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InvivoGen

2012 - 2013

CATALOG I

Innate Immunity

Cover image: Glowing fiber optics, a metaphor to conceptualize the signaling transduction elements of the catalog

INNOVATION WITHIN REACH

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Catalog 2: Mammalian Cell Expression 2012-2013

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INNATE IMMUNITY

The innate immune system is an evolutionarily conserved system acting as a first-line of defense against invading microbial pathogens and other potential threats to the host. A range of pattern recognition receptors (PRRs) recognize specific pathogen-associated molecular patterns (PAMPs) exclusively present on microbes such as viruses, bacteria, parasites and fungi. In addition, PRRs are involved in sensing endogenous 'danger' signals by recognizing danger-associated molecular patterns (DAMPs). The recognition of PAMPs or DAMPs by the PRRs triggers an inflammatory response. Innate inflammatory responses include the secretion of cytokines/chemokines, the induction of antimicrobial peptides, pyroptotic cell death and the recruitment of phagocytic cells. Exquisite coordination of the multiple innate immune pathways is crucial to the efficient destruction and clearance of invading pathogens and other molecular threats. Importantly, the innate immune system not only precedes but empowers the highly specialized adaptive immune system to confer long-lasting immunological memory. The main PRRs families of the innate immune system are the Toll-Like receptors (TLRs), the NOD-Like receptors (NLRs), the RIG-I-Like receptors (RLRs), cytosolic DNA sensors (CDS), the C-type lectin receptors (CLRs), and also playing central roles are inflammasomes and autophagy.

Toll-Like Receptors (TLRs)

TLRs are the first identified and the best characterized receptors among the signaling PRRs. Signaling by these receptors initiate key inflammatory responses and also shape adaptive immunity. All TLRs (10 in humans and 11 in mice) are type I transmembrane proteins characterized by an extracellular leucine-rich domain and a cytoplasmic tail that contains a conserved Toll/IL-1 receptor (TIR) domain. TLRs recognize a variety of PAMPs from bacteria, fungi, parasites, and viruses, including lipid-based bacterial cell wall components such as lipopolysaccharide (LPS) and lipopeptides, microbial protein components such as flagellin, and nucleic acids such as single-stranded or double-stranded RNA and CpG DNA. TLR ligands include host endogenous DAMPs liberated from damaged tissues and cells. TLRs initiate shared and distinct signaling pathways by recruiting different combinations of four TIR-domain-containing adaptor molecules: MyD88, TIRAP (Mal), TRIF and TRAM. These signaling pathways activate the transcription factors NF-κB and AP-1 leading to the production of inflammatory cytokines and chemokines. They also activate interferon regulatory factors leading to the production of type I interferons.

Nod-Like Receptors (NLRs)

NLRs constitute a recently identified family of intracellular pattern recognition receptors (PRRs), which contains more than 20 members in mammals. Although the ligands and functions of many of these receptors are not known, their primary role is to recognize cytoplasmic pathogen-associated molecular patterns (PAMPs) and/or endogenous danger signals, inducing immune responses. NLRs are characterized by a tripartite-domain organization with a conserved nucleotide binding oligomerization domain (NACHT/NOD), leucine-rich repeats (LRRs) involved in microbial sensing and an N-terminal effector region comprising a protein-protein interaction domain such as the CARD, pyrin or BIR domain. A standardization in nomenclature of NLRs is based on the effector domain, and gives rise to 5 subfamilies; NLRA, NLRB, NLRC, NLRP and NLRX. NLRCs include NODs and NLRC4 (or IPAF) and contain CARD effector domains, whereas NLRPs (or NALPs) contain pyrin effector domains and NLRBs are the NAIPs containing BIR domains.

RIG-I-Like Receptors (RLRs) and Cytosolic DNA Sensors (CDS)

RLRs constitute a family of cytoplasmic RNA helicases that are critical for host antiviral responses. The RLRs include the RNA sensors RIG-I, MDA-5 and LGP2, which when activated lead to the activation of transcription factors that also control the transcription of genes encoding interferons and other cytokines. RIG-I and MDA-5 sense double-stranded RNA, a replication intermediate for RNA viruses, leading to production of type I interferons in infected cells. LGP2 contains a RNA binding domain and acts as a negative feedback regulator of RIG-I and MDA-5. Recent advances in the recognition of nucleic acids have identified a family of cytosolic DNA sensors. Among some of the known DNA sensors are DAI, LRRFIP1, AIM2, IFI16 and the negative regulator p202. Cytosolic DNA sensors detect exogenous double stranded DNA leading to the induction of interferons and/or the processing of pro-inflammatory cytokines.

