods, mice are DNA immunized with a plasmid expressing the antigen, and IgA- or IgG-producing hybridomas are screened using a neutralizing assay based on engineered cell lines (HEK-Blue™ Cells). IgA antibodies are purified by Protein L affinity chromatography. Protein L is a bacterial protein that binds antibodies through κ light chain interactions. These techniques have been utilized to generate a first series of IgA MAbs that target the extra-cellular TLRs and key cytokines of the innate immune system. These IgA MAbs display potent neutralizing activities and can be used for flow cytometry, and thus represent useful research tools. Many more IgA MAbs are in the pipeline, some with potential therapeutic applications.

1. Cerutti A. *et al.*, 2010. Immunoglobulin Responses at the Mucosal Interfaces. Annu Rev Immunol. 29:273-93. **2. Woof JM. & Kerr MA., 2007.** The function of immunoglobulin A in immunity. J Pathol. 208(2):270-82. Review. **3. Fagarasan *et al.*, 2010.** Adaptive immune regulation in the gut: T cell-dependent and T cell-independent IgA synthesis. Annu. Rev. Immnuol. 28: 740-273. **4. Wei M. *et al.*, 2011.** Mice carrying a knock-in mutation of Aicda resulting in a defect in somatic hypermutation have impaired gut homeostasis and compromised mucosal defense. Nat Immunol. 12(3):264-70. **5. Tokuhara D. *et al*, 2010.** Secretory IgA-mediated protection against V. cholerae and heat-labile enterotoxin-producing enterotoxigenic Escherichia coli by rice-based vaccine. PNAS 107(19):8794-9. **6. Planque S. *et al.*, 2010.** Neutralization of genetically diverse HIV-1 strains by IgA antibodies to the gp120-CD4-binding site from long-term survivors of HIV infection. AIDS 24(6): 875-84. **7. Mantis NJ. *et al.*, 2007.** Inhibition of HIV-1 Infectivity and Epithelial Cell Transfer by Human Monoclonal IgG and IgA Antibodies Carrying the b12 V Region. J. Immunol. 179: 3144 - 3152. **8. Cogne M. *et al.*, 2007.** Non-Human Transgenic Mammal for the Constant Region of the Class a Human Immunoglobulin Heavy Chain and Applications Thereof. US2007248601.

IgA Product Line

➢ IgA Antibodies

➢ IgA Purification

➢ IgA Detection & Quantification

IgA Antibodies

InvivoGen provides human IgA antibodies that have been generated by recombinant DNA technology. These IgA antibodies are chimeric monoclonal antibodies composed of the constant domains of the human IgA2 molecule and variable regions of different origins. They have been selected for their ability to efficiently neutralize the biological activity of selected cytokines and Toll-Like Receptors. The neutralizing activity of these IgA antibodies was determined using InvivoGen's HEK-Blue™ reporter cells. Some of these IgA antibodies can also be used for flow cytometry.

ANTIBODY	REACTIVITY	APPLICATIONS	QUANTITY	CATALOG CODE
Anti-Cytokine IgAs				
Anti-hCD40L-hIgA2	Human CD40L	Neutralization	100 µg	maba-hcd40l
Anti-hIFNα-hIgA2	Human IFN-α	Neutralization, FC	100 µg	maba-hifna
Anti-hIFNγ-hIgA2	Human IFN-γ	Neutralization	100 µg	maba-hifng
Anti-hIL-1β-hIgA2	Human IL-1β	Neutralization	100 µg	maba-hil1b
Anti-hIL-4-hIgA2	Human IL-4	Neutralization	100 µg	maba-hil4
Anti-hIL-6-hIgA2	Human IL-6	Neutralization, FC	100 µg	maba-hil6
Anti-hIL-13-hIgA2	Human IL-13	Neutralization, FC	100 µg	maba-hil13
Anti-hIL-18-hIgA2	Human IL-18	Neutralization	100 µg	maba-hil18
Anti-hTGFβ-hIgA2	Human TGF-β	Neutralization	100 µg	maba-htgfb
Anti-hTNFα-hIgA2	Human TNF-α	Neutralization, FC	100 µg	htnfa-mab7
Anti-TLR IgAs				
Anti-hCD14-hIgA(2)	Human CD14	Neutralization of TLR2 and TLR4 , FC	100 µg	maba-hcd14
Anti-hTLR2-hIgA(2)	Human TLR2	Neutralization, FC	100 µg	maba2-htlr2
Anti-hTLR3-hIgA(2)	Human TLR3	FC	100 µg	maba-htlr3
Anti-hTLR5-hIgA(2)	Human TLR5	Neutralization, FC	100 µg	maba2-htlr5
Control IgA				
IgA2 Isotype Control	Human IgA2	Control	100 µg	maba2-ctrl

Contents and Storage

Each IgA antibody is provided lyophilized from a 0.2 µm filtered solution in PBS. Product should be reconstituted in sterile water. Lyophilized antibodies are stable at least six months when stored at -20°C. Reconstituted IgAs are stable 1 month when stored at 4°C and 6 months when aliquoted and stored at -20°C.

Anti-Human IgA Secondary Antibodies

InvivoGen provides F(ab')2 fragment secondary antibodies, to avoid non-specific binding through Fc receptors, that react with human IgA. These goat antibodies are conjugated with fluorescein (FITC) or biotin for immunodetection or cell sorting applications.

PRODUCT	QTY	CAT. CODE
Goat F(ab')2 Anti-Human IgA - Biotin	500 µg	chiga-biot
Goat F(ab')2 Anti-Human IgA - FITC	500 µg	chiga-fitc
Goat F(ab')2 IgG Isotype Control - FITC	100 tests	cgig-fitc

Contents and Storage

Secondary anti-human IgA antibodies are supplied in 1 ml PBS/NaN_3. Store at 4°C.

IgA Antibody Purification

Protein G and Protein A are currently used to purify IgG antibodies, but these supports are not appropriate for IgA antibody purification. For fast and efficient purification of IgA antibodies from biological samples, InvivoGen provides now four different methods, Peptide M, SSL7, Jacalin, and Protein L. The correct choice of purification method depends upon the IgA subclass of the antibody, the species in which it was raised and the intended use of the antibodies. Antibodies from tissue culture supernatant, serum or ascites can be purified using one or more of the following antibody binding proteins offered by InvivoGen.

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[1].

Binding capacity: 6 mg human IgA per ml of gel

SSL7 / Agarose - IgA1 and IgA2 purification

SSL7 (*Staphylococcus aureus* superantigen-like protein 7; formally known as SET1), is a staphylococcus toxin isolated from *Staphylococcus aureus*. SSL7 binds with a high affinity to the monomeric form of human IgA1 and IgA2. SSL7 has no affinity for human IgG, therefore, it can be used to purify human IgA from human sera, milk and other biological samples[2,3]. SSL7 also binds the secretory form of IgA found in milk from humans, cows, and sheep. SSL7 will bind bovine IgA antibodies present in milk, but not bovine IgA present in serum. SSL7 does not bind mouse, rabbit, sheep or goat IgA present in serum[2].

Binding capacity: 1 mg human IgA per ml of gel

Jacalin / Agarose - IgA1 purification

Jacalin is an a D-galactose binding lectin extracted from jack-fruit seeds (*Artocarpus integrifolia*). Jacalin immobilized on supports such as agarose is useful for the purification of human serum or secretory IgA1[4,5]. IgA can be separated from human IgG and IgM in human serum or colostrums using Immobilized Jacalin. This support is also useful for removing contaminating IgA from IgG samples. Additionally, Jacalin binds IgD[4]. Jacalin can also be used to separate IgA1 subclass from IgA2[5].

Binding capacity: 1-3 mg human IgA per ml of gel

Protein L / Agarose - κ light chain specific

Protein L is an immunoglobulin-binding protein expressed by the anaerobic bacterial species *Peptostreptococcus magnus*[6]. 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[7]. 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

PRODUCT	Human κ light chain	Human λ light chain	Human IgA1	Human IgA2	Human IgG	Human IgM	Human IgE	Human IgD	Mouse IgA	Rat IgA	Bovine IgA
Peptide M	-	-	++++	++++	-	-	n/a	n/a	-	n/a	+
SSL7	-	-	++++	++++	-	-	n/a	-	-	++++	- (serum) + (milk)
Jacalin	-	-	++++	-/+	-	n/a	n/a	+++	-	-	-
Protein L	++++	-	++++	++++	++++	++++	++++	++++	++++	++++	-

1. Sandin C. *et al.*, 2002. Isolation and detection of human IgA using a streptococcal IgA-binding peptide. J Immunol. 169(3):1357-64. 2. Langley *et al.*, 2005. The staphylococcal superantigen-like protein 7 binds IgA and complement C5 and inhibits IgA-Fc alpha RI binding and serum killing of bacteria. J Immunol 174 :2926-2933. 3. Ramsland PA. *et al.*, 2007. Structural basis for evasion of IgA immunity by Staphylococcus aureus revealed in the complex of SSL7 with Fc of human IgA1. PNAS 104:15051-15056. 4. Aucouturier P. *et al.*, 1998. Jacalin, the human IgA1 and IgD precipitating lectin, also binds IgA2 of both allotypes. J Immnuol Methods 113:185-191. 5. Gregory RL. *et al.*, 1987. Separation of human IgA1 and IgA2 using jacalin-agarose chromatography. J Immunol Meth 99:101-106. 6. 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. 7. 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.

PRODUCT	QUANTITY	CAT. CODE
Peptide M / Agarose	2 ml 5 ml	gel-pdm-2 gel-pdm-5
SSL7 / Agarose	2 ml 10 ml	gel-ssl-2 gel-ssl-10
Jacalin / Agarose	2 ml 5 ml	gel-jac-2 gel-jac-5
Protein L / Agarose	2 ml 10 ml	gel-protl-2 gel-protl-10

IgA Detection and Quantification

InvivoGen provides all the reagents necessary to detect and quantify the light and heavy chains of IgA antibodies by sandwich ELISA: capture antibodies, revelation antibodies and IgA standards. InvivoGen offers also a polyclonal rabbit antiserum to human J chain for the detection of dimeric IgA.

IgA κ Light Chain for Sandwich ELISA

Goat F(ab')2 anti-human kappa - Capture antibody
Goat F(ab')2 anti-human kappa was generated from antibodies isolated from antisera of goats hyperimmunized with human myeloma proteins containing κ light chains. Antibodies were purified by affinity chromatography and digested by pepsin to remove the Fc portion, to avoid non-specific binding through Fc receptors.
Goat F(ab')2 anti-human kappa allows the capture of κ light chain-containing human IgA antibodies.

Goat anti-human kappa - HRP - Revelation antibody
Goat anti-human kappa-HRP is conjugated with horseradish peroxidase to allow the detection of human IgA kappa light chain by ELISA.

Human IgA kappa - IgA light chain standard
Human IgA kappa is a human IgAκ isotype control purified from human myeloma serum.

Contents and Storage

Goat F(ab')2 anti-human kappa is provided as 0.5 mg of purified immunoglobulin in 1.0 ml of 100 mM borate buffered saline, pH 8.0.
Goat anti-human kappa-HRP is supplied as 1.0 ml of stock solution in 50% glycerol/50% PBS, pH 7.4.
Human IgA kappa is provided filtered sterile at a concentration of 0.5 mg/ml in 1.0 ml of 100 mM borate buffered saline, pH 8.0.
Products are shipped at room temperature and should be stored at 4°C.

PRODUCT	QTY	CAT. CODE
Goat F(ab')2 anti-human kappa	0.5 mg	fab-igak
Goat anti-human kappa - HRP	1 ml	hrp-igak
Human IgA kappa	0.5 mg	ctrl-igak

IgA Heavy Chain for Sandwich ELISA

Goat F(ab')2 anti-human IgA - Capture antibody
Goat F(ab')2 anti-human IgA was generated from antibodies isolated from antisera of goats hyperimmunized with human IgA paraproteins. Antibodies were purified by affinity chromatography and digested by pepsin to remove the Fc portion, to avoid non-specific binding through Fc receptors.
Goat F(ab')2 anti-human IgA allows the capture of the heavy chain of human IgA antibodies.

Goat anti-human IgA - HRP - Revelation antibody
Goat anti-human IgA-HRP is conjugated with horseradish peroxidase to allow the detection of human IgA heavy chain by ELISA.

Human IgA from colostrum - IgA standard
Human IgA is isolated from pooled normal human colostrum by fractionation and ion-exchange chromatography.

Contents and Storage

Goat F(ab')2 anti-human IgA is provided as 0.5 mg of purified immunoglobulin in 1.0 ml of 100 mM borate buffered saline, pH 8.0.
Goat anti-human IgA-HRP is supplied as 1.0 ml of stock solution in 50% glycerol/50% PBS, pH 7.4.
Human IgA is supplied as an essentially salt-free, lyophilized powder.
Products are shipped at room temperature and should be stored at 4°C.

PRODUCT	QTY	CAT. CODE
Goat F(ab')2 anti-human IgA	0.5 mg	fab-iga
Goat anti-human IgA - HRP	1 ml	hrp-iga
Human IgA	0.5 mg	ctrl-iga

J Chain Antiserum

J chain antiserum was prepared by injection of purified human J chain in rabbits. Human J chain is distinct from all other chain components of polymeric IgA. J chain antiserum can be used for the detection of dimeric IgA by Western blotting (working dilution 1:1,000).

Contents and Storage

J chain antiserum is provided as 100 µg lyophilized antiserum. J chain antiserum is sterile and azide-free. Product is shipped at room temperature and should be stored at -20°C or 4°C once resuspended.

PRODUCT	QTY	CAT. CODE
J Chain Antiserum	100 µg	pab-jc

Antibody Collection

InvivoGen offers a selection of monoclonal and polyclonal antibodies. Most of them target pattern recognition receptors. 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.

Anti-TLR-IgA and Anti-cytokine-IgA2 antibodies are recombinant monoclonal IgA2 antibodies against TLRs. They have been developed by InvivoGen using proprietary techniques. They have been selected for their ability to efficiently block the biological activity of these TLRs. They can also be used for flow cytometry (FC).

Anti-TLR-IgG antibodies are mouse monoclonal antibodies against TLRs. They have been generated by InvivoGen using DNA vaccination and screened for their ability to neutralize TLR activity.

MAb-TLR and MAb-Dectin-1 antibodies are monoclonal mouse IgG antibodies. They can be used for various applications but have been tested in our laboratories only for neutralization and flow cytometry. MAb-TLR antibodies are also available conjugated with FITC.

PAb-TLR antibodies are polyclonal antibodies against human extracellular TLRs, developed by InvivoGen. These antibodies have been generated by DNA vaccination in rats. They were obtained by purification of the IgG fraction from the sera by Protein G affinity chromatography.

Contents and Storage

Anti-TLR-IgA, Anti-cytokine-IgA2 and **Anti-TLR-IgG** antibodies are provided lyophilized from a 0.2 µm filtered solution in PBS.
MAb-TLR antibodies are purified and provided as 100 µg lyophilized powder.
PAb-TLR antibodies are provided as 200 µg lyophilized sera. PAb-TLRs are sterile, azide-free (contain Pen/Strep), endotoxin-tested (<0.001 EU/µg).
Store all lyophilized antibodies at -20°C.

TARGET	ANTIBODY	SPECIFICITY	DESCRIPTION	APPLICATIONS*	QTY	CAT. CODE
CD14	Anti-hCD14-IgA	Human CD14	Monoclonal human IgA2	Neutralization, FC	100 µg	maba-hcd14
CD14 NEW	MAb-mCD14	Mouse CD14	Monoclonal mouse IgG2a	Neutralization, FC	100 µg	mab-mcd14
CD20	Anti-hCD20-hIgG1	Human CD20	Monoclonal human IgG1	Neutralization, FC	100 µg	hcd20-mab1
CD40L	Anti-hCD40L-hIgA2	Human CD40L	Monoclonal human IgA2	Neutralization	100 µg	maba-h40l
DC-SIGN NEW	MAb-hDC-SIGN	Human DC-SIGN	Monoclonal mouse IgG1	Neutralization, FC	100 µg	mab-hdcsg
Dectin-1 NEW	Anti-hDectin-1-IgG	Human Dectin-1	Monoclonal mouse IgG1	Neutralization	100 µg	mabg-hdect
Dectin-1	MAb-mDectin-1	Mouse Dectin-1	Monoclonal rat IgG2b (clone 2A11)	Neutralization, FC	100 µg	mab-mdect
FliC	Anti-Flagellin FliC	*S. typhimurium* flagellin	Monoclonal mouse IgG1	WB	100 µg	mabg-flic
HA Tag	Anti-HA Tag	Hemagglutinin epitope	Monoclonal mouse IgG1	WB, IP	250 µl	ab-hatag
IFN-α	Anti-hIFNα-IgA2	Human Interferon α	Monoclonal human IgA2	Neutralization	100 µg	maba-hifna
IFN-γ	Anti-hIFNγ-IgA2	Human Interferon γ	Monoclonal human IgA2	Neutralization	100 µg	maba-hifng
IL-1β	Anti-hIL-1β-IgA2	Human Interleukin 1β	Monoclonal human IgA2	Neutralization	100 µg	maba-hil1b
IL-4	Anti-hIL-4-IgA2	Human Interleukin 4	Monoclonal human IgA2		100 µg	maba-hil4
IL-6	Anti-hIL-6-IgA2	Human Interleukin 6	Monoclonal human IgA2	Neutralization	100 µg	maba-hil6
IL-13	Anti-hIL-13-IgA2	Human Interleukin 13	Monoclonal human IgA2	Neutralization	100 µg	maba-hil13
IL-18	Anti-hIL-18-IgA2	Human Interleukin 18	Monoclonal human IgA2	Neutralization	100 µg	maba-hil18
IL-28 NEW	Anti-hIL-28-IgG	Human Interleukin 28	Monoclonal mouse IgG1	Neutralization	100 µg	mabg-hil28
MD2 NEW	MAb-hMD2	Human MD-2	Monoclonal mouse IgG2b	Neutralization, FC	100 µg	mab-hmd2
MR NEW	MAb-hMR	Human Mannose Receptor	Monoclonal mouse IgG1	Neutralization, FC, W	100 µg	mab-hmr
Mincle NEW	Anti-hMincle-IgG	Human Mincle	Monoclonal mouse IgG2b	Neutralization, FC	100 µg	mabg-hmcl
Mincle NEW	Anti-mMincle-IgG	Mouse Mincle	Monoclonal rat IgG2b	Neutralization, FC	100 µg	mabg-mmcl
TGF-β	Anti-hTGFβ-IgA2	Human TGF-beta	Monoclonal human IgA2	Neutralization	100 µg	maba-htgfb
TLR1	Anti-hTLR1-IgG	Human TLR1	Monoclonal mouse IgG1 (clone H2G2)	Neutralization	100 µg	mabg-htlr1
TLR1	MAb-hTLR1	Human TLR1	Monoclonal mouse IgG1 (clone GD2.F4)	FC	100 µg	mab-htlr1

TARGET	ANTIBODY	SPECIFICITY	DESCRIPTION	APPLICATIONS*	QTY	CAT. CODE
TLR1	MAb-hTLR1-FITC	Human TLR1	Monoclonal mouse IgG1 FITC, (clone GD2.F4)	FC	100 µg	mab-htlr1f
TLR1	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
TLR2	MAb-hTLR2	Human TLR2	Monoclonal mouse IgG2a (clone TL2.1)	FC, IHC, WB	100 µg	mab-htlr2
TLR2	MAb-hTLR2-FITC	Human TLR2	Monoclonal mouse IgG2a FITC, (clone TL2.1)	FC, IHC	100 µg	mab-htlr2f
TLR2	PAb-hTLR2	Human TLR2	Polyclonal rat IgG	Neutralization	200 µg	pab-hstlr2
TLR2	Anti-mTLR2-IgG	Mouse TLR2	Monoclonal mouse IgG2a (clone C9A12)	Neutralization	100 µg	mabg-mtlr2
TLR2	MAb-mTLR2	Human/mouse TLR2	Monoclonal mouse IgG1 (clone T2.5)	Neutralization·FC, IHC	100 µg	mab-mtlr2
TLR2	MAb-mTLR2-FITC	Human/mouse TLR2	Monoclonal mouse IgG1 FITC, (clone T2.5)	FC	100 µg	mab-mtlr2f
TLR3	Anti-hTLR3-IgA	Human TLR3	Monoclonal human IgA2	FC	100 µg	maba-htlr3
TLR3	MAb-hTLR3	Human TLR3	Monoclonal mouse IgG1 (clone TLR3.7)	FC, WB	100 µg	mab-htlr3
TLR3	MAb-hTLR3-FITC	Human TLR3	Monoclonal mouse IgG1 FITC, (TLR3.7)	FC, WB	100 µg	mab-htlr3f
TLR4	Anti-hTLR4-IgG	Human TLR4	Monoclonal mouse IgG1	Neutralization	100 µg	mabg-htlr4
TLR4	MAb-hTLR4	Human/monkey TLR4	Monoclonal mouse IgG2a (clone HTA125)	FC, IHC	100 µg	mab-htlr4
TLR4	MAb-hTLR4-FITC	Human/monkey TLR4	Monoclonal mouse IgG2a FITC, (clone HTA125)	FC	100 µg	mab-htlr4f
TLR4 NEW	MAb2-hTLR4	Human TLR4	Monoclonal mouse IgG1	Neutralization, FC	100 µg	mab2-htlr4
TLR4	PAb-hTLR4	Human TLR4	Polyclonal rat IgG	Neutralization	200 µg	pab-hstlr4
TLR4/MD2 NEW	MAb-hTLR4/MD2	Human TLR4/MD2	Monoclonal mouse IgG1	Neutralization, FC	100 µg	mab-htlr4md2
TLR4/MD2	MAb-mTLR4/MD2	Mouse TLR4/MD2	Monoclonal rat IgG2a (clone MTS510)	FC, IHC	100 µg	mab-mtlr4md2
TLR4/MD2	MAb-mTLR4/MD2-FITC	Mouse TLR4/MD2	Monoclonal rat IgG2a FITC (clone MTS510)	FC	100 µg	mab-mtlr4md2f
TLR5	Anti-hTLR5-IgA	Human TLR5	Monoclonal human IgA2	Neutralization, FC	100 µg	maba2-htlr5
TLR5	Anti-mTLR5-IgG	Mouse TLR5	Monoclonal rat IgG2a (clone Q23D11)	Neutralization	100 µg	mabg-mtlr5
TLR5	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
TLR6	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, WB	100 µg	mab-mtlr9
TLR9	MAb-mTLR9-FITC	Human/mouse TLR9	Monoclonal mouse IgG2a FITC, (clone 5G5)	FC	100 µg	mab-mtlr9f
TNF-α	Anti-hTNF-α-hIgG1	Human TNF-alpha	Monoclonal human IgG1	Neutralization	100 µg	htnfa-mab1
TNF-RI NEW	MAb-hTNF-RI	Human TNF receptor I	Monoclonal mouse IgG2a	Neutralization, FC, WB	100 µg	mab-htnfr1

TARGET	ANTIBODY	DESCRIPTION	APPLICATIONS	QTY	CAT. CODE
Control	Human IgA2 Isotype Control	Monoclonal human IgA2, (*E. coli* β-Gal)	Isotype control	100 µg	maba2-ctrl
Control	Mouse IgG1 Isotype Control	Monoclonal mouse IgG1, (*E. coli* β-Gal)	Isotype control	100 µg	mabg1-ctrlm
Control	Mouse IgG2a Isotype Control	Monoclonal mouse IgG2a, (*E. coli* β-Gal)	Isotype control	100 µg	mabg2a-ctrlm
Control	Mouse IgG2b Isotype Control	Monoclonal mouse IgG2b, (*E. coli* β-Gal)	Isotype control	100 µg	mabg2b-ctrlm
Control	PAb Control	Polyclonal rat IgG	Control	200 µg	pab-sctr

* FC, flow cytometry; IHC, immunohistochemistry; IP, immunoprecipitation; WB, Western blot

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 a 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, these cells play a major role in the induction of apoptosis in tumors and 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 critical in the defense against extracellular pathogens (helminthes, 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 in 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 the 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 the innate immunity, predominantly targeting the APCs and consequently influencing the adaptative 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 the immune responses. Activation of some members of the NLR family, such as NLRP3 and NLRC4, triggers the formation of a protein complex, called inflammasome, implicated in the induction of the pro-inflammatory cytokines IL-1β[2] and IL-18. The NLRP3 and NLRC4 inflammasomes have been involved in the innate immunity induced by certain adjuvants but their mechanism of action remains unclear.

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[3]. Alum provokes a strong Th2 response, but is rather ineffective against pathogens that require Th1–cell-mediated immunity. Alum induces the immune response by a depot effect and activation of APCs. Recently, the NLRP3 inflammasome has been linked to the immunostimulatory properties of alum[2] 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 both cellular (Th1) and 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 pathogens 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].

TLR2 Ligands

Several TLR2 agonists, in particular lipopeptides, have been evaluated as vaccine adjuvants. Pam3CSK4, a synthetic bacterial lipopetide recognized by TLR2 and TLR1, has been proven to be a potent adjuvant for various vaccines, including a sublingual allergy vaccine[7], flu vaccine[8] and leishmaniasis vaccine[9]. It was shown to increase antigen-specific IgG titers and Th1 cytokine production.

TLR3 and RLR Ligands

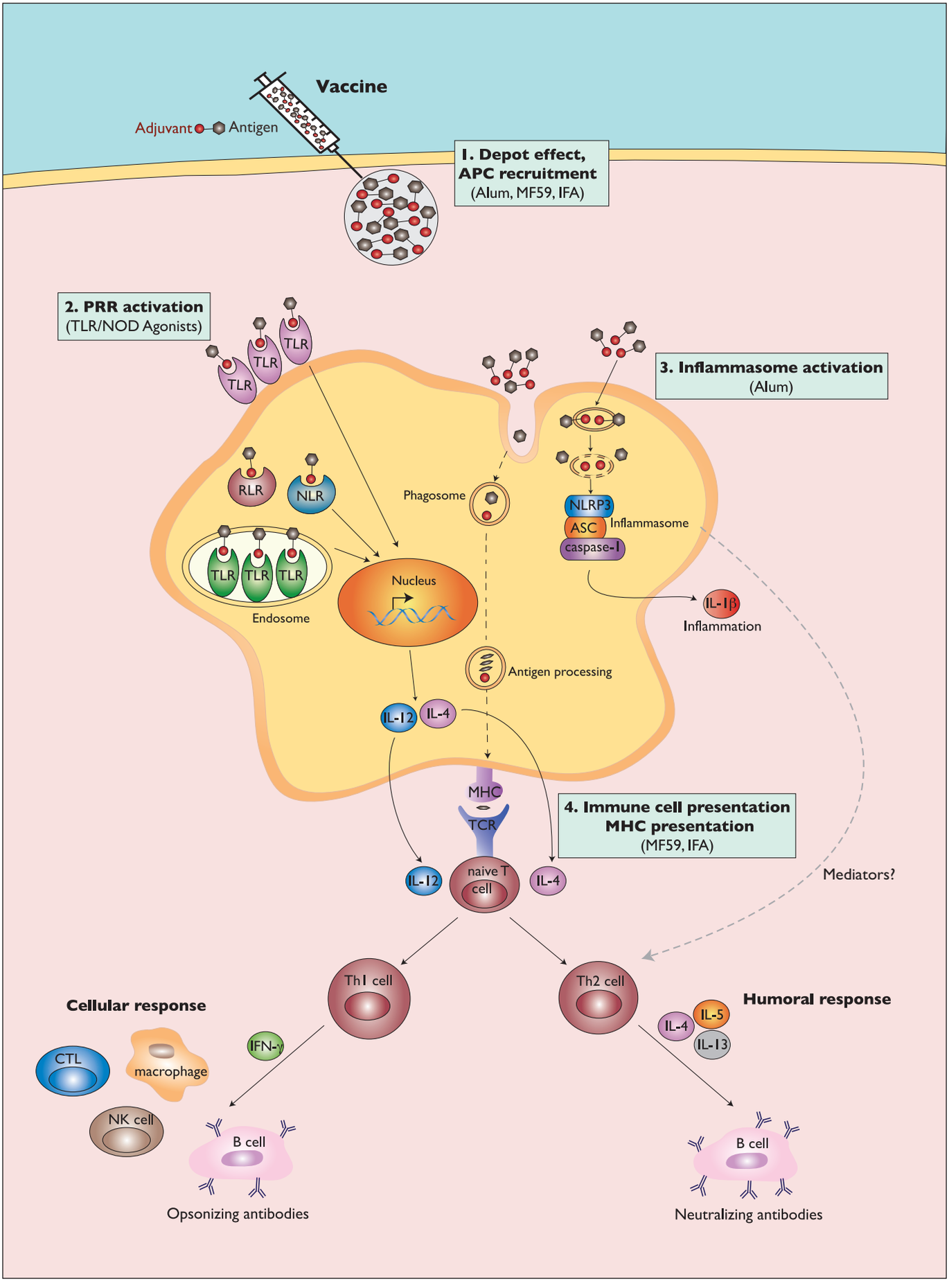
Double-stranded RNA (dsRNA), which is produced during the replication of most viruses, is a potent inducer of innate immunity. Synthetic analogs of dsRNA, such as poly(I:C), have been tested as adjuvants. They act through TLR3 and RIG-I/MDA-5, inducing IL-12 and type I IFNs production, facilitating antigen cross-presentation to MHC class II molecules, and improving generation of cytotoxic T cells[10].

TLR4 Ligands

Bacterial lipopolysaccharides (LPS), which are ligands for TLR4, have long been recognized as potent adjuvants, but their pyrogenic activity have prevented their clinical use. The development of less toxic derivatives led to the production of monophosphoryl lipid A (MPLA). MPL®, a modified MPLA formulated with alum (AS04), triggers a polarized Th1 response[11] and is approved for clinical use in Europe[10].

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 papilloma virus (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)


Vaccine
Adjuvant
Antigen
1. Depot effect,
APC recruitment
(Alum, MF59, IFA)
2. PRR activation
(TLR/NOD Agonists)
TLR
TLR
TLR
RLR
NLR
TLR
TLR
TLR
Endosome
Nucleus
Phagosome
3. Inflammasome activation
(Alum)
NLRP3
ASC
caspase-1
Inflammasome
IL-1β
Inflammation
Antigen processing
IL-12
IL-4
MHC
TCR
4. Immune cell presentation
MHC presentation
(MF59, IFA)
IL-12
naive T cell
IL-4
Mediators?
Th1 cell
Th2 cell
Cellular response
Humoral response
IFN-γ
IL-5
IL-4
IL-13
CTL
macrophage
NK cell
B cell
B cell
Opsonizing antibodies
Neutralizing antibodies

TLR5 Ligands

The TLR5 ligand, bacterial flagellin, 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. Flagellin can be used as an adjuvant mixed with the antigen but it is more frequently fused to a recombinant vaccine antigen[12,13].

TLR7/8 Ligands

The TLR7/8 pathway, specialized in the recognition of single stranded viral RNA, has demonstrated promising pre-clinical results as a target for potential vaccine adjuvants. Imidazoquinolines (i.e. imiquimod and R848) are synthetic componds that activate TLR7/8 in multiple subsets of dendritic cells leading to the production of IFN-α and IL-12 thus promoting a Th1 response[5].

TLR9 Ligands

TLR9 recognizes unmethylated CpG motifs present in bacterial DNA and in synthetic oligodeoxynucleotides named CpG ODNs. Preclinical and clinical studies have demonstrated that CpG ODNs can increase both the humoral and cellular responses to various vaccines[14]. CpG ODNs promote the induction of Th1 and pro-inflammatory cytokines and support the maturation/activation of professional antigen presenting cells[15].

NOD2 Ligands

Muramyl dipeptide (MDP) is a NOD2 ligand that was first identified in bacterial peptidoglycan as an active component in Freund's complete adjuvant. MDP and its derivatives boost vaccine potency by promoting the production of Th1 cytokines and maturation of APCs[16].

Mincle Ligand

Trehalose-6,6-dibehenate (TDB), a synthetic analog of the mycobacterial cord factor, was recently identified as a ligand for the pattern recognition receptor Mincle[17]. Incorporation of TDB into cationic liposomes composed of DDA produce a potent adjuvant, known as CAF01[18]. This glycolipid adjuvant elicits protective T cell immunity against *M. tuberculosis* and other pathogens by inducing a mixed Th1/Th17 response that requires the Syk-Card9–Bcl10-Malt1 signaling axis after binding to Mincle.

STING Ligands

Cyclic dinucleotides are secondary messengers produced by bacteria and are important in many bacterial processes, including biofilm formation, motility, and virulence. Two cyclic dinucleotides, cyclic diadenylate monophosphate (c-di-AMP) and cyclic diguanylate monophosphate (c-di-GMP), are being investigated as potential vaccine adjuvants. Mucosal delivery of c-di-AMP and c-di-GMP induces a balanced Th1/Th2 profile and Th17 response[19,20]. Recently, both c-di-AMP and c-di-GMP were found to directly bind STING, a critical regulator of the innate immune response to a variety of RNA viruses and pathogenic DNA, leading to the activation of TBK1 and IRF3, and the production of type I interferons[21].

Recent advances in our understanding of innate immunity has greatly boosted adjuvant research, and it is now clear that many effective adjuvants are ligands for specific innate immune receptors. Currently, much effort is devoted to the development of adjuvants with the ability to increase cell-mediated immunity and trigger multiple immunological pathways. This new type of adjuvants is needed to protect against challenging diseases such as malaria, HIV-AIDS and cancer. Combination aproaches, in which particulate and immunostimulatory adjuvants are combined, are showing great promise. Better knowledge of the cellular and molecular mechanisms of immunopotentiation will hopefully facilitate the development of more potent and safer adjuvants.

Adjuvants licensed for use in human vaccines

PRODUCT	DESCRIPTION	INDICATION
TLR3		
Poly-ICLC	Poly (I:C) with poly-lysine	Cancer, HIV, Respiratory Viral Infections
TLR4		
MPL®	Monophosphoryl lipid A and QS21 with a liposome (AS01) or a water-in-oil emulsion (AS02)	Malaria, Cancer, Tuberculosis
GLA (MPLAs)	Synthetic MPLA used alone, with Alhydrogel or as a stable emulsion	Schistosomiasis, Flu, Hookworm, Malaria
TLR7/TLR8		
Imiquimod, R848	Imidazoquinoline compounds	Cancer
TLR9		
CpG7909 (ODN2006)	Type B CpG ODN used alone or with Alhydrogel	Hepatitis B, HIV, Cancer, Malaria
1018 ISS	Type B CpG ODN	Cancer
Mincle		
TDB	Synthetic cord factor with DDA (CAF01)	Tuberculosis, HIV

1. Leroux-Roels G., 2010. Unmet needs in modern vaccinology adjuvants to improve the immune response. Vaccine. 28S(3):C25-3. **2. 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. **3. Marrack P. *et al.*, 2009.** Towards an understanding of the adjuvant action of aluminium. Nat Rev Immunol. 9(4):287-93. **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. **7. Lombardi V. *et al.*, 2008.** Toll-like receptor 2 agonist Pam3CSK4 enhances the induction of antigen-specific tolerance via the sublingual route. Clin Exp Allergy. 38(11):1819-29. innate immunity to work. Immunity 33(4):492-503. **8. Caproni E. *et al.*, 2012.** MF59 and Pam3CSK4 Boost Adaptive Responses to Influenza Subunit Vaccine through an IFN Type I-Independent Mechanism of Action. J Immunol. [Epub ahead of print]. **9. Jayakumar A. *et al.*, 2011.** TLR1/2 activation during heterologous prime-boost vaccination (DNA-MVA) enhances CD8+ T Cell responses providing protection against Leishmania (Viannia). PLoS Negl Trop Dis. 5(6):e1204. **10. Coffman R. *et al.*, 2010.** Vaccine adjuvants: Putting innate immunity to work. Immunity 33(4):492-503. **11. Didierlaurent A. *,et al.*, 2009.** AS04, an aluminum salt- and TLR4 agonist-based adjuvant system, induces a transient localized innate immune response leading to enhanced adaptive immunity. J Immunol 183(10): 6186-97. **12. Huleatt J. *et al.*, 2007.** Vaccination with recombinant fusion proteins incorporating Toll-like receptor ligands induces rapid cellular and humoral immunity. Vaccine 25(4): 763-75. **13. Mizel S. & Bates JT., 2010.** Flagellin as an adjuvant: Cellular mechanisms and potential. J Immunol. 175(10):5677-82. **14. Vollmer J & Krieg AM., 2009.** Immunotherapeutic applications of CpG oligodeoxynucleotide TLR9 agonists. Adv Drug Deliv Rev. 61(3):195-204. **15. Klinman DM. *et al.*, 2004.** CpG oligonucleotides improve the protective immune response induced by the anthrax vaccination of rhesus macaques. Vaccine. 22(21-22):2881-6. **16. Ogawa C. *et al.*, 2011.** Muramyl dipeptide and its derivatives: peptide adjuvant in immunological disorders and cancer therapy. Curr Bioact Compd. 7(3):180-197. **17. Schoenen H, *et al.*, 2010.** Cutting edge: Mincle is essential for recognition and adjuvanticity of the mycobacterial cord factor and its synthetic analog trehalose-dibehenate. J Immunol. ;184(6):2756-60. **18. Davidsen J, *et al.*, 2005.** Characterization of cationic liposomes based on dimethyldioctadecylammonium and synthetic cord factor from M. tuberculosis (trehalose 6,6'-dibehenate)-a novel adjuvant inducing both strong CMI and antibody responses. Biochim Biophys Acta. 1718(1-2):22-31. **19. Ebensen T. *et al.*, 2011.** Bis-(3',5')-cyclic dimeric adenosine monophosphate: strong Th1/Th2/Th17 promoting mucosal adjuvant. Vaccine. 29(32):5210-20. **20. Madhun AS. *et al.*, 2011.** Intranasal c-di-GMP-adjuvanted plant-derived H5 influenza vaccine induces multifunctional Th1 CD4+ cells and strong mucosal and systemic antibody responses in mice. Vaccine. 29(31):4973-82. **21. Burdette DL. *et al.*, 2010.** STING is a direct innate immune sensor of cyclic di-GMP. Nature. 478(7370):515-8.

Vaccine Adjuvants

InvivoGen provides different classes of vaccine adjuvants that are either already approved for use in human vaccination, such as alum, or under investigation such as the TLR agonists 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- Oil-in-water	Th2	1:1 (AddaVax™ : antigen)	2 ml 10 ml	vac-adx-2 vac-adx-10
Alhydrogel 2%	Aluminium hydroxide gel	Th2	1:9 - 1:1 (alhydrogel : antigen)	50 ml 250 ml	vac-alu-50 vac-alu-250
IFA	Incomplete Freund's adjuvant Water-in-oil	Th2	1:1 (IFA : antigen)	10 ml 6 x 10 ml	vac-ifa-10 vac-ifa-60
PRR Ligands					
c-di-AMP VacciGrade™ **NEW**	Cyclic diadenylate monophosphate - STING agonist	Th1 / Th2	5 - 50 µg/mouse	1 mg	vac-cda
c-di-GMP VacciGrade™ **NEW**	Cyclic diguanylate monophosphate - STING agonist	Th1 / Th2	5 - 50 µg/mouse	1 mg	vac-cdg
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
MPLA VacciGrade™	Detoxified monophosphoryl Lipid A - TLR4 agonist	Th1	2 - 20 µg/mouse	1 mg	vac-mpl
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™ **NEW**	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™ **NEW**	Synthetic analog of the cord factor - Mincle agonist	Th1	1 - 100 µg/ml	2 mg	vac-tdb

Contents and Storage

AddaVax™ is provided as a ready-to-use sterile emulsion. Alhydrogel 2% is provided as a ready-to-use, sterile wet gel suspension. IFA is provided as a sterile ready-to-use liquid. VacciGrade™ PRR Ligands are provided lyophilized.

Products are shipped at room temperature.
AddaVax™, Alhydrogel 2% and IFA should be stored at 4°C. DO NOT FREEZE. VacciGrade™ PRR Ligands should be stored at 4°C or -20°C according to the product label.

Alum and 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 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 cytokines and chemokines production by macrophages and granulocytes[1].

Alhydrogel 2%

Alhydrogel is an aluminium hydroxide (referred to as alum) wet gel suspension. Alum improves attraction and uptake of antigen by APCs. 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.

IFA

IFA (Incomplete Freund's adjuvant), a water-in-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].

1.Ott G. *et al.*, 2000. The adjuvant MF59: a 10-year perspective. Methods in Molecular Medicine, Vol 42, 211-228 **2. Coffman RL. *et al.*, 2010.** Vaccine adjuvants: Putting innate immunity to work. Immunity 33(4):492-503. **3. Marrack P. *et al.*, 2009.** Towards an understanding of adjuvant action of aluminium. Nat Rev Immunol. 9(4): 287-93. **4. Petrovsky N. & Aguilar JC., 2004.** Vaccine adjuvants: Current state and future trends. Immunol Cell Biol. 82(5): 488-96. **5. Moreira L0. *et al.*, 2008.** Modulation of adaptive immunity by different adjuvant-antigen combinations in mice lacking Nod2. Vaccine 26(46): 5808-13.

PRR Ligands

NEW! 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 c-di-AMP and c-di-GMP can induce a strong immune response *in vitro* and *in vivo*. It was recently found that these cyclic dinucleotides induce the production of cytokines, such as type I interferons, through the STING/TBK1/IRF3 pathway[1]. Due to their immunostimulatory properties, these molecules have been investigated as vaccine adjuvants. Mucosal delivery of c-di-AMP[2] and c-di-GMP[3] elicits a balanced Th1/Th2 profile and Th17 response, which is crucial against intracellular pathogens. These adjuvants act through the recruitment of monocytes and granulocytes, and the maturation of dendritic cells[2,4].

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[5]. It has been demonstrated that flagellin can act as a potent adjuvant in flu vaccines[6, 7]. Furthermore, flagellin can also signal through the NLRC4 inflammasome[8], 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[9]. More specifically, R848 (Resiquimod) activates NF-κB and MAP kinase pathways in B cells, thereby promoting the production of antibodies[10]. R848 is a good inducer of IFN-related innate immunity pathways[11].

MPLA & MPLAs VacciGrade™

The TLR4 agonist, MPLA (monophosphoryl lipid A) is a detoxified derivative of lipid A from *S. minnesota* lipopolysaccharide (LPS or endotoxin). MPLA is considerably less toxic than LPS while maintaining the immunostimulatory activity[12]. Preclinical studies indicate that MPLA induces a strong Th1 response[13]. 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 adsorbed to 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[14, 15].

N-glycolyl-MDP VacciGrade™

MDP (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[16]. 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[17]. Furthermore MDP leads to the activation of the NLRP3 inflammasome[18].

ODN 1585, ODN 1826, ODN 2006 & ODN 2395 VacciGrade™

Synthetic oligodeoxynucleotides containing unmethylated CpG motifs (CpG ODNs) have been extensively studied as adjuvants. Three types of CpG ODNs exist: type A, B and C. All CpG ODN types are recognized by TLR9, which is expressed exclusively on 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[13]. 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[19]. 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[20,21]. ODN 1585, a class A CpG ODN, was recently shown to provide protective immunity against HPV16-associated-tumors in mice when combined with a peptide vaccine[22]. *In vivo* studies have demonstrated that type C ODNs which combine the effects of types A and B ODNs, such ODN 2395, are very potent Th1 adjuvants[23].

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[11]. 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*[24]. Pam3CSK4 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 the immune response through two distinct PRRs[6]. 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[10]. 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[10]. Promising results have been obtained using poly(I:C) as an adjuvant in flu vaccine delivered intranasally[9].

NEW! TDB VacciGrade™

Trehalose-6,6-dibehenate (TDB) is a non-toxic synthetic analogue 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[25]. 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[26]. Adjuvanticity of the cationic DDA:TDB liposomes and sustained protection against disease challenge has been demonstrated in particular with a tuberculosis vaccine candidate[27, 28], and has good potential for application in a range of other diseases[29].

1. Burdette DL. *et al.*, 2010. STING is a direct innate immune sensor of cyclic di-GMP. Nature. 478(7370):515-8. **2. Ebensen T. *et al.*, 2011.** Bis-(3',5')-cyclic dimeric adenosine monophosphate: strong Th1/Th2/Th17 promoting mucosal adjuvant. Vaccine. 29(32):5210-20. **3. Madhun AS. *et al.*, 2011.** Intranasal c-di-GMP-adjuvanted plant-derived H5 influenza vaccine induces multifunctional Th1 CD4+ cells and strong mucosal and systemic antibody responses in mice. Vaccine. 29(31):4973-82. **4. Karaolis DK. *et al.*, 2007.** Bacterial c-di-GMP is an immunostimulatory molecule. J Immunol. 1786:2171-81. **5 Huleatt J. *et al.*, 2007.** Vaccination with recombinant fusion proteins incorporating Toll-like receptor ligands induces rapid cellular and humoral immunity. Vaccine 25(4): 763-75. **6. Mbow ML. *et al.*, 2010.** New adjuvants for human vaccines. Curr Opin Immunol. 22(3): 411-6. **7. Skountzou I. *et al.*, 2010.** Salmonella flagellins are potent adjuvants for intranasally administered whole inactivated influenza vaccine. Vaccine 28(4): 4103-12. **8. Miao EA. & Warren SE., 2010.** Innate immune detection of bacterial virulence factors via the NLRC4 inflammasome. J Clin Immunol. 30(4): 502-6. **9. Steinhagen F. *et al.*, 2010.** TLR-based immune adjuvants. Vaccine. 29(17):3341-55. **10. Coffman RL. *et al.*, 2010.** Vaccine adjuvants: Putting innate immunity to work. Immunity 33(4):492-503. **11. Caproni E. *et al.*, 2012.** MF59 and Pam3CSK4 Boost Adaptive Responses to Influenza Subunit Vaccine through an IFN Type I-Independent Mechanism of Action. J Immunol. 188(7):3088-98. **12. Casella CR. *et al.*, 2008.** Putting endotoxin to work for us: monophosphoryl lipid A as a safe and effective vaccine adjuvant. Cell Mol Life Sci. 65(20):3231-40. **13. Fransen F. *et al.*, 2007.** Agonists of Toll-like receptors 3, 4, 7, and 9 are candidates for use as adjuvants in an outer membrane vaccine against Neisseria meningitidis serogroup . Infect Immun. 75(12) :5939-46. **14. Baldwin SL.. *et al.*, 2009.** Enhanced humoral and Type 1 cellular immune responses with Fluzone adjuvanted with a synthetic TLR4 agonist formulated in an emulsion. Vaccine, 27: 5956–5963. **15. Lousada-Dietrich S. *et al.*, 2011.** A synthetic TLR4 agonist formulated in an emulsion enhances humoral and Type 1 cellular immune responses against GMZ2--a GLURP-MSP3 fusion protein malaria vaccine candidate. Vaccine. 29(17):3284-92. **16. Girardin S. *et al.*, 2003.** Nod2 is a general sensor of peptidoglycan through muramyl dipeptide (MDP) detection. J Biol Chem. 278(11):8869-72. **17. Coulombe F. *et al.*, 2009.** Increased NOD2-mediated recognition of N-glycolyl muramyl dipeptide. J Exp Med. 206(8):1709-16. **18. Martinon F. *et al.*, 2004.** Identification of bacterial muramyl dipeptide as activator of the NALP3/cryopyrin inflammasome. Curr Biol 14 (21): 1929-34. **19. Krieg AM., 2006.** Therapeutic potential of Toll-like receptor 9 activation. Nat Rev Drug Discov ;5:471-484. **20. Geary SM. *et al.*,** Tumor immunotherapy using adenovirus vaccines in combination with intratumoral doses of CpG ODN. Cancer Immunol Immunother. 60(9):1309-17. **21. Naarding MA. *et al.*, 2011.** Hepatitis C virus soluble E2 in combination with QuilA and CpG ODN induces neutralizing antibodies in mice. Vaccine. 29(16):2910-7. **22. Reinis M. *et al.*, 2010.** Induction of protective immunity against MHC class I-deficient, HPV16-associated tumours with peptide and dendritic cell-based vaccines. Int J Oncol. 36(3):545-51. **23. Vollmer J. *et al.*, 2004.** Characterization of three CpG oligodeoxynucleotide classes with distinct immunostimulatory activities. Eur J Immunol. 34(1):251-62. **24. Jayakumar A. *et al.*, 2011.** TLR1/2 activation during heterologous prime-boost vaccination (DNA-MVA) enhances CD8+ T Cell responses providing protection against Leishmania (Viannia). PLoS Negl Trop Dis. 5(6):e1204. **25. Schoenen H, *et al.*, 2010.** Cutting edge: Mincle is essential for recognition and adjuvanticity of the mycobacterial cord factor and its synthetic analog trehalose-dibehenate. J Immunol. ;184(6):2756-60. **26. Davidsen J, *et al.*, 2005.** Characterization of cationic liposomes based on dimethyldioctadecylammonium and synthetic cord factor from M. tuberculosis (trehalose 6,6'-dibehenate)-a novel adjuvant inducing both strong CMI and antibody responses. Biochim Biophys Acta. 1718(1-2):22-31. **27. Holten-Andersen L. *et al.*, 2004.** Combination of the cationic surfactant dimethyl dioctadecyl ammonium bromide and synthetic mycobacterial cord factor as an efficient adjuvant for tuberculosis subunit vaccines. Infect Immun. 72(3):1608-17. **28. Christensen D. *et al.*, 2011.** Cationic liposomes as vaccine adjuvants. Expert Rev Vaccines. 10(4):513-21. **29. Agger EM. *et al.*, 2008.** Cationic liposomes formulated with synthetic mycobacterial cordfactor (CAF01): a versatile adjuvant for vaccines with different immunological requirements. PLoS One. 3(9):e3116.

OVA Antigens

Ovalbumin (OVA) is a key reference protein for immunization and biochemical studies (Western blots, ELISA). Commercially available ovalbumin is often contaminated with endotoxins altering the results *in vivo*. InvivoGen provides two grades of ovalbumin and two standard OVA peptides.

EndoFit™ Ovalbumin: Endotoxin level < 1 EU/mg, for *in vivo* use, minimum 98% protein

Ovalbumin: for detection use (Western blot, ELISA), minimum 98% protein

OVA 257-264: an H-2Kb-restricted OVA class I epitope, for detection use (ELISPOT) - Sequence : SIINFEKL (MW 963.2)

OVA 323-339: an H-2b-restricted OVA class II epitope, for detection use (ELISPOT) - Sequence : ISQAVHAAHAEINEAGR (MW 1773.9)

Contents and Storage

Products are provided lyophilized and shipped at room temperature. Store at 4°C or -20°C according to the product label.

PRODUCT	QTY	CAT. CODE
EndoFit™ Ovalbumin	10 mg	vac-efova
Ovalbumin	1 g	vac-ova
OVA 257-264	1 mg	vac-sin
OVA 323-339	1 mg	vac-isq

pBOOST - DNA Vaccine Adjuvants

pBOOST plasmids were developed as genetic adjuvants for DNA vaccines to potentiate the immune response to a specific antigen. The method of plasmid DNA vaccine delivery is known to bias the immune response to a specific antigen towards a Th1 or Th2 response. These biases can be further enhanced by the codelivery of interferon regulatory factors (IRFs) or TANK-binding kinase 1 (TBK1) to increase the efficacy of the vaccination.

Description

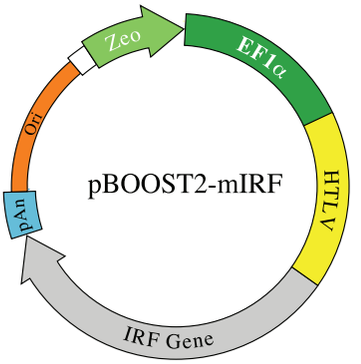
pBOOST2-mIRF - Th1 or Th2 polarization

IRF-1, IRF-3 and IRF-7 have been shown to be able to bias T cells towards type 1 or type 2 immune responses, leading to the activation of cytotoxic T cells and/or the production of antibodies.

pBOOST2-mIRF plasmids carry the murine IRF-1, IRF-3 or IRF-7 genes. These genes are either wild-type or mutant.

- **Wild-type IRF-1:** IRF-1 primarily increases Th2 antibody responses[1]. Following intramuscular or gene gun injections of DNA vaccines, IRF-1 can increase the titers of antibodies up to 10-fold.
- **Mutant IRF-3:** IRF-3 primarily increases Th1 T-cell responses[1]. A constitutively active form of IRF-3 was generated by creating a single point mutation of Ser^{396} to Asp. This super-activated IRF-3 presents a >10-fold enhanced transactivating potential over the wild-type IRF-3 for the IFN-α and IFN-β promoters[2].
- **Chimeric IRF7/3:** IRF-3 and IRF-7 increase both Th1 and Th2 responses by transactivating different target promoters[1]. To exploit the biological features of both IRFs, a chimeric form of IRF-7 and IRF-3 was generated by combining the DNA binding specificity of IRF-7 with the strong transactivation capacity of super-activated IRF-3. IRF-7/3 chimera provides >10-fold greater induction of IFN-α and IFN-β promoters than super-activated IRF-3 alone[3].

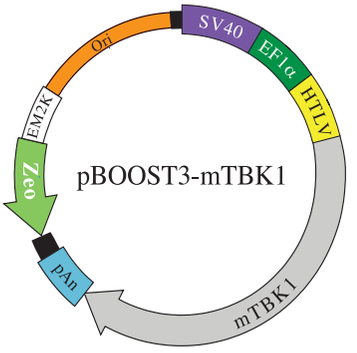
pBOOST2-mIRF plasmids express the transgene under the control of the strong and ubiquitous EF-1α/HTLV composite promoter and are selectable in *E. coli* with Zeocin™.



pBOOST3-mTBK1 - Enhancement of DNA vaccine immunogenicity

pBOOST3-mTBK1 plasmid expresses the mouse TBK1 gene. TANK-binding kinase 1 (TBK1), a non-canonical IκB kinase, was recently shown to mediate the adjuvant effect of DNA vaccines[4]. Administration of DNA vaccines induces the production of type I interferons and inflammatory cytokines in a CpG-independent manner but in a TBK1-dependent manner. Therefore, co-administration of a TBK1-expressing plasmid is expected to further boost DNA vaccine-induced immunogenicity.

pBOOST3-mTBK1 contains the EF-1α/HTLV composite promoter coupled to the SV40 enhancer and is selectable in *E. coli* with Zeocin™.



1. Sasaki S. *et al.*, 2002. Regulation of DNA-raised immune responses by cotransfected interferon regulatory factors. J Virol.76(13):6652-9. **2. Servant MJ. *et al.*, 2003.** Identification of the minimal phosphoacceptor site required for in vivo activation of interferon regulatory factor 3 in response to virus and double-stranded RNA. J Biol Chem. 278(11):9441-7. **3. Bramson JL. *et al.*, 2003.** Super-activated interferon-regulatory factors can enhance plasmid immunization. Vaccine. 21(13-14):1363-70. **4. Ishii KJ. *et al.*, 2008.** TANK-binding kinase-1 delineates innate and adaptive immune responses to DNA vaccines. Nature 451: 725-729

Contents and Storage

Each pBOOST plasmid is provided as 20 µg of lyophilized DNA. Product is shipped at room temperature. Store at -20°C. Plasmids are stable up to one year when properly stored. pBOOST plasmids come with 4 pouches of *E. coli* Fast-Media® Zeo (2 TB and 2 Agar, see pages 48-49).

PRODUCT	QTY	CAT. CODE
pBOOST2-wtmIRF1	20 µg	pbst2-wtmirf1
pBOOST2-samIRF3	20 µg	pbst2-samirf3
pBOOST2-samIRF7/3	20 µg	pbst2-samirf73
pBOOST2-mcs (control)	20 µg	pbst2-mcs
pBOOST3-mTBK1	20 µg	pbst3-mtbk1
pBOOST3-mcs (control)	20 µg	pbst3-mcs

pVAC - Induction of Neutralizing Antibodies

pVAC is a DNA vaccine plasmid family specifically designed to stimulate a humoral immune response by intramuscular injection. Antigenic proteins are targeted and anchored to the cell surface by cloning the gene of interest in frame between the IL2 signal sequence and the C-terminal transmembrane anchoring domain of human placental alkaline phosphatase. The antigenic peptide produced on the surface of muscle cells is thought to be taken up by antigen presenting cells (APCs) and processed through the major histocompatibility complex (MHC) class II pathway[1,2,3].

Description

Cell Surface Expression of the Antigenic Protein

Two pVAC backbones are available:

- **pVAC1-mcs** is designed for the cloning of an antigenic gene that is not naturally secreted. The MCS is flanked by the 5' IL2 signal sequence and the 3' glycosylphosphatidylinositol (GPI) anchoring domain of placental alkaline phosphatase (PLAP).
- **pVAC2-mcs** is designed for the cloning of an antigenic gene that already possesses a signal sequence. The MCS is located upstream of the GPI anchoring domain of PLAP.

High-level Expression of the Antigenic Gene

pVAC plasmids utilize the promoter region of the rhesus monkey EF1α gene to achieve high levels of expression in skeletal muscle cells and antigen presenting cells. Expression levels are further increased by the addition of the SV40 enhancer that heightens the ability of the plasmid to be transported into the nucleus, especially in non-dividing cells.

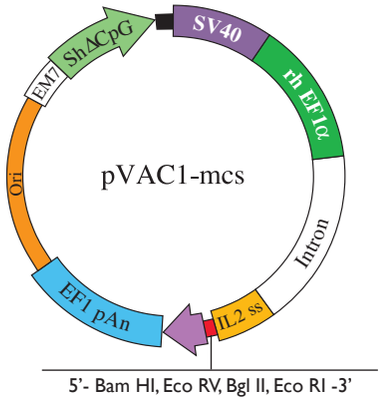
Sustained Expression of the Antigenic Gene

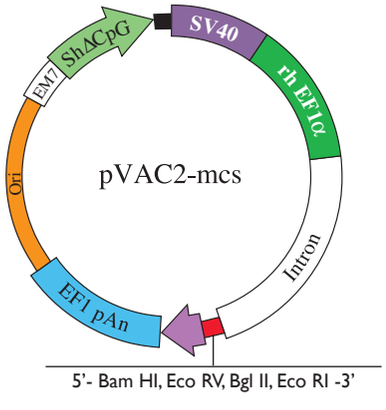
The bacterial region required for replication and selection of pVAC in *E. coli* contains a reduced number of immunogenic CpG motifs by featuring a minimized origin of replication (Ori) and a CpG-free Zeocin™-resistance gene (Sh-ΔCpG).

Convenient Cloning of the Antigenic Gene

pVAC contains a multiple cloning site (MCS) with several commonly used restriction sites.

1. Corr M. *et al.* 1999. In vivo priming by DNA injection occurs predominantly by antigen transfer. J Immunol. 163(9):4721-7. 2. Forns X. *et al.* 1999. DNA immunization of mice and macaques with plasmids encoding hepatitis C virus envelope E2 protein expressed intracellularly and on the cell surface. Vaccine 17:1992-2002. 3. McCluskie MJ *et al.* 1999. Route and method of delivery of DNA vaccine influence immune responses in mice and non-human primates. Mol Med 5:287-300.





Contents and Storage

Each pVAC 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 up to one year when properly stored. Each pVAC is provided with 4 pouches of *E. coli* Fast-Media® Zeo (2 TB and 2 Agar, see pages 48-49).

PRODUCT	QTY	CAT. CODE
pVAC1-mcs	20 µg	pvac1
pVAC2-mcs	20 µg	pvac2

Articles using InvivoGen's pVAC plasmids

Buck CB. *et al.*, 2005. Maturation of papillomavirus capsids. J Virol. 79(5):2839-46.

Ghochikyan A. *et al.*, 2003. Generation and characterization of the humoral immune response to DNA immunization with a chimeric beta-amyloid-interleukin-4 minigene. Eur J Immunol. 33(12):3232-41.

Yang DH. *et al.*, 2006. DNA versus protein immunisation for production of monoclonal antibodies against Choristoneura fumiferana ecdysone receptor (CfEcR). Vaccine. 24(16):3115-26.

7 CUSTOM SERVICES

Large Scale Plasmid DNA Production

If you are conducting gene therapy research, DNA vaccination research, transfection or pre-clinical trials, we can provide you with large quantities of plasmid DNA. We offer a comprehensive custom service. In order to supply the most suitable and ready-to-use product, we manufacture plasmid DNA at standard research and pre-clinical grades.

- Technological Expertise
- Personalized Technical Support
- High Quality Plasmid DNA
- Cost-Effective
- Short Turn-Around Time

Two purification grades of plasmid DNA

If you are conducting gene therapy research, DNA vaccination research, transfection or pre-clinical trials, we can provide you with large quantities of plasmid DNA. We offer a comprehensive custom-made service. In order to supply the most suitable and ready-to-use product, we manufacture plasmid DNA at standard research and pre-clinical grades.

- Standard Research Grade

The level of endotoxin is reduced to less than 0.1 EU/µg plasmid DNA (FDA specifications).

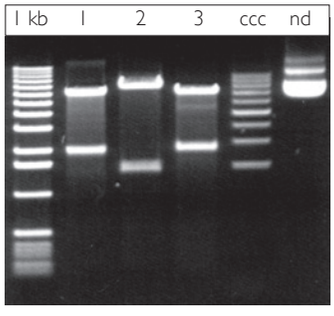
- Pre-Clinical Grade

The purification is guaranteed with a protein level less than 1% and with an absence of microorganisms (verified by Bioburden Assay).

High Standard Quality Control

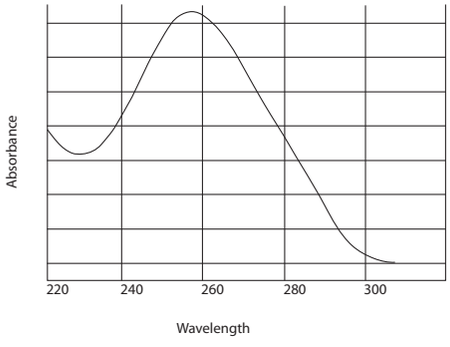
Our processing and our rigorous quality procedures allow us to provide certified plasmid DNA. Each batch of plasmid DNA is delivered with a certificate of analysis and meets the characteristics listed below.

Analysis on agarose gel 0.8%



1 kb : linear DNA ladder
1 : Pac I (6306-2109)
2 : Xba I/Ase I (1497-6921)
3 : Bst XI (2148-6270)
ccc : circular DNA ladder
nd : not digested

Absorbance Curve (220 nm - 320 nm)



CRITERIA TESTED	VALUE	ASSAY METHOD
Appearance in water solution	Clear, colorless	Visual inspection
Plasmid identity	Restriction pattern	Agarose gel electrophoresis
DNA homogeneity	>95% supercoiled form	Agarose gel electrophoresis
Purity	1.7-2.0 A260/A280	Optical density measurements
Presence of *E. coli* DNA	<5%	Agarose gel electrophoresis
Presence of RNA and ss DNA	Undetectable	Agarose gel electrophoresis
Presence of endotoxin	< 0.1 EU / µg plasmid DNA	BioWhittaker LAL test
Presence of protein*	<1%	Micro BCA assay
Sterility*	Absence of microorganisms	Bioburden assay

*pre-clinical grade only

PRODUCT	QTY	CAT. CODE
DNA Standard Research Grade	Up to 1 g	p-custom
DNA Pre-Clinical Grade	Up to 1 g	p-custom

Contact us for more information
info@invivogen.com

CUSTOM CLONING

Many Combinations Possible According to Your Aims

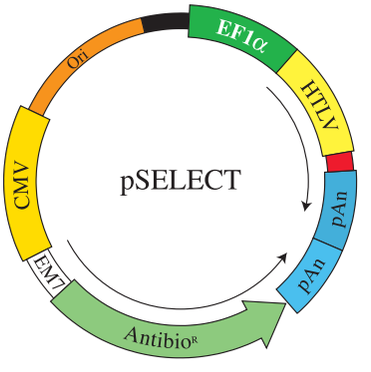
Recombinant DNA cloning is not always easy and often time-consuming. Cloning projects can be complicated due to issues such as gene toxicity, large vector or insert size, unstable DNA elements or the presence of DNA secondary structures. We have the experience and expertise to help you build the constructs you desire. InvivoGen provides cloning and subcloning services to free your energy and time from routine subcloning for more creative research. Our cloning service provides expert advice tailored to your needs at very competitive rates and short turn-around time. All custom-built constructs are sequence verified with annotated plasmid maps.

- Wide Expertise and Consultation Available
- Customized Scientific Support
- Your Product Ready-to-Use
- Rapid Processing and Cost-Effective

Contact us for more information
info@invivogen.com

Custom-Made pSELECT

pORF and pMOD plasmids are not selectable in mammalian cells. If you are planning on stably expressing an open reading frame provided in a pORF or pMOD, our experts can subclone this open reading frame into a pSELECT plasmid carrying the selectable marker of your choice:



Features and Benefits

Two transcription Units
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.

Strong and Ubiquitous Expression of the Transgene
pSELECT plasmids feature the strong EF-1α/HTLV composite promoter that combines the elongation factor 1 alpha core promoter and the 5'untranslated region of the Human T-cell Leukemia Virus.

Variety of Selection Options
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.

Contents and Storage

Custom-made pSELECT plasmids are provided as 20 µg of lyophilized DNA. Products are shipped at room temperature. Store at -20°C.

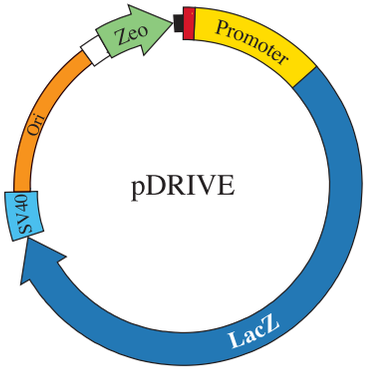
pSELECT Plasmids Available

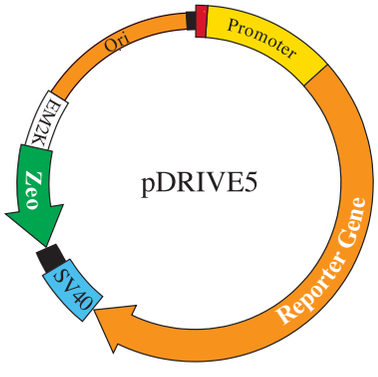
pSELECT-blasti	**pSELECT-puro**
pSELECT-hygro	**pSELECT-zeo**
pSELECT-neo	**pSELECT-gfpzeo**

PRODUCT	QTY	CAT. CODE
Custom-made pSELECT	20 µg	p-custom

Custom-Made pDRIVE

You can create your own composite promoter by combining the enhancer, core promoter and 5'UTR of your choice. We will assemble this composite promoter in a pDRIVE plasmid





Description

Customize Your Promoter

Choose the enhancer, the core promoter and 5'UTR from InvivoGen's lists, and we will generate the composite promoter of your choice.

- **Enhancer:** InvivoGen provides a choice of ubiquitous or specific enhancers. Choose any enhancer from the list below:
- **Core promoter:** You can choose the core promoter from the list of ubiquitous or specific native promoters (see pages 42-43).
- **5'UTR:** The 5' untranslated region can be chosen from the list below:

Choose Your Selection

Custom-made pDRIVE carry the Zeocin™ resistance gene for selection and amplification in *E. coli*. We can replace the bacterial selection cassette with a mammalian selection cassette of your choice derived from a pSELECT plasmid (see page 30).

Choose Your Reporter

- **LacZ** which expression can be determined using chromogenic, luminescent or histochemical detection.
- **Lucia®,** a new secreted synthetic coelenterazine-utilizing luciferase that can be directly measured in the cell culture supernatant in bioluminescent assays using QUANTI-Luc™ (see page 23).
- **GFP** which expression can be assessed qualitatively by fluorescence microscopy and quantitatively using a fluorometer or flow cytometry.
- **SEAP** (secreted embryonic alkaline phosphatase) which expression levels can be assayed with luminescent or chromogenic methods, such as QUANTI-Blue™ (see page 20).

Enhancer	Name	Origin	Specificity
AFP	Alpha-Fetoprotein	Human	AFP-positive cells
CEA	Carcinoembryonic antigen	Human	CEA-positive cells
CMV	Cytomegalovirus	Viral	Ubiquitous
P2	Adipocyte P2	Mouse	Adipose tissue
PSA	Prostate specific antigen	Human	PSA-positive cells only
SV40	Simian virus	Viral	Ubiquitous
Tie2	Angiopoietin receptor	Mouse	Vascular ECs
Tyr	Tyrosinase	Mouse	Melanocytes and melanoma

5'UTR	Name	Origin	Activity
AdTp	Adenotripartite	Viral	Enhances translation
EF-1α	Elongation factor 1 alpha	Chimpanzee Mouse	Increases mRNA translation
eIF4g	Eukaryotic initiation factor 4g	Human	Contains a putative IRES
HSP70	Heat shock protein 70	Human	Increases mRNA translation
NRF	NF-κB repressing factor	Mouse	Acts as a potent IRES
HTLV RU5'	Human T-cell leukemia virus	Human	Increases mRNA translation
Tie2	Angiopoietin receptor	Mouse	Vascular ECs
Tyr	Tyrosinase	Mouse	Melanocytes and melanoma

pDRIVE Plasmids Available

pDRIVE-LacZ
pDRIVE5-Lucia™
pDRIVE5-GFP
pDRIVE5-SEAP

Contents and Storage

Custom-made pDRIVE plasmids are provided as 20 µg of lyophilized DNA. Products are shipped at room temperature. Store at -20°C.

PRODUCT	QTY	CAT. CODE
Custom-made pDRIVE	20 µg	p-custom

Custom-Made CpG-Free Genes

InvivoGen is an expert in developing CpG-Free genes and now offers a CpG-Free gene custom service. Send us the sequence of your gene of interest, and our specialists will design, synthesize and clone into an expression vector a CpG-Free allele of this gene. We guarantee that the sequence of the synthetic gene will 100% match the designed sequence.

Description

Sequence Design

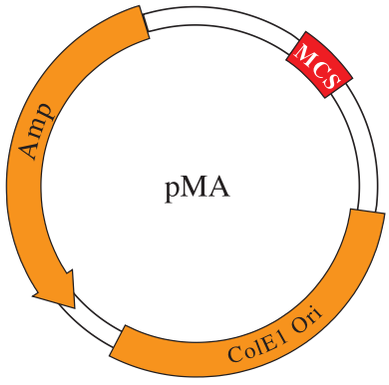
Our experts will design the sequence of a CpG-free allele of your gene of interest using a proprietary software that allows the elimination of all CpG dinucleotides, the humanization of the sequence, the elimination of secondary structures, and the elimination/reduction of *dam* and *dcm* methylations to diminish *E. coli* signature

Cloning and Sequencing

The Custom-made CpG-free Gene is cloned into the pMA plasmid. We can clone it in the vector of your choice for an additional charge.
The Custom-made CpG-free Gene is 100% sequence verified, both strands are sequenced.

Processing

Send us the nucleic (Genbank accession number) or proteic sequence of your gene of interest. The synthesis of the CpG-free allele will start after you confirm its sequence.
The turnaround time is 2-3 weeks for sequences shorter than 1 kb.
We can process sequences up to 5 kb.



Contents and Storage

Custom-made CpG-free genes are provided as 20 µg of lyophilized DNA. Products are shipped at room temperature. Store at -20°C.

PRODUCT	QTY	CAT. CODE
Custom-made CpG-free Gene	20 µg	p-custom

Custom-Made psiRNA

InvivoGen provides a complete siRNA service to help you speed-up your gene silencing experiments. Just give us the accession number of your gene of interest and we will take care of all the steps.
Construction of custom-made psiRNA plasmids includes selection of the siRNA sequence for the target gene, synthesis of the siRNA insert oligonucleotides, cloning of the annealed oligonucleotides in psiRNA and sequencing of the siRNA insert.

Features and Benefits

Choose your siRNA sequence

You can either send us your siRNA sequence or, using the siRNA Wizard software, InvivoGen will select the best candidate siRNA sequence for your target gene.

Choose your psiRNA Plasmid

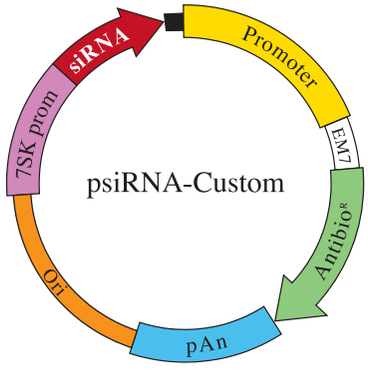
The siRNA insert is cloned into the psiRNA-h7SK plasmid (see page 62). We can also clone two siRNA inserts in the psiRNA-DUO plasmid (see page 63).

Choose your selectable marker

psiRNA plasmids are available with various selectable markers that confer antibiotic resistance in both *E. coli* and mammalian cells: blasticidin, hygromycin B, Kanamycin / G418, Zeocin™ or GFP::Zeo.

Contents and Storage

Custom-made psiRNA are provided as 20 µg of lyophilized DNA. They can also be provided as a kit that contains in addition: 20 µg of psiRNA-EGFP or psiRNA-LucGL3 as a control, 1 vial of LyoComp GT116, 4 pouches of the corresponding Fast-Media®. Products are shipped at room temperature. Store at -20°C.



psiRNA Plasmids Available

psiRNA-h7SKblasti
psiRNA-h7SKhygro
psiRNA-h7SKgfpzeo
psiRNA-h7SKneo
psiRNA-h7SKzeo

PRODUCT	QTY	CAT. CODE
Custom-made psiRNA	20 µg	p-custom
Custom-made psiRNA Kit	1 kit	k-custom

PRODUCT INFORMATION

TERMS AND CONDITIONS

Prices

Written price quotes are firm for purchase orders received within 30 days. Prices are subject to change without notice.

Payment Terms

Payment terms are net 30 days from the invoice date. Pre-payments may be required for initial orders with completion of credit application. InvivoGen does not require a minimum order quantity.

Shipping

Product is shipped F.O.B. from InvivoGen, San Diego, CA. Domestic orders are shipped 2-3 day express by our designated carrier. Orders can be expedited to overnight service for an additional fee. European orders are shipped from our affiliate in France, Cayla. Please include Value Added Tax (VAT) registration number when placing the order. For non-U.S. orders, other charges such as import duties and value added taxes may apply. Shipping days are Monday through Friday.

Warranty

InvivoGen warrants that the products sold will meet our specifications at the time of delivery. InvivoGen's sole liability shall be limited to, at our option, replacement of material(s) that does not meet our specification or refund of the purchase price. By acceptance of the product, Buyer indemnifies and holds InvivoGen harmless against, and assumes all liability for any direct, incidental, special or consequential loss, damage or expense directly or indirectly arising from the use of the product, even if InvivoGen knew of the possibility of such loss, damage or expense.

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Limited Use License

pCpGfree plasmids & HEK-Blue™ Products: The purchase of these product conveys to the buyer the non-transferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

The buyer agrees that any activity undertaken with the product and replicates or derivatives will be conducted in compliance with all applicable guidelines, laws and regulations.

Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. "Replicate" means any biological or chemical material that represents a substantially unmodified copy of the Material such as, but not limited to, material produced by growth of cells. "Derivative" means material created from the Material that is substantially modified to have new properties such as, but not limited to, recombinant DNA modified clones.

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, 3950 Sorrento Valley Blvd. Suite 100, San Diego California 92121. Tel:858-457-5873. Fax: 858-457-5843.

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

AddaVax™ - EndoFit™ - Fast-Media® - Fungin™ - Gene A-List™ - HEK-Blue™ - HygroGold™ - InvivoGen® - LENTI-Smart™ - LipoGen™ - Lucia® - LyoVec™ - Normocin™ - Plasmocin™ - Plasmocure™ - PlasmoTest™ - Primocin™ - Prom A-List™ - PromTest™ - psiRNA™ - QUANTI-Blue™ - QUANTI-Luc™ - VacciGrade™ - Zeocin™

PRODUCT INFORMATION

Alphabetical List by Product Name

PRODUCT (QUANTITY)	CAT. CODE	PAGE
17-AAG (5 mg)	ant-agl-5	81
17-AAG (25 mg)	ant-agl-25	81
17-AEP-GA (1 mg)	ant-egl-1	81
17-AEP-GA (5 mg)	ant-egl-5	81
17-DMAG (5 mg)	ant-dgl-5	81
17-DMAG (25 mg)	ant-dgl-25	81
17-DMAP-GA (1 mg)	ant-mgl-1	81
17-DMAP-GA (5 mg)	ant-mgl-5	81
17-GMB-APA-GA (1 mg)	gmbapa-ga	82
17-NHS-ALA-GA (1 mg)	ant-nhgl-1	82
2-Aminopurine (250 mg)	tlrl-apr	73
3-Methyladenine (50 mg)	tlrl-3ma	77
5-Aza-2'-deoxycytidine (10 mg)	met-adc-1	79
5-Aza-2'-deoxycytidine (50 mg)	met-adc-5	79
5-Aza-cytidine (100 mg)	inh-aza	79
5-Fluorocytosine (250 mg)	sud-5fc	79
5-Fluorouracil (250 mg)	sud-5fu	79
AddaVax™ (2 ml)	vac-adx-2	99
AddaVax™ (10 ml)	vac-adx-10	99
AG490 (10 mg)	tlrl-ag4	78
Alhydrogel 2% (50 ml)	vac-alu-50	99
Alhydrogel 2% (250 ml)	vac-alu-250	99
Anti-[anti-hTNF-α] (100 µg)	mab-idtnf	89
Anti-Flagellin FliC (100 µg)	mabg-flic	94
Anti-HA Tag (250 µl)	ab-hatag	94
Anti-hCD14-IgA (100 µg)	maba-hcd14	94
Anti-hCD20-hIgA1 (100 µg)	hcd20-mab6	89
Anti-hCD20-hIgA2 (100 µg)	hcd20-mab7	89
Anti-hCD20-hIgE (100 µg)	hcd20-mab8	89
Anti-hCD20-hIgG1 (100 µg)	hcd20-mab1	89
Anti-hCD20-hIgG2 (100 µg)	hcd20-mab2	89
Anti-hCD20-hIgG3 (100 µg)	hcd20-mab3	89
Anti-hCD20-hIgG4 (100 µg)	hcd20-mab4	89
Anti-hCD20-hIgM (100 µg)	hcd20-mab5	89
Anti-hCD20-mIgG1 (100 µg)	hcd20-mab9	89
Anti-hCD20-mIgG2a (100 µg)	hcd20-mab10	89
Anti-hCD20-mIgA (100 µg)	hcd20-mab11	89
Anti-hCD40L-IgA2 (100 µg)	maba-h40l	94
Anti-hDectin-1-IgG (100 µg)	mabg-hdect	94
Anti-hIFN-α-IgA2 (100 µg)	maba-hifna	94
Anti-hIFN-γ-IgA2 (100 µg)	maba-hifng	94
Anti-hIL-1β-IgA2 (100 µg)	maba-hil1b	94
Anti-hIL-4-IgA2 (100 µg)	maba-hil4	94
Anti-hIL-6-IgA2 (100 µg)	maba-hil6	94
Anti-hIL-13-IgA2 (100 µg)	maba-hil13	94
Anti-hIL-18-IgA2 (100 µg)	maba-hil18	94

PRODUCT (QUANTITY)	CAT. CODE	PAGE
Anti-hIL-28-IgG (100 µg)	mabg-hil28	94
Anti-hMincle-IgG (100 µg)	mabg-hmcl	94
Anti-hTGFβ-IgA2 (100 µg)	maba-htgfb	94
Anti-hTLR1-IgG (100 µg)	mabg-htlr1	94
Anti-hTLR2-IgA (100 µg)	maba2-htlr2	95
Anti-hTLR3-IgA (100 µg)	maba-htlr3	95
Anti-hTLR4-IgG (100 µg)	mabg-htlr4	95
Anti-hTLR5-IgA (100 µg)	maba2-htlr5	95
Anti-hTLR6-IgG (100 µg)	mabg-htlr6	95
Anti-hTNF-α-hIgA1 (100 µg)	htnfa-mab6	89
Anti-hTNF-α-hIgA2 (100 µg)	htnfa-mab7	89
Anti-hTNF-α-hIgE (100 µg)	htnfa-mab8	89
Anti-hTNF-α-hIgG1 (100 µg)	htnfa-mab1	89
Anti-hTNF-α-hIgG2 (100 µg)	htnfa-mab2	89
Anti-hTNF-α-hIgG3 (100 µg)	htnfa-mab3	89
Anti-hTNF-α-hIgG4 (100 µg)	htnfa-mab4	89
Anti-hTNF-α-hIgM (100 µg)	htnfa-mab5	89
Anti-hTNF-α-mIgG1 (100 µg)	htnfa-mab9	89
Anti-hTNF-α-mIgG2 (100 µg)	htnfa-mab10	89
Anti-hTNF-α-mIgA (100 µg)	htnfa-mab11	89
Anti-Lucia-IgG (100 µg)	mabg-lucia	22
Anti-mMincle-IgG (100 µg)	mabg-mmcl	94
Anti-mTLR2-IgG (100 µg)	mabg-mtlr2	95
Anti-mTLR5-IgG (100 µg)	mabg-mtlr5	95
Bafilomycin A1 (10 µg)	tlrl-baf	73
Bay11-7082 (10 mg)	tlrl-b82	75
Biotin-GA (1 mg)	ant-bgl-1	82
Biotin-GA (5 mg)	ant-bgl-5	82
Bix-01294 (2 mg)	inh-bix	79
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	73
c-di-AMP VacciGrade (1 mg)	vac-cda	99
c-di-GMP VacciGrade (1 mg)	vac-cdg	99
Celastrol (1 mg)	ant-cls	75
ChemiComp GT115 (5 x 0.1 ml)	gt115-11	47
ChemiComp GT115 (5 x 0.2 ml)	gt115-21	47
ChemiComp GT116 (5 x 0.1 ml)	gt116-11	47
ChemiComp GT116 (5 x 0.2 ml)	gt116-21	47
Chloroquine (250 mg)	tlrl-chq	73
CI-994 (10 mg)	inh-ci59	79
CLI-095 (1 mg)	tlrl-cli95	73
CP-690550 (5 mg)	tlrl-cp69	78
Custom-made CpG-free Gene (20 µg)	p-custom	57

PRODUCT INFORMATION

Alphabetical List by Product Name

PRODUCT (QUANTITY)	CAT. CODE	PAGE
Custom-made pDRIVE (20 µg)	p-custom	45
Custom-made psiRNA (20 µg)	p-custom	69
Custom-made psiRNA kit	k-custom	69
Custom-made pSELECT (20 µg)	p-custom	106
Cyclosporin A (100 mg)	tlrl-cyca	77
Dexamethasone (100 mg)	tlrl-dex	75
DNA Standard Research Grade	p-custom	105
DNA Pre-Clinical Grade	p-custom	105
EndoFit™ Ovalbumin (10 mg)	vac-efova	101
Everolimus (5 mg)	tlrl-eve	77
Fast-Media® Amp Agar (30 pouches)	fas-am-s	49
Fast-Media® Amp Agar (500 pouches)	fas-am-s500	49
Fast-Media® Amp LB (30 pouches)	fas-am-b	49
Fast-Media® Amp LB (500 pouches)	fas-am-b500	49
Fast-Media® Amp TB (30 pouches)	fas-am-l	49
Fast-Media® Amp TB (500 pouches)	fas-am-l500	49
Fast-Media® Amp XGal (20 pouches)	fas-am-x	49
Fast-Media® Base Agar (30 pouches)	fas-s	49
Fast-Media® Base Agar (500 pouches)	fas-s500	49
Fast-Media® Base TB (30 pouches)	fas-l	49
Fast-Media® Base TB (500 pouches)	fas-l500	49
Fast-Media® Blas Agar (20 pouches)	fas-bl-s	49
Fast-Media® Blas TB (20 pouches)	fas-bl-l	49
Fast-Media® Blas XGal (20 pouches)	fas-bl-x	49
Fast-Media® Hygro Agar (20 pouches)	fas-hg-s	49
Fast-Media® Hygro TB (20 pouches)	fas-hg-l	49
Fast-Media® Hygro XGal (20 pouches)	fas-hg-x	49
Fast-Media® Kan Agar (30 pouches)	fas-kn-s	49
Fast-Media® Kan Agar (500 pouches)	fas-kn-s500	49
Fast-Media® Kan LB (30 pouches)	fas-kn-b	49
Fast-Media® Kan LB (500 pouches)	fas-kn-b500	49
Fast-Media® Kan TB (30 pouches)	fas-kn-l	49
Fast-Media® Kan TB (500 pouches)	fas-kn-l500	49
Fast-Media® Kan XGal (20 pouches)	fas-kn-x	49
Fast-Media® Puro Agar (20 pouches)	fas-pr-s	49
Fast-Media® Puro TB (20 pouches)	fas-pr-l	49
Fast-Media® Zeo Agar (20 pouches)	fas-zn-s	49
Fast-Media® Zeo TB (20 pouches)	fas-zn-l	49
Fast-Media® Zeo XGal (20 pouches)	fas-zn-x	49
Fast-Media® Zeo X-Gluc (10 pouches)	fas-zn-g	49
FK506 (10 mg)	tlrl-fk5	77
Flagellin FliC VacciGrade (50 µg)	vac-fla	99
Fungin™ (75 mg)	ant-fn-1	11
Fungin™ (200 mg)	ant-fn-2	11
G418 Sulfate (1 g)	ant-gn-1	13
G418 Sulfate (5 g)	ant-gn-5	13

PRODUCT (QUANTITY)	CAT. CODE	PAGE
Ganciclovir (250 mg)	sud-gcv	80
Gardiquimod VacciGrade (5 mg)	vac-gdq	99
Gefitinib (10 mg)	tlrl-gef	73
Geldanamycin (5 mg)	ant-gl-5	81
Geldanamycin (25 mg)	ant-gl-25	81
Glybenclamide (1 g)	tlrl-gly	73
Goat anti-human IgA - HRP (1 ml)	hrp-iga	93
Goat anti-human kappa - HRP (1 ml)	hrp-igak	93
Goat F(ab')2 anti-human IgA (0.5 mg)	fab-iga	93
Goat F(ab')2 anti-human IgA - Biotin (0.5 mg)	chiga-biot	91
Goat F(ab')2 anti-human IgA - FITC (0.5 mg)	chiga-fitc	91
Goat F(ab')2 anti-human kappa (0.5 mg)	fab-igak	93
Goat F(ab')2 IgG isotype control - FITC (100 tests)	cgig-fitc	91
H-89 (5 mg)	tlrl-h89	73
HEK-Blue™ Detection (5 pouches)	hb-det2	20
HEK-Blue™ Detection (10 pouches)	hb-det3	20
HEK-Blue™ Selection (5 x 2 ml)	hb-sel	9
Human IgA (0.5 mg)	ctrl-iga	93
Human IgA kappa (0.5 mg)	ctrl-igak	93
Human IgA2 Isotype Control (100 µg)	maba2-ctrl	95
HygroGold™ (1 g)	ant-hg-1	14
HygroGold™ (5 g)	ant-hg-5	14
HygroGold™ (10 g powder)	ant-hg-10p	14
Hygromycin B (1 g)	ant-hm-1	14
Hygromycin B (5 g)	ant-hm-5	14
IFA (10 ml)	vac-ifa-10	99
IFA (6 x 10 ml)	vac-ifa-60	99
IFNr qRT-Primers (kit)	rts-hifnr	65
Imiquimod VacciGrade (5 mg)	vac-imq	99
Jacalin / Agarose (2 ml)	gel-jac-2	92
Jacalin / Agarose (5 ml)	gel-jac-5	92
J Chain Antiserum (100 µg)	pab-jc	93
LacZ Cell Staining Kit	rep-lz-c	18
LacZ Tissue Staining Kit	rep-lz-t	18
LENTI-Smart™ (INT) (5 vials)	ltsint-5	37
LENTI-Smart™ (INT) (10 vials)	ltsint-10	37
LENTI-Smart™ NIL (5 vials)	ltsnil-5	38
LENTI-Smart™ NIL (10 vials)	ltsnil-10	38
LENTI-Smart™ Starter Kit (10 vials)	lts-str	38
Leptomycin B (5 µg)	tlrl-lep	80
LY294002 (5 mg)	tlrl-ly29	77
LyoComp GT115 (5 x 0.1 ml)	lyo-115-11	47
LyoComp GT115 (5 x 0.2 ml)	lyo-115-21	47
LyoComp GT116 (4 x 0.5 ml)	lyo-116-11	47
LyoComp GT116 (4 x 1 ml)	lyo-116-21	47
LyoVec™ (8 ml, 160 reactions)	lyec-12	16

PRODUCT INFORMATION

Alphabetical List by Product Name

PRODUCT (QUANTITY)	CAT. CODE	PAGE
LyoVec™ (18 ml, 360 reactions)	lyec-22	16
MAb-hDC-SIGN (100 µg)	mab-hdcsg	94
MAb-hMD2 (100 µg)	mab-hmd2	94
MAb-hMR (100 µg)	mab-hmr	94
MAb-hTLR1 (100 µg)	mab-htlr1	94
MAb-hTLR1-FITC (100 µg)	mab-htlr1f	95
MAb-hTLR2 (100 µg)	mab-htlr2	95
MAb-hTLR2-FITC (100 µg)	mab-htlr2f	95
MAb-hTLR3 (100 µg)	mab-htlr3	95
MAb-hTLR3-FITC (100 µg)	mab-htlr3f	95
MAb2-hTLR4 (100 µg)	mab2-htlr4	95
MAb-hTLR4 (100 µg)	mab-htlr4	95
MAb-hTLR4-FITC (100 µg)	mab-htlr4f	95
MAb-hTLR4/MD2 (100 µg)	mab-htlr4md2	95
MAb-hTNF-R1 (100 µg)	mab-htnfr1	95
MAb-mCD14 (100 µg)	mab-mcd14	94
MAb-mDectin-1 (100 µg)	mab-mdect	94
MAb-mTLR2 (100 µg)	mab-mtlr2	95
MAb-mTLR2-FITC (100 µg)	mab-mtlr2f	95
MAb-mTLR4/MD2 (100 µg)	mab-mtlr4md2	95
MAb-mTLR4/MD2-FITC (100 µg)	mab-mtlr4md2f	95
MAb-mTLR9 (100 µg)	mab-mtlr9	95
MAb-mTLR9-FITC (100 µg)	mab-mtlr9f	95
Metformin (1 mg)	tlrl-metf	77
MG-132 (5 mg)	tlrl-mg132	75
MPLA VacciGrade (1 mg)	vac-mpl	99
MPLAs VacciGrade (1 mg)	vac-mpls	99
Mouse IgG1 Isotype Control (100 µg)	mabg1-ctrlm	95
Mouse IgG2a Isotype Control (100 µg)	mabg2a-ctrlm	95
Mouse IgG2b Isotype Control (100 µg)	mabg2b-ctrlm	95
N-Glycolyl-MDP VacciGrade (5 mg)	vac-gmdp	99
Normocin™ (500 mg)	ant-nr-1	11
Normocin™ (1 g)	ant-nr-2	11
ODN 1585 VacciGrade (1 mg)	vac-1585-1	99
ODN 1826 VacciGrade (1 mg)	vac-1826-1	99
ODN 2006 VacciGrade (1 mg)	vac-2006-1	99
ODN 2395 VacciGrade (1 mg)	vac-2395-1	99
OVA 257-264 (1 mg)	vac-sin	101
OVA 323-339 (1 mg)	vac-isq	101
Ovalbumin (1 g)	vac-ova	101
Ovalbumin EndoFit™ (10 mg)	vac-efova	101
OxPAPC (1 mg)	tlrl-oxp1	74
PAb Control (200 µg)	pab-sctr	95
PAb-hTLR1 (200 µg)	pab-hstlr1	95
PAb-hTLR2 (200 µg)	pab-hstlr2	95
PAb-hTLR4 (200 µg)	pab-hstlr4	95

PRODUCT (QUANTITY)	CAT. CODE	PAGE
PAb-hTLR5 (200 µg)	pab-hstlr5	95
PAb-hTLR6 (200 µg)	pab-hstlr6	95
Pam3CSK4 VacciGrade (1 mg)	vac-pms	99
pBLAST-<Gene> (20 µg)	pbla-<gene>	40
pBLAST-mcs (20 µg)	pbla-mcs	40
pBOOST2-mcs (20 µg)	pbst2-mcs	102
pBOOST2-msaIRF3 (20 µg)	pbst2-samirf3	102
pBOOST2-msaIRF7/3 (20 µg)	pbst2-samirf73	102
pBOOST2-wtmIRF1 (20 µg)	pbst2-wtmirf1	102
pBOOST3-mTBK1 (20 µg)	pbst3-mtbk1	102
pBOOST3-mcs (20 µg)	pbst3-mcs	102
pCpGfree-basic (mSEAP) (20 µg)	pcpgf-bas	53
pCpGfree-basic-Lucia (20 µg)	pcpgf-baslc	53
pCpGfree-LacZ (20 µg)	pcpgf-lacz	52
pCpGfree-Lucia (20 µg)	pcpgf-lucia	52
pCpGfree-mcs (20 µg)	pcpgf-mcs	52
pCpGfree-mSEAP (20 µg)	pcpgf-mseap	52
pCpGfree-promoter (mSEAP) (20 µg)	pcpgf-prom	53
pCpGfree-promoter-Lucia (20 µg)	pcpgf-promlc	53
pCpGfree-siRNA (kit)	kcpgf-sirna	55
pCpGfree-siRNADUO (kit)	kcpg-sirna2	55
pCpGfree-vitroBLacZ (20 µg)	pcpgvtb-lz	54
pCpGfree-vitroBmcs (20 µg)	pcpgvtb-mcsg2	54
pCpGfree-vitroHLacZ (20 µg)	pcpgvth-lz	54
pCpGfree-vitroHmcs (20 µg)	pcpgvth-mcsg2	54
pCpGfree-vitroNLacZ (20 µg)	pcpgvtn-lz	54
pCpGfree-vitro-Nmcs (20 µg)	pcpgvtn-mcsg2	54
pCpGrich-mcs (20 µg)	pcpgr-mcs	52
PD0325901 (2 mg)	inh-pd32	75
PD98059 (10 mg)	tlrl-pd98	75
pDRIVE(LacZ)-<Native Prom> (*E.coli* disk)	pdrive-<prom>	41
pDRIVE(LacZ)-<Composite Prom> (*E.coli* disk)	pdrive-<prom>	41
pDRIVE-custom (20 µg)	p-custom	45
pDRIVE5-GFP-n (20 µg)	pdv5-gfp-n	44
pDRIVE5-SEAP-<Native Prom> (*E.coli* disk)	pdrive5s-<prom>	41
pDRIVE5-SEAP-<Composite Prom> (*E.coli* disk)	pdrive5s-<prom>	41
pDRIVE5-Lucia-<Native Prom> (*E.coli* disk)	pdrive5lc-<prom>	41
pDRIVE5-Lucia-<Composite Prom> (*E.coli* disk)	pdrive5lc-<prom>	41
Pepinh-Control (2 mg)	tlrl-pictrl	74
Pepinh-MYD (2 mg)	tlrl-pimyd	74
Pepinh-TRIF (2 mg)	tlrl-pitrif	74
Peptide M / Agarose (2 ml)	gel-pdm-2	92
Peptide M / Agarose (5 ml)	gel-pdm-5	92
Perifosine (5 mg)	tlrl-peri	77
pFUSE(ss)-CHIg-hA1 (20 µg)	pfuse(ss)-hcha1	88
pFUSE(ss)-CHIg-hA2m1 (20 µg)	pfuse(ss)-hcha2m1	88

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pFUSE(ss)-CHIg-hD (20 µg)	pfuse(ss)-hchd	88
pFUSE(ss)-CHIg-hE (20 µg)	pfuse(ss)-hche	88
pFUSE(ss)-CHIg-hG1 (20 µg)	pfuse(ss)-hchg1	88
pFUSE(ss)-CHIg-hG2 (20 µg)	pfuse(ss)-hchg2	88
pFUSE(ss)-CHIg-hG3 (20 µg)	pfuse(ss)-hchg301	88
pFUSE(ss)-CHIg-hG4 (20 µg)	pfuse(ss)-hchg4	88
pFUSE(ss)-CHIg-hM (20 µg)	pfuse(ss)-hchm	88
pFUSE(ss)-CHIg-mA (20 µg)	pfuse(ss)-mcha	88
pFUSE(ss)-CHIg-mD (20 µg)	pfuse(ss)-mchd	88
pFUSE(ss)-CHIg-mE (20 µg)	pfuse(ss)-mche	88
pFUSE(ss)-CHIg-mG1 (20 µg)	pfuse(ss)-mchg1	88
pFUSE(ss)-CHIg-mG2a (20 µg)	pfuse(ss)-mchg2a	88
pFUSE(ss)-CHIg-mG2b (20 µg)	pfuse(ss)-mchg2b	88
pFUSE(ss)-CHIg-mG3 (20 µg)	pfuse(ss)-mchg3	88
pFUSE(ss)-CHIg-mM (20 µg)	pfuse(ss)-mchm	88
pFUSE(ss)-CHIg-rG (20 µg)	pfuse(ss)-rchg	88
pFUSE2(ss)-CLIg-hk (20 µg)	pfuse2(ss)-hclk	88
pFUSE2(ss)-CLIg-hl2 (20 µg)	pfuse2(ss)-hcll2	88
pFUSE2(ss)-CLIg-mk (20 µg)	pfuse2(ss)-mclk	88
pFUSE2(ss)-CLIg-ml1 (20 µg)	pfuse2(ss)-mcll1	88
pFUSE2(ss)-CLIg-ml2 (20 µg)	pfuse2(ss)-mcll2	88
pFUSE2(ss)-CLIg-rk1 (20 µg)	pfuse2(ss)-rclk1	88
pFUSE2(ss)-CLIg-rk2 (20 µg)	pfuse2(ss)-rclk2	88
pFUSE-Lucia-CHIg-hG1 (20 µg)	pfuselc-hchg1	88
pFUSE-Lucia-CHIg-hG2 (20 µg)	pfuselc-hchg2	88
pFUSE-Lucia-CHIg-hG3 (20 µg)	pfuselc-hchg3	88
pFUSE-Lucia-CHIg-hG4 (20 µg)	pfuselc-hchg4	88
pFUSE-Lucia-CHIg-mG1 (20 µg)	pfuselc-mchg1	88
pFUSE-Lucia-CHIg-mG2a (20 µg)	pfuselc-mchg2a	88
pFUSE-Lucia-CHIg-mG2b (20 µg)	pfuselc-mchg2b	88
pFUSE-Lucia-CHIg-mG3 (20 µg)	pfuselc-mchg3	88
pFUSE-hIgG1-Fc(1/2) (20 µg)	pfuse-hg1fc(1/2)	28
pFUSE-hIgG2-Fc(1/2) (20 µg)	pfuse-hfc(1/2)	28
pFUSE-hIgG3-Fc(1/2) (20 µg)	pfuse-hg3fc(1/2)	28
pFUSE-hIgG4-Fc(1/2) (20 µg)	pfuse-hg4fc(1/2)	28
pFUSE-mIgG1-Fc(1/2) (20 µg)	pfuse-mg1fc(1/2)	28
pFUSE-mIgG2a-Fc(1/2) (20 µg)	pfuse-mg2afc(1/2)	28
pFUSE-mIgG2b-Fc(1/2) (20 µg)	pfuse-mg2bfc(1/2)	28
pFUSE-mIgG3-Fc(1/2) (20 µg)	pfuse-mg3fc(1/2)	28
pFUSE-rIgG-Fc(1/2) (20 µg)	pfuse-rfc(1/2)	28
pFUSE-rtIgG2b-Fc(1/2) (20 µg)	pfuse-rtg2bfc(1/2)	28
pFUSE-hIgG1e1-Fc(1/2) (20 µg)	pfc(1/2)-hg1e1	29
pFUSE-hIgG1e2-Fc(1/2) (20 µg)	pfc(1/2)-hg1e2	29
pFUSE-hIgG1e3-Fc(1/2) (20 µg)	pfc(1/2)-hg1e3	29
pFUSE-hIgG1e4-Fc(1/2) (20 µg)	pfc(1/2)-hg1e4	29
pFUSE-hIgG1e5-Fc(1/2) (20 µg)	pfc(1/2)-hg1e5	29

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pFUSE-hIgG1e6-Fc(1/2) (20 µg)	pfc(1/2)-hg1e6	29
pFUSE-hIgG1e7-Fc(1/2) (20 µg)	pfc(1/2)-hg1e7	29
pFUSE-hIgG1e9-Fc(1/2) (20 µg)	pfc(1/2)-hg1e9	29
pFUSE-hIgG2e1-Fc(1/2) (20 µg)	pfc(1/2)-hg2e1	29
pFUSE-hIgG4e1-Fc(1/2) (20 µg)	pfc(1/2)-hg4e1	29
pFUSE-mIgG2ae1-Fc(1/2) (20 µg)	pfc(1/2)-mg2ae1	29
pFUSE-Lucia-hG1Fc (20 µg)	pfuse-hg1lc	28
pFUSE-Lucia-mG2aFc (20 µg)	pfuse-mg2alc	28
pFUSE-SEAP-hG1Fc (20 µg)	pfuse-hg1sp	28
pFUSE-SEAP-hG2Fc (20 µg)	pfuse-hsp	28
pFUSE-SEAP-hG3Fc (20 µg)	pfuse-hg3sp	28
pFUSE-SEAP-hG4Fc (20 µg)	pfuse-hg4sp	28
pFUSE-SEAP-mG2aFc (20 µg)	pfuse-mg2asp	28
pFUSE-SEAP-mG2bFc (20 µg)	pfuse-mg2bsp	28
pFUSE-SEAP-mG3Fc (20 µg)	pfuse-mg3sp	28
pFUSE-SEAP-rFc (20 µg)	pfuse-rsp	28
pFUSE-SEAP-rtFc (20 µg)	pfuse-rtsp	28
Phleomycin (100 mg)	ant-ph-1	15
Phleomycin (500 mg)	ant-ph-5	15
Phleomycin (250 mg powder)	ant-ph-2p	15
Phleomycin (500 mg powder)	ant-ph-5p	15
Phleomycin (1 g powder)	ant-ph-10p	15
Piceatannol (5 mg)	tlrl-pct	74
pINFUSE-hIgG1-Fc(1/2) (20 µg)	pfc(1/2)-hgin1	28
pINFUSE-hIgG2-Fc(1/2) (20 µg)	pfc(1/2)-hgin2	28
pINFUSE-hIgG3-Fc(1/2) (20 µg)	pfc(1/2)-hgin3	28
pINFUSE-hIgG4-Fc(1/2) (20 µg)	pfc(1/2)-hgin4	28
pINFUSE-mIgG2b-Fc(1/2) (20 µg)	pfc(1/2)-mgin2	28
Plasmocin™ prophylactic (25 mg)	ant-mpp	10
Plasmocin™ treatment (50 mg)	ant-mpt	10
Plasmocure™ (100 mg)	ant-pc	10
PlasmoTest™ (kit)	rep-pt2	9
PlasmoTest™ Controls (200 tests)	pt-ctr2	9
PlasmoTest™ Reagent Kit (500 samples)	rep-ptrk	9
pMONO-blasti/GFP (20 µg)	pmonob-gfp	30
pMONO-blasti/mcs (20 µg)	pmonob-mcs	30
pMONO-hygro/GFP (20 µg)	pmonoh-gfp	30
pMONO-hygro/mcs (20 µg)	pmonoh-mcs	30
pMONO-neo/GFP (20 µg)	pmonon-gfp	30
pMONO-neo/mcs (20 µg)	pmonon-mcs	30
pMONO-zeo/GFP (20 µg)	pmonoz-gfp	30
pMONO-zeo/mcs (20 µg)	pmonoz-mcs	30
Poly(I:C) (HMW) VacciGrade (10 mg)	vac-pic	99
Polymyxin B (100 mg)	tlrl-pmb	74
pORF-<Gene> (*E.coli* disk)	porf-<gene>	40
pORF-mcs (*E.coli* disk)	porf-mcs	40

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Primocin™ (500 mg)	ant-pm-1	11
Primocin™ (1 g)	ant-pm-2	11
PromTest (10 x 5 µg)	prom-test	44
Protein L / Agarose (2 ml)	gel-protl-2	92
Protein L / Agarose (10 ml)	gel-protl-10	92
pSELECT-blasti/LacZ (20 µg)	psetb-lacz	30
pSELECT-blasti/mcs (20 µg)	psetb-mcs	30
pSELECT-CGFP-blasti (20 µg)	psetb-cgfp	31
pSELECT-CGFP-zeo (20 µg)	psetz-cgfp	31
pSELECT-CHA-blasti (20 µg)	psetb-cha	31
pSELECT-CHA-zeo (20 µg)	psetz-cha	31
pSELECT-CHis-blasti (20 µg)	psetb-chis	31
pSELECT-CHis-zeo (20 µg)	psetz-chis	31
pSELECT-GFPzeo-LacZ (20 µg)	psetgz-lacz	30
pSELECT-GFPzeo-mcs (20 µg)	psetgz-mcs	30
pSELECT-hygro-LacZ (20 µg)	pseth-lacz	30
pSELECT-hygro-mcs (20 µg)	pseth-mcs	30
pSELECT-neo-LacZ (20 µg)	psetn-lacz	30
pSELECT-neo-mcs (20 µg)	psetn-mcs	30
pSELECT-NGFP-blasti (20 µg)	psetb-ngfp	31
pSELECT-NGFP-zeo (20 µg)	psetz-ngfp	31
pSELECT-NHA-blasti (20 µg)	psetb-nha	31
pSELECT-NHA-zeo (20 µg)	psetz-nha	31
pSELECT-NHis-blasti (20 µg)	psetb-nhis	31
pSELECT-NHis-zeo (20 µg)	psetz-nhis	31
pSELECT-NLucia-blasti (20 µg)	psetb-nlucia	31
pSELECT-NLucia-zeo (20 µg)	psetz-nlucia	31
pSELECT-puro-LacZ (20 µg)	psetp-lacz	30
pSELECT-puro-mcs (20 µg)	psetp-mcs	30
pSELECT-zeo-<Gene> (20 µg)	psetz-<gene>	56
pSELECT-zeo-LacZ (20 µg)	psetz-lacz	18
pSELECT-zeo-Lucia (20 µg)	psetz-lucia	22
pSELECT-zeo-mcs (20 µg)	psetz-mcs	30
pSELECT-zeo-seap (20 µg)	psetz-seap	19
psiRNA-DUO Kit	ksirna4-gz3	63
psiRNA-h7SKblasti Kit	ksirna3-b21	62
psiRNA-h7SKGFPzeo Kit	ksirna4-gz21	62
psiRNA-h7SKhygro Kit	ksirna3-h21	62
psiRNA-h7SKneo Kit	ksirna3-n21	62
psiRNA-h7SKzeo Kit	ksirna3-z21	62
psiTEST Kit	ksitest	64
pUNO-<Gene> (*E.coli* disk)	puno-<gene>	40
pUNO-mcs (*E.coli* disk)	puno-mcs	40
Puromycin (100 mg)	ant-pr-1	14
Puromycin (500 mg)	ant-pr-5	14
pVAC1-mcs (20 µg)	pvac1	103

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pVAC2-mcs (20 µg)	pvac2	103
pVITRO1-blasti-GFP/LacZ (20 µg)	pvitro1-bgfplacz	33
pVITRO1-blasti-GFP/SEAP (20 µg)	pvitro1-bgfpsp	33
pVITRO1-blasti-Lucia/SEAP (20 µg)	pvitro1-blucsp	33
pVITRO1-blasti-mcs (20 µg)	pvitro1-bmcs	33
pVITRO1-hygro-GFP/LacZ (20 µg)	pvitro1-gfplacz	33
pVITRO1-hygro-GFP/SEAP (20 µg)	pvitro1-gfpsp	33
pVITRO1-hygro-Lucia/SEAP (20 µg)	pvitro1-lucsp	33
pVITRO1-hygro-mcs (20 µg)	pvitro1-mcs	33
pVITRO1-neo-GFP/LacZ (20 µg)	pvitro1-ngfplacz	33
pVITRO1-neo-GFP/SEAP (20 µg)	pvitro1-ngfpsp	33
pVITRO1-neo-Lucia/SEAP (20 µg)	pvitro1-nlucsp	33
pVITRO1-neo-mcs (20 µg)	pvitro1-nmcs	33
pVITRO2-blasti-GFP/LacZ (20 µg)	pvitro2-bgfplacz	33
pVITRO2-blasti-GFP/SEAP (20 µg)	pvitro2-bgfpsp	33
pVITRO2-blasti-Lucia/SEAP (20 µg)	pvitro2-blucsp	33
pVITRO2-blasti-mcs (20 µg)	pvitro2-bmcs	33
pVITRO2-hygro-GFP/LacZ (20 µg)	pvitro2-gfplacz	33
pVITRO2-hygro-GFP/SEAP (20 µg)	pvitro2-gfpsp	33
pVITRO2-hygro-Lucia/SEAP (20 µg)	pvitro2-lucsp	33
pVITRO2-hygro-mcs (20 µg)	pvitro2-mcs	33
pVITRO2-neo-GFP/LacZ (20 µg)	pvitro2-ngfplacz	33
pVITRO2-neo-GFP/SEAP (20 µg)	pvitro2-ngfpsp	33
pVITRO2-neo-Lucia/SEAP (20 µg)	pvitro2-nlucsp	33
pVITRO2-neo-mcs (20 µg)	pvitro2-nmcs	33
pVIVO1-GFP/LacZ (20 µg)	pvivo1-gfplacz	35
pVIVO1-GFP/SEAP (20 µg)	pvivo1-gfpsp	35
pVIVO1-Lucia/SEAP (20 µg)	pvivo1-lucsp	35
pVIVO1-mcs (20 µg)	pvivo1-mcs	35
pVIVO2-GFP/LacZ (20 µg)	pvivo2-gfplacz	35
pVIVO2-GFP/SEAP (20 µg)	pvivo2-gfpsp	35
pVIVO2-Lucia/SEAP (20 µg)	pvivo2-lucsp	35
pVIVO2-mcs (20 µg)	pvivo2-mcs	35
QUANTI-Blue™ (5 pouches)	rep-qb1	20
QUANTI-Blue™ (10 pouches)	rep-qb2	20
QUANTI-Luc™ (2 pouches)	rep-qlc1	23
QUANTI-Luc™ (5 pouches)	rep-qlc2	23
R848 VacciGrade (5 mg)	vac-r848	99
Rapamycin (5 mg)	tlrl-rap	77
Ready-Made psiRNA plasmid (20 µg)	psirna42-<gene>	67
Ready-Made psiRNA kit	ksirna42-<gene>	67
Recombinant Lucia® Protein (1 µg)	rec-lucia	22
Recombinant SEAP Protein (10 µg)	rec-hseap	19
Resveratrol (100 mg)	tlrl-resv	75
Ruxolitinib (5 mg)	tlrl-rux	78
SAHA (25 mg)	inh-saha	80

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SB202190 (5 mg)	tlrl-sb90	75
SB203580 (5 mg)	tlrl-sb20	76
SB431542 (5 mg)	inh-sb43	76
SEAP Reporter Assay Kit	rep-sap	19
SP600125 (10 mg)	tlrl-sp60	76
SSL7 / Agarose (2 ml)	gel-ssl-2	92
SSL7 / Agarose (10 ml)	gel-ssl-10	92
Tamoxifen (200 mg)	tlrl-txf	77
TDB VacciGrade (2 mg)	vac-tdb	99
Trichostatin A (1 mg)	met-tsa-1	80
Trichostatin A (5 mg)	met-tsa-5	80
Triptolide (1 mg)	ant-tpl	76
U0126 (5 mg)	tlrl-u0126	76
Valproic Acid (5 g)	inh-vpa	80
Wortmannin (5 mg)	tlrl-wtm	78
Zeocin™ (1 g)	ant-zn-1	15
Zeocin™ (1 g powder)	ant-zn-1p	15
Zeocin™ (5 g)	ant-zn-5	15
Zeocin™ (5 g, bottle)	ant-zn-5b	15
Zeocin™ (5 g powder)	ant-zn-5p	15
Z-VAD-FMK (1 mg)	tlrl-vad	74

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ab-hatag	**Anti-HA Tag** (250 µl)	94
ant-agl-5	**17-AAG** (5 mg)	81
ant-agl-25	**17-AAG** (25 mg)	81
ant-bgl-1	**Biotin-GA** (1 mg)	82
ant-bgl-5	**Biotin-GA** (5 mg)	82
ant-bl-1	**Blasticidin** (100 mg)	13
ant-bl-5	**Blasticidin** (500 mg)	13
ant-bl-5b	**Blasticidin** (500 mg, bottle)	13
ant-bl-10p	**Blasticidin** (1 g powder)	13
ant-cls	**Celastrol** (1 mg)	75
ant-dgl-5	**17-DMAG** (5 mg)	81
ant-dgl-25	**17-DMAG** (25 mg)	81
ant-egl-1	**17-AEP-GA** (1 mg)	81
ant-egl-5	**17-AEP-GA** (5 mg)	81
ant-gl-5	**Geldanamycin** (5 mg)	81
ant-gl-25	**Geldanamycin** (25 mg)	81
ant-hg-1	**HygroGold™** (1 g)	14
ant-hg-5	**HygroGold™** (5 g)	14
ant-hg-10p	**HygroGold™** (10 g powder)	14
ant-hm-1	**Hygromycin B** (1 g)	14
ant-hm-5	**Hygromycin B** (5 g)	14
ant-fn-1	**Fungin™** (75 mg)	11
ant-fn-2	**Fungin™** (200 mg)	11
ant-gn-1	**G418 Sulfate** (1 g)	13
ant-gn-5	**G418 Sulfate** (5 g)	13
ant-mgl-1	**17-DMAP-GA** (1 mg)	81
ant-mgl-5	**17-DMAP-GA** (5 mg)	81
ant-mpp	**Plasmocin™ prophylactic** (25 mg)	10
ant-mpt	**Plasmocin™ treatment** (50 mg)	10
ant-nhgl-1	**17-NHS-ALA-GA** (1 mg)	82
ant-nr-1	**Normocin™** (500 mg)	11
ant-nr-2	**Normocin™** (1 g)	11
ant-pc	**Plasmocure™** (100 mg)	10
ant-ph-1	**Phleomycin** (100 mg)	15
ant-ph-5	**Phleomycin** (500 mg)	15
ant-ph-2p	**Phleomycin** (250 mg powder)	15
ant-ph-5p	**Phleomycin** (500 mg powder)	15
ant-ph-10p	**Phleomycin** (1 g powder)	15
ant-pm-1	**Primocin™** (500 mg)	11
ant-pm-2	**Primocin™** (1 g)	11
ant-pr-1	**Puromycin** (100 mg)	14
ant-pr-5	**Puromycin** (500 mg)	14
ant-tpl	**Triptolide** (1 mg)	76
ant-zn-1	**Zeocin™** (1 g)	15
ant-zn-1p	**Zeocin™** (1 g powder)	15
ant-zn-5	**Zeocin™** (5 g)	15
ant-zn-5b	**Zeocin™** (5 g, bottle)	15
ant-zn-5p	**Zeocin™** (5 g powder)	15
chiga-biot	**Goat F(ab')2 anti-human IgA - Biotin** (0.5 mg)	91
chiga-fitc	**Goat F(ab')2 anti-human IgA - FITC** (0.5 mg)	91
cgig-fitc	**Goat F(ab')2 IgG isotype control - FITC** (100 tests)	91
ctrl-iga	**Human IgA** (0.5 mg)	93
ctrl-igak	**Human IgA kappa** (0.5 mg)	93
fab-iga	**Goat F(ab')2 anti-human IgA** (0.5 mg)	93
fab-igak	**Goat F(ab')2 anti-human kappa** (0.5 mg)	93
fas-am-s	**Fast-Media® Amp Agar** (30 pouches)	49
fas-am-s500	**Fast-Media® Amp Agar** (500 pouches)	49
fas-am-b	**Fast-Media® Amp LB** (30 pouches)	49
fas-am-b500	**Fast-Media® Amp LB** (500 pouches)	49
fas-am-l	**Fast-Media® Amp TB** (30 pouches)	49
fas-am-l500	**Fast-Media® Amp TB** (500 pouches)	49
fas-am-x	**Fast-Media® Amp XGal** (20 pouches)	49
fas-s	**Fast-Media® Base Agar** (30 pouches)	49
fas-s500	**Fast-Media® Base Agar** (500 pouches)	49
fas-l	**Fast-Media® Base TB** (30 pouches)	49
fas-l500	**Fast-Media® Base TB** (500 pouches)	49
fas-bl-s	**Fast-Media® Blas Agar** (20 pouches)	49
fas-bl-l	**Fast-Media® Blas TB** (20 pouches)	49
fas-bl-x	**Fast-Media® Blas XGal** (20 pouches)	49
fas-hg-s	**Fast-Media® Hygro Agar** (20 pouches)	49
fas-hg-l	**Fast-Media® Hygro TB** (20 pouches)	49
fas-hg-x	**Fast-Media® Hygro XGal** (20 pouches)	49
fas-kn-s	**Fast-Media® Kan Agar** (30 pouches)	49
fas-kn-s500	**Fast-Media® Kan Agar** (500 pouches)	49
fas-kn-b	**Fast-Media® Kan LB** (30 pouches)	49
fas-kn-b500	**Fast-Media® Kan LB** (500 pouches)	49
fas-kn-l	**Fast-Media® Kan TB** (30 pouches)	49
fas-kn-l500	**Fast-Media® Kan TB** (500 pouches)	49
fas-kn-x	**Fast-Media® Kan XGal** (20 pouches)	49
fas-pr-s	**Fast-Media® Puro Agar** (20 pouches)	49
fas-pr-l	**Fast-Media® Puro TB** (20 pouches)	49
fas-zn-s	**Fast-Media® Zeo Agar** (20 pouches)	49
fas-zn-l	**Fast-Media® Zeo TB** (20 pouches)	49
fas-zn-x	**Fast-Media® Zeo XGal** (20 pouches)	49
fas-zn-g	**Fast-Media® Zeo X-Gluc** (10 pouches)	49
gel-jac-2	**Jacalin / Agarose** (2 ml)	92
gel-jac-5	**Jacalin / Agarose** (5 ml)	92
gel-pdm-2	**Peptide M / Agarose** (2 ml)	92
gel-pdm-5	**Peptide M / Agarose** (5 ml)	92
gel-protl-2	**Protein L / Agarose** (2 ml)	92
gel-protl-10	**Protein L / Agarose** (10 ml)	92
gel-ssl-2	**SSL7 / Agarose** (2 ml)	92

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gel-ssl-10	**SSL7 / Agarose** (10 ml)	92
gmbapa-ga	**17-GMB-APA-GA** (1 mg)	82
gt115-11	**ChemiComp GT115** (5 x 0.1 ml)	47
gt115-21	**ChemiComp GT115** (5 x 0.2 ml)	47
gt116-11	**ChemiComp GT116** (5 x 0.1 ml)	47
gt116-21	**ChemiComp GT116** (5 x 0.2 ml)	47
hb-det2	**HEK-Blue™ Detection** (5 pouches)	20
hb-det3	**HEK-Blue™ Detection** (10 pouches)	20
hb-sel	**HEK-Blue™ Selection** (5 x 2 ml)	9
hcd20-mab1	**Anti-hCD20-hIgG1** (100 µg)	89
hcd20-mab2	**Anti-hCD20-hIgG2** (100 µg)	89
hcd20-mab3	**Anti-hCD20-hIgG3** (100 µg)	89
hcd20-mab4	**Anti-hCD20-hIgG4** (100 µg)	89
hcd20-mab5	**Anti-hCD20-hIgM** (100 µg)	89
hcd20-mab6	**Anti-hCD20-hIgA1** (100 µg)	89
hcd20-mab7	**Anti-hCD20-hIgA2** (100 µg)	89
hcd20-mab8	**Anti-hCD20-hIgE** (100 µg)	89
hcd20-mab9	**Anti-hCD20-mIgG1** (100 µg)	89
hcd20-mab10	**Anti-hCD20-mIgG2a** (100 µg)	89
hcd20-mab11	**Anti-hCD20-mIgA** (100 µg)	89
hrp-iga	**Goat anti-human IgA - HRP** (1 ml)	93
hrp-igak	**Goat anti-human kappa - HRP** (1 ml)	93
htnfa-mab1	**Anti-hTNF-α-hIgG1** (100 µg)	89
htnfa-mab2	**Anti-hTNF-α-hIgG2** (100 µg)	89
htnfa-mab3	**Anti-hTNF-α-hIgG3** (100 µg)	89
htnfa-mab4	**Anti-hTNF-α-hIgG4** (100 µg)	89
htnfa-mab5	**Anti-hTNF-α-hIgM** (100 µg)	89
htnfa-mab6	**Anti-hTNF-α-hIgA1** (100 µg)	89
htnfa-mab7	**Anti-hTNF-α-hIgA2** (100 µg)	89
htnfa-mab8	**Anti-hTNF-α-hIgE** (100 µg)	89
htnfa-mab9	**Anti-hTNF-α-mIgG1** (100 µg)	89
htnfa-mab10	**Anti-hTNF-α-mIgG2** (100 µg)	89
htnfa-mab11	**Anti-hTNF-α-mIgA** (100 µg)	89
inh-aza	**5-Aza-cytidine** (100 mg)	79
inh-bix	**Bix-01294** (2 mg)	79
inh-ci59	**CI-994** (10 mg)	79
inh-pd32	**PD0325901** (2 mg)	75
inh-saha	**SAHA** (25 mg)	80
inh-sb43	**SB431542** (5 mg)	76
inh-vpa	**Valproic Acid** (5 g)	80
k-custom	**Custom-made psiRNA kit**	69
kcpgf-sirna	**pCpGfree-siRNA** (kit)	55
kcpg-sirna2	**pCpGfree-siRNADUO** (kit)	55
ksirna4-gz3	**psiRNA-DUO Kit**	63
ksirna3-b21	**psiRNA-h7SKblasti Kit**	62
ksirna4-gz21	**psiRNA-h7SKGFPzeo Kit**	62
ksirna3-h21	**psiRNA-h7SKhygro Kit**	62
ksirna3-n21	**psiRNA-h7SKneo Kit**	62
ksirna3-z21	**psiRNA-h7SKzeo Kit**	62
ksirna42-<gene>	**Ready-Made psiRNA kit**	67
ksitest	**psiTEST Kit**	64
ltsint-5	**LENTI-Smart™ (INT)** (5 vials)	37
ltsint-10	**LENTI-Smart™ (INT)** (10 vials)	37
ltsnil-5	**LENTI-Smart™ NIL** (5 vials)	38
ltsnil-10	**LENTI-Smart™ NIL** (10 vials)	38
lts-str	**LENTI-Smart™ Starter Kit** (10 vials)	38
lyo-115-11	**LyoComp GT115** (5 x 0.1 ml)	47
lyo-115-21	**LyoComp GT115** (5 x 0.2 ml)	47
lyo-116-11	**LyoComp GT116** (4 x 0.5 ml)	47
lyo-116-21	**LyoComp GT116** (4 x 1 ml)	47
lyec-12	**LyoVec™** (8 ml, 160 reactions)	16
lyec-22	**LyoVec™** (18 ml, 360 reactions)	16
mab-hdcsg	**MAb-hDC-SIGN** (100 µg)	94
mab-hmd2	**MAb-hMD2** (100 µg)	94
mab-hmr	**MAb-hMR** (100 µg)	94
mab-htlr1	**MAb-hTLR1** (100 µg)	94
mab-htlr1f	**MAb-hTLR1-FITC** (100 µg)	95
mab-htlr2	**MAb-hTLR2** (100 µg)	95
mab-htlr2f	**MAb-hTLR2-FITC** (100 µg)	95
mab-htlr3	**MAb-hTLR3** (100 µg)	95
mab-htlr3f	**MAb-hTLR3-FITC** (100 µg)	95
mab-htlr4	**MAb-hTLR4** (100 µg)	95
mab-htlr4f	**MAb-hTLR4-FITC** (100 µg)	95
mab-htlr4md2	**MAb-hTLR4/MD2** (100 µg)	95
mab-htnfr1	**MAb-hTNF-R1** (100 µg)	95
mab-idtnf	**Anti-[anti-hTNF-α]** (100 µg)	89
mab-mcd14	**MAb-mCD14** (100 µg)	94
mab-mdect	**MAb-mDectin-1** (100 µg)	94
mab-mtlr2	**MAb-mTLR2** (100 µg)	95
mab-mtlr2f	**MAb-mTLR2-FITC** (100 µg)	95
mab-mtlr4md2	**MAb-mTLR4/MD2** (100 µg)	95
mab-mtlr4md2f	**MAb-mTLR4/MD2-FITC** (100 µg)	95
mab-mtlr9	**MAb-mTLR9** (100 µg)	95
mab-mtlr9f	**MAb-mTLR9-FITC** (100 µg)	95
mab2-htlr4	**MAb2-hTLR4** (100 µg)	95
maba-h40l	**Anti-hCD40L-IgA2** (100 µg)	94
maba-hcd14	**Anti-hCD14-IgA** (100 µg)	94
maba-hifna	**Anti-hIFN-α-IgA2** (100 µg)	94
maba-hifng	**Anti-hIFN-γ-IgA2** (100 µg)	94
maba-hil1b	**Anti-hIL-1β-IgA2** (100 µg)	94
maba-hil4	**Anti-hIL-4-IgA2** (100 µg)	94
maba-hil6	**Anti-hIL-6-IgA2** (100 µg)	94

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maba-hil13	**Anti-hIL-13-IgA2** (100 µg)	94
maba-hil18	**Anti-hIL-18-IgA2** (100 µg)	94
maba-htgfb	**Anti-hTGFβ-IgA2** (100 µg)	94
maba-htlr3	**Anti-hTLR3-IgA** (100 µg)	95
maba2-ctrl	**Human IgA2 Isotype Control** (100 µg)	95
maba2-htlr2	**Anti-hTLR2-IgA** (100 µg)	95
maba2-htlr5	**Anti-hTLR5-IgA** (100 µg)	95
mabg-flic	**Anti-Flagellin FliC** (100 µg)	94
mabg-hdect	**Anti-hDectin-1-IgG** (100 µg)	94
mabg-hil28	**Anti-hIL-28-IgG** (100 µg)	94
mabg-hmcl	**Anti-hMincle-IgG** (100 µg)	94
mabg-htlr1	**Anti-hTLR1-IgG** (100 µg)	94
mabg-htlr4	**Anti-hTLR4-IgG** (100 µg)	95
mabg-htlr6	**Anti-hTLR6-IgG** (100 µg)	95
mabg-lucia	**Anti-Lucia-IgG** (100 µg)	22
mabg-mmcl	**Anti-mMincle-IgG** (100 µg)	94
mabg-mtlr2	**Anti-mTLR2-IgG** (100 µg)	95
mabg-mtlr5	**Anti-mTLR5-IgG** (100 µg)	95
mabg1-ctrlm	**Mouse IgG1 Isotype Control** (100 µg)	95
mabg2a-ctrlm	**Mouse IgG2a Isotype Control** (100 µg)	95
mabg2b-ctrlm	**Mouse IgG2b Isotype Control** (100 µg)	95
met-adc-1	**5-Aza-2'-deoxycytidine** (10 mg)	79
met-adc-5	**5-Aza-2'-deoxycytidine** (50 mg)	79
met-tsa-1	**Trichostatin A** (1 mg)	80
met-tsa-5	**Trichostatin A** (5 mg)	80
p-custom	**Custom-made CpG-free Gene** (20 µg)	57
p-custom	**Custom-made pDRIVE** (20 µg)	45
p-custom	**Custom-made psiRNA** (20 µg)	69
p-custom	**Custom-made pSELECT** (20 µg)	106
p-custom	**DNA Standard Research Grade**	105
p-custom	**DNA Pre-Clinical Grade**	105
p-custom	**pDRIVE-custom** (20 µg)	45
pab-hstlr1	**PAb-hTLR1** (200 µg)	95
pab-hstlr2	**PAb-hTLR2** (200 µg)	95
pab-hstlr4	**PAb-hTLR4** (200 µg)	95
pab-hstlr5	**PAb-hTLR5** (200 µg)	95
pab-hstlr6	**PAb-hTLR6** (200 µg)	95
pab-jc	**J Chain Antiserum** (100 µg)	93
pab-sctr	**PAb Control** (200 µg)	95
pbla-<gene>	**pBLAST-<Gene>** (20 µg)	40
pbla-mcs	**pBLAST-mcs** (20 µg)	40
pbst2-mcs	**pBOOST2-mcs** (20 µg)	102
pbst2-samirf3	**pBOOST2-msaIRF3** (20 µg)	102
pbst2-samirf73	**pBOOST2-msaIRF7/3** (20 µg)	102
pbst2-wtmirf1	**pBOOST2-wtmIRF1** (20 µg)	102
pbst3-mcs	**pBOOST3-mcs** (20 µg)	102

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pbst3-mtbk1	**pBOOST3-mTBK1** (20 µg)	102
pcpgf-bas	**pCpGfree-basic (mSEAP)** (20 µg)	53
pcpgf-baslc	**pCpGfree-basic-Lucia** (20 µg)	53
pcpgf-lacz	**pCpGfree-LacZ** (20 µg)	52
pcpgf-lucia	**pCpGfree-Lucia** (20 µg)	52
pcpgf-mcs	**pCpGfree-mcs** (20 µg)	52
pcpgf-mseap	**pCpGfree-mSEAP** (20 µg)	52
pcpgf-prom	**pCpGfree-promoter (mSEAP)** (20 µg)	53
pcpgf-promlc	**pCpGfree-promoter-Lucia** (20 µg)	53
pcpgr-mcs	**pCpGrich-mcs** (20 µg)	52
pcpgvtb-lz	**pCpGfree-vitroBLacZ** (20 µg)	54
pcpgvtb-mcsg2	**pCpGfree-vitroBmcs** (20 µg)	54
pcpgvth-lz	**pCpGfree-vitroHLacZ** (20 µg)	54
pcpgvth-mcsg2	**pCpGfree-vitroHmcs** (20 µg)	54
pcpgvtn-lz	**pCpGfree-vitroNLacZ** (20 µg)	54
pcpgvtn-mcsg2	**pCpGfree-vitro-Nmcs** (20 µg)	54
pdrive-<prom>	**pDRIVE(LacZ)-<Native Prom>** (*E.coli* disk)	41
pdrive-<prom>	**pDRIVE(LacZ)-<Composite Prom>** (*E.coli* disk)	41
pdrive5lc-<prom>	**pDRIVE5-Lucia-<Native Prom>** (*E.coli* disk)	41
pdrive5lc-<prom>	**pDRIVE5-Lucia-<Composite Prom>** (*E.coli* disk)	41
pdrive5s-<prom>	**pDRIVE5-SEAP-<Native Prom>** (*E.coli* disk)	41
pdrive5s-<prom>	**pDRIVE5-SEAP-<Composite Prom>** (*E.coli* disk)	41
pdv5-gfp-n	**pDRIVE5-GFP-n** (20 µg)	44
pfc(1/2)-hg1e1	**pFUSE-hIgG1e1-Fc(1/2)** (20 µg)	29
pfc(1/2)-hg1e2	**pFUSE-hIgG1e2-Fc(1/2)** (20 µg)	29
pfc(1/2)-hg1e3	**pFUSE-hIgG1e3-Fc(1/2)** (20 µg)	29
pfc(1/2)-hg1e4	**pFUSE-hIgG1e4-Fc(1/2)** (20 µg)	29
pfc(1/2)-hg1e5	**pFUSE-hIgG1e5-Fc(1/2)** (20 µg)	29
pfc(1/2)-hg1e6	**pFUSE-hIgG1e6-Fc(1/2)** (20 µg)	29
pfc(1/2)-hg1e7	**pFUSE-hIgG1e7-Fc(1/2)** (20 µg)	29
pfc(1/2)-hg1e9	**pFUSE-hIgG1e9-Fc(1/2)** (20 µg)	29
pfc(1/2)-hg2e1	**pFUSE-hIgG2e1-Fc(1/2)** (20 µg)	29
pfc(1/2)-hg4e1	**pFUSE-hIgG4e1-Fc(1/2)** (20 µg)	29
pfc(1/2)-hgin1	**pINFUSE-hIgG1-Fc(1/2)** (20 µg)	28
pfc(1/2)-hgin2	**pINFUSE-hIgG2-Fc(1/2)** (20 µg)	28
pfc(1/2)-hgin3	**pINFUSE-hIgG3-Fc(1/2)** (20 µg)	28
pfc(1/2)-hgin4	**pINFUSE-hIgG4-Fc(1/2)** (20 µg)	28
pfc(1/2)-mg2ae1	**pFUSE-mIgG2ae1-Fc(1/2)** (20 µg)	29
pfc(1/2)-mgin2	**pINFUSE-mIgG2b-Fc(1/2)** (20 µg)	28
pfuse-hfc(1/2)	**pFUSE-hIgG2-Fc(1/2)** (20 µg)	28
pfuse-hg1fc(1/2)	**pFUSE-hIgG1-Fc(1/2)** (20 µg)	28
pfuse-hg1lc	**pFUSE-Lucia-hG1Fc** (20 µg)	28
pfuse-hg1sp	**pFUSE-SEAP-hG1Fc** (20 µg)	28
pfuse-hsp	**pFUSE-SEAP-hG2Fc** (20 µg)	28
pfuse-hg3fc(1/2)	**pFUSE-hIgG3-Fc(1/2)** (20 µg)	28
pfuse-hg3sp	**pFUSE-SEAP-hG3Fc** (20 µg)	28

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pfuse-hg4fc(1/2)	**pFUSE-hIgG4-Fc(1/2)** (20 µg)	28
pfuse-hg4sp	**pFUSE-SEAP-hG4Fc** (20 µg)	28
pfuse-mg1fc(1/2)	**pFUSE-mIgG1-Fc(1/2)** (20 µg)	28
pfuse-mg2afc(1/2)	**pFUSE-mIgG2a-Fc(1/2)** (20 µg)	28
pfuse-mg2alc	**pFUSE-Lucia-mG2aFc** (20 µg)	28
pfuse-mg2asp	**pFUSE-SEAP-mG2aFc** (20 µg)	28
pfuse-mg2bfc(1/2)	**pFUSE-mIgG2b-Fc(1/2)** (20 µg)	28
pfuse-mg2bsp	**pFUSE-SEAP-mG2bFc** (20 µg)	28
pfuse-mg3fc(1/2)	**pFUSE-mIgG3-Fc(1/2)** (20 µg)	28
pfuse-mg3sp	**pFUSE-SEAP-mG3Fc** (20 µg)	28
pfuse-rfc(1/2)	**pFUSE-rIgG-Fc(1/2)** (20 µg)	28
pfuse-rsp	**pFUSE-SEAP-rFc** (20 µg)	28
pfuse-rtg2bfc(1/2)	**pFUSE-rtIgG2b-Fc(1/2)** (20 µg)	28
pfuse-rtsp	**pFUSE-SEAP-rtFc** (20 µg)	28
pfuse2(ss)-hclk	**pFUSE2(ss)-CLIg-hk** (20 µg)	88
pfuse2(ss)-hcll2	**pFUSE2(ss)-CLIg-hl2** (20 µg)	88
pfuse2(ss)-mclk	**pFUSE2(ss)-CLIg-mk** (20 µg)	88
pfuse2(ss)-mcll1	**pFUSE2(ss)-CLIg-ml1** (20 µg)	88
pfuse2(ss)-mcll2	**pFUSE2(ss)-CLIg-ml2** (20 µg)	88
pfuse2(ss)-rclk1	**pFUSE2(ss)-CLIg-rk1** (20 µg)	88
pfuse2(ss)-rclk2	**pFUSE2(ss)-CLIg-rk2** (20 µg)	88
pfuselc-hchg1	**pFUSE-Lucia-CHIg-hG1** (20 µg)	88
pfuselc-hchg2	**pFUSE-Lucia-CHIg-hG2** (20 µg)	88
pfuselc-hchg3	**pFUSE-Lucia-CHIg-hG3** (20 µg)	88
pfuselc-hchg4	**pFUSE-Lucia-CHIg-hG4** (20 µg)	88
pfuselc-mchg1	**pFUSE-Lucia-CHIg-mG1** (20 µg)	88
pfuselc-mchg2a	**pFUSE-Lucia-CHIg-mG2a** (20 µg)	88
pfuselc-mchg2b	**pFUSE-Lucia-CHIg-mG2b** (20 µg)	88
pfuselc-mchg3	**pFUSE-Lucia-CHIg-mG3** (20 µg)	88
pfuse(ss)-hcha1	**pFUSE(ss)-CHIg-hA1** (20 µg)	88
pfuse(ss)-hcha2m1	**pFUSE(ss)-CHIg-hA2m1** (20 µg)	88
pfuse(ss)-hchd	**pFUSE(ss)-CHIg-hD** (20 µg)	88
pfuse(ss)-hche	**pFUSE(ss)-CHIg-hE** (20 µg)	88
pfuse(ss)-hchg1	**pFUSE(ss)-CHIg-hG1** (20 µg)	88
pfuse(ss)-hchg2	**pFUSE(ss)-CHIg-hG2** (20 µg)	88
pfuse(ss)-hchg301	**pFUSE(ss)-CHIg-hG3** (20 µg)	88
pfuse(ss)-hchg4	**pFUSE(ss)-CHIg-hG4** (20 µg)	88
pfuse(ss)-hchm	**pFUSE(ss)-CHIg-hM** (20 µg)	88
pfuse(ss)-mcha	**pFUSE(ss)-CHIg-mA** (20 µg)	88
pfuse(ss)-mchd	**pFUSE(ss)-CHIg-mD** (20 µg)	88
pfuse(ss)-mche	**pFUSE(ss)-CHIg-mE** (20 µg)	88
pfuse(ss)-mchg1	**pFUSE(ss)-CHIg-mG1** (20 µg)	88
pfuse(ss)-mchg2a	**pFUSE(ss)-CHIg-mG2a** (20 µg)	88
pfuse(ss)-mchg2b	**pFUSE(ss)-CHIg-mG2b** (20 µg)	88
pfuse(ss)-mchg3	**pFUSE(ss)-CHIg-mG3** (20 µg)	88
pfuse(ss)-mchm	**pFUSE(ss)-CHIg-mM** (20 µg)	88

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pfuse(ss)-rchg	**pFUSE(ss)-CHIg-rG** (20 µg)	88
pmonob-gfp	**pMONO-blasti/GFP** (20 µg)	30
pmonob-mcs	**pMONO-blasti/mcs** (20 µg)	30
pmonoh-gfp	**pMONO-hygro/GFP** (20 µg)	30
pmonoh-mcs	**pMONO-hygro/mcs** (20 µg)	30
pmonon-gfp	**pMONO-neo/GFP** (20 µg)	30
pmonon-mcs	**pMONO-neo/mcs** (20 µg)	30
pmonoz-gfp	**pMONO-zeo/GFP** (20 µg)	30
pmonoz-mcs	**pMONO-zeo/mcs** (20 µg)	30
porf-<gene>	**pORF-<Gene>** (*E.coli* disk)	40
porf-mcs	**pORF-mcs** (*E.coli* disk)	40
prom-test	**PromTest** (10 x 5 µg)	44
psetb-cgfp	**pSELECT-CGFP-blasti** (20 µg)	31
psetb-cha	**pSELECT-CHA-blasti** (20 µg)	31
psetb-chis	**pSELECT-CHis-blasti** (20 µg)	31
psetb-lacz	**pSELECT-blasti/LacZ** (20 µg)	30
psetb-mcs	**pSELECT-blasti/mcs** (20 µg)	30
psetb-ngfp	**pSELECT-NGFP-blasti** (20 µg)	31
psetb-nha	**pSELECT-NHA-blasti** (20 µg)	31
psetb-nhis	**pSELECT-NHis-blasti** (20 µg)	31
psetb-nlucia	**pSELECT-NLucia-blasti** (20 µg)	31
psetgz-lacz	**pSELECT-GFPzeo-LacZ** (20 µg)	30
psetgz-mcs	**pSELECT-GFPzeo-mcs** (20 µg)	30
pseth-lacz	**pSELECT-hygro-LacZ** (20 µg)	30
pseth-mcs	**pSELECT-hygro-mcs** (20 µg)	30
psetn-lacz	**pSELECT-neo-LacZ** (20 µg)	30
psetn-mcs	**pSELECT-neo-mcs** (20 µg)	30
psetp-lacz	**pSELECT-puro-LacZ** (20 µg)	30
psetp-mcs	**pSELECT-puro-mcs** (20 µg)	30
psetz-<gene>	**pSELECT-zeo-<Gene>** (20 µg)	56
psetz-cgfp	**pSELECT-CGFP-zeo** (20 µg)	31
psetz-cha	**pSELECT-CHA-zeo** (20 µg)	31
psetz-chis	**pSELECT-CHis-zeo** (20 µg)	31
psetz-lacz	**pSELECT-zeo-LacZ** (20 µg)	18
psetz-lucia	**pSELECT-zeo-Lucia** (20 µg)	22
psetz-mcs	**pSELECT-zeo-mcs** (20 µg)	30
psetz-ngfp	**pSELECT-NGFP-zeo** (20 µg)	31
psetz-nha	**pSELECT-NHA-zeo** (20 µg)	31
psetz-nhis	**pSELECT-NHis-zeo** (20 µg)	31
psetz-nlucia	**pSELECT-NLucia-zeo** (20 µg)	31
psetz-seap	**pSELECT-zeo-seap** (20 µg)	19
psirna42-<gene>	**Ready-Made psiRNA plasmid** (20 µg)	67
pt-ctr2	**PlasmoTest™ Controls** (200 tests)	9
puno-<gene>	**pUNO-<Gene>** (*E.coli* disk)	40
puno-mcs	**pUNO-mcs** (*E.coli* disk)	40
pvac1	**pVAC1-mcs** (20 µg)	103

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pvac2	**pVAC2-mcs** (20 µg)	103
pvitro1-bgfplacz	**pVITRO1-blasti-GFP/LacZ** (20 µg)	33
pvitro1-bgfpsp	**pVITRO1-blasti-GFP/SEAP** (20 µg)	33
pvitro1-blucsp	**pVITRO1-blasti-Lucia/SEAP** (20 µg)	33
pvitro1-bmcs	**pVITRO1-blasti-mcs** (20 µg)	33
pvitro1-gfplacz	**pVITRO1-hygro-GFP/LacZ** (20 µg)	33
pvitro1-gfpsp	**pVITRO1-hygro-GFP/SEAP** (20 µg)	33
pvitro1-lucsp	**pVITRO1-hygro-Lucia/SEAP** (20 µg)	33
pvitro1-mcs	**pVITRO1-hygro-mcs** (20 µg)	33
pvitro1-ngfplacz	**pVITRO1-neo-GFP/LacZ** (20 µg)	33
pvitro1-ngfpsp	**pVITRO1-neo-GFP/SEAP** (20 µg)	33
pvitro1-nlucsp	**pVITRO1-neo-Lucia/SEAP** (20 µg)	33
pvitro1-nmcs	**pVITRO1-neo-mcs** (20 µg)	33
pvitro2-bgfplacz	**pVITRO2-blasti-GFP/LacZ** (20 µg)	33
pvitro2-bgfpsp	**pVITRO2-blasti-GFP/SEAP** (20 µg)	33
pvitro2-blucsp	**pVITRO2-blasti-Lucia/SEAP** (20 µg)	33
pvitro2-bmcs	**pVITRO2-blasti-mcs** (20 µg)	33
pvitro2-gfplacz	**pVITRO2-hygro-GFP/LacZ** (20 µg)	33
pvitro2-gfpsp	**pVITRO2-hygro-GFP/SEAP** (20 µg)	33
pvitro2-lucsp	**pVITRO2-hygro-Lucia/SEAP** (20 µg)	33
pvitro2-mcs	**pVITRO2-hygro-mcs** (20 µg)	33
pvitro2-ngfplacz	**pVITRO2-neo-GFP/LacZ** (20 µg)	33
pvitro2-ngfpsp	**pVITRO2-neo-GFP/SEAP** (20 µg)	33
pvitro2-nlucsp	**pVITRO2-neo-Lucia/SEAP** (20 µg)	33
pvitro2-nmcs	**pVITRO2-neo-mcs** (20 µg)	33
pvivo1-gfplacz	**pVIVO1-GFP/LacZ** (20 µg)	35
pvivo1-gfpsp	**pVIVO1-GFP/SEAP** (20 µg)	35
pvivo1-lucsp	**pVIVO1-Lucia/SEAP** (20 µg)	35
pvivo1-mcs	**pVIVO1-mcs** (20 µg)	35
pvivo2-gfplacz	**pVIVO2-GFP/LacZ** (20 µg)	35
pvivo2-gfpsp	**pVIVO2-GFP/SEAP** (20 µg)	35
pvivo2-lucsp	**pVIVO2-Lucia/SEAP** (20 µg)	35
pvivo2-mcs	**pVIVO2-mcs** (20 µg)	35
rec-hseap	**Recombinant SEAP Protein** (10 µg)	19
rec-lucia	**Recombinant Lucia® Protein** (1 µg)	22
rep-lz-c	**LacZ Cell Staining Kit**	18
rep-lz-t	**LacZ Tissue Staining Kit**	18
rep-pt2	**PlasmoTest™** (kit)	9
rep-ptrk	**PlasmoTest™ Reagent Kit** (500 samples)	9
rep-qb1	**QUANTI-Blue™** (5 pouches)	20
rep-qb2	**QUANTI-Blue™** (10 pouches)	20
rep-qlc1	**QUANTI-Luc™** (2 pouches)	23
rep-qlc2	**QUANTI-Luc™** (5 pouches)	23
rep-sap	**SEAP Reporter Assay Kit**	19
rts-hifnr	**IFNr qRT-Primers** (kit)	65
sud-5fc	**5-Fluorocytosine** (250 mg)	79
sud-5fu	**5-Fluorouracil** (250 mg)	79
sud-gcv	**Ganciclovir** (250 mg)	80
tlrl-3ma	**3-Methyladenine** (50 mg)	77
tlrl-ag4	**AG490** (10 mg)	78
tlrl-apr	**2-Aminopurine** (250 mg)	73
tlrl-b82	**Bay11-7082** (10 mg)	75
tlrl-baf	**Bafilomycin A1** (10 µg)	73
tlrl-bx7	**BX795** (5 mg)	73
tlrl-chq	**Chloroquine** (250 mg)	73
tlrl-cli95	**CLI-095** (1 mg)	73
tlrl-cp69	**CP-690550** (5 mg)	78
tlrl-cyca	**Cyclosporin A** (100 mg)	77
tlrl-dex	**Dexamethasone** (100 mg)	75
tlrl-eve	**Everolimus** (5 mg)	77
tlrl-fk5	**FK506** (10 mg)	77
tlrl-gef	**Gefitinib** (10 mg)	73
tlrl-gly	**Glybenclamide** (1 g)	73
tlrl-h89	**H-89** (5 mg)	73
tlrl-lep	**Leptomycin B** (5 µg)	80
tlrl-ly29	**LY294002** (5 mg)	77
tlrl-metf	**Metformin** (1 mg)	77
tlrl-mg132	**MG-132** (5 mg)	75
tlrl-oxp1	**OxPAPC** (1 mg)	74
tlrl-pct	**Piceatannol** (5 mg)	74
tlrl-pd98	**PD98059** (10 mg)	75
tlrl-peri	**Perifosine** (5 mg)	77
tlrl-pictrl	**Pepinh-Control** (2 mg)	74
tlrl-pimyd	**Pepinh-MYD** (2 mg)	74
tlrl-pitrif	**Pepinh-TRIF** (2 mg)	74
tlrl-pmb	**Polymyxin B** (100 mg)	74
tlrl-rap	**Rapamycin** (5 mg)	77
tlrl-resv	**Resveratrol** (100 mg)	75
tlrl-rux	**Ruxolitinib** (5 mg)	78
tlrl-sb20	**SB203580** (5 mg)	76
tlrl-sb90	**SB202190** (5 mg)	75
tlrl-sp60	**SP600125** (10 mg)	76
tlrl-txf	**Tamoxifen** (200 mg)	77
tlrl-u0126	**U0126** (5 mg)	76
tlrl-vad	**Z-VAD-FMK** (1 mg)	74
tlrl-wtm	**Wortmannin** (5 mg)	78
vac-1585-1	**ODN 1585 VacciGrade** (1 mg)	99
vac-1826-1	**ODN 1826 VacciGrade** (1 mg)	99
vac-2006-1	**ODN 2006 VacciGrade** (1 mg)	99
vac-2395-1	**ODN 2395 VacciGrade** (1 mg)	99
vac-adx-2	**AddaVax™** (2 ml)	99
vac-adx-10	**AddaVax™** (10 ml)	99

Alphabetical List by Catalog Code

CAT. CODE	PRODUCT (QUANTITY)	PAGE
vac-alu-50	**Alhydrogel 2%** (50 ml)	99
vac-alu-250	**Alhydrogel 2%** (250 ml)	99
vac-cda	**c-di-AMP VacciGrade** (1 mg)	99
vac-cdg	**c-di-GMP VacciGrade** (1 mg)	99
vac-efova	**EndoFit™ Ovalbumin** (10 mg)	101
vac-fla	**Flagellin FliC VacciGrade** (50 µg)	99
vac-gdq	**Gardiquimod VacciGrade** (5 mg)	99
vac-gmdp	**N-Glycolyl-MDP VacciGrade** (5 mg)	99
vac-ifa-10	**IFA** (10 ml)	99
vac-ifa-60	**IFA** (6 x 10 ml)	99
vac-imq	**Imiquimod VacciGrade** (5 mg)	99
vac-isq	**OVA 323-339** (1 mg)	101
vac-mpl	**MPLA VacciGrade** (1 mg)	99
vac-mpls	**MPLAs VacciGrade** (1 mg)	99
vac-ova	**Ovalbumin** (1 g)	101
vac-pic	**Poly(I:C) (HMW) VacciGrade** (10 mg)	99
vac-pms	**Pam3CSK4 VacciGrade** (1 mg)	99
vac-r848	**R848 VacciGrade** (5 mg)	99
vac-sin	**OVA 257-264** (1 mg)	101
vac-tdb	**TDB VacciGrade** (2 mg)	99

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Cover image: The colorful continuum of hexagonal patterning to signify the building blocks of creation

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