C-Type Lectin Receptors (CLRs)

CLRs, also called the C-type lectins, encompass a large family of proteins that act as phagocytic receptors that bind carbohydrate moieties of various pathogens. The significance of the CLRs in shaping the adaptive immune response is becoming apparent. The lectin activity of these receptors is mediated by conserved carbohydrate-recognition domains (CRDs). These receptors are involved in fungal recognition and the modulation of the innate immune response for the clearance of microbes and antigen presentation to T lymphocytes. CLRs include Dectin-1, Dectin-2, Mincle, DC-SIGN, DNGR-1 and the soluble MBL.

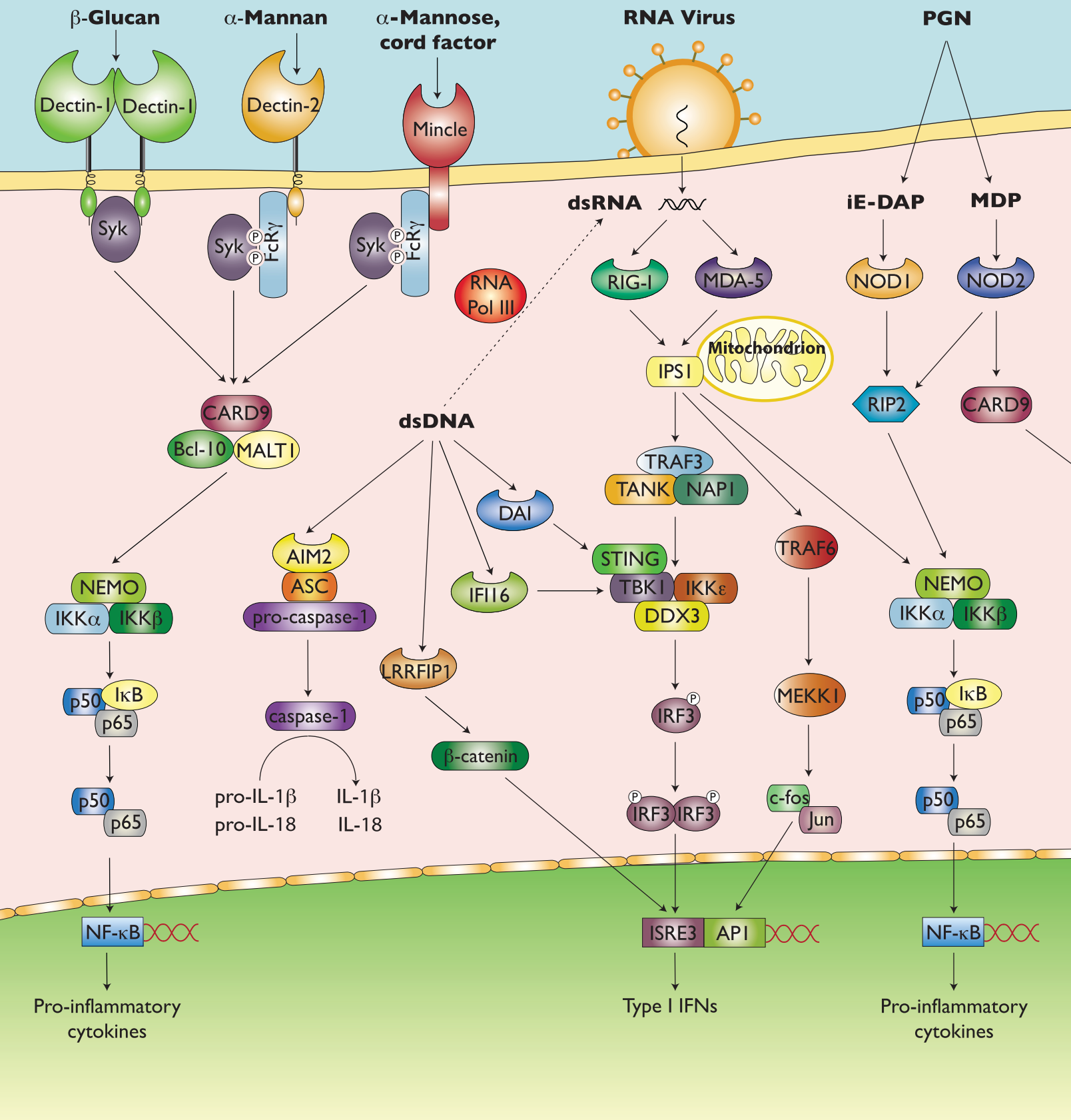
Inflammasomes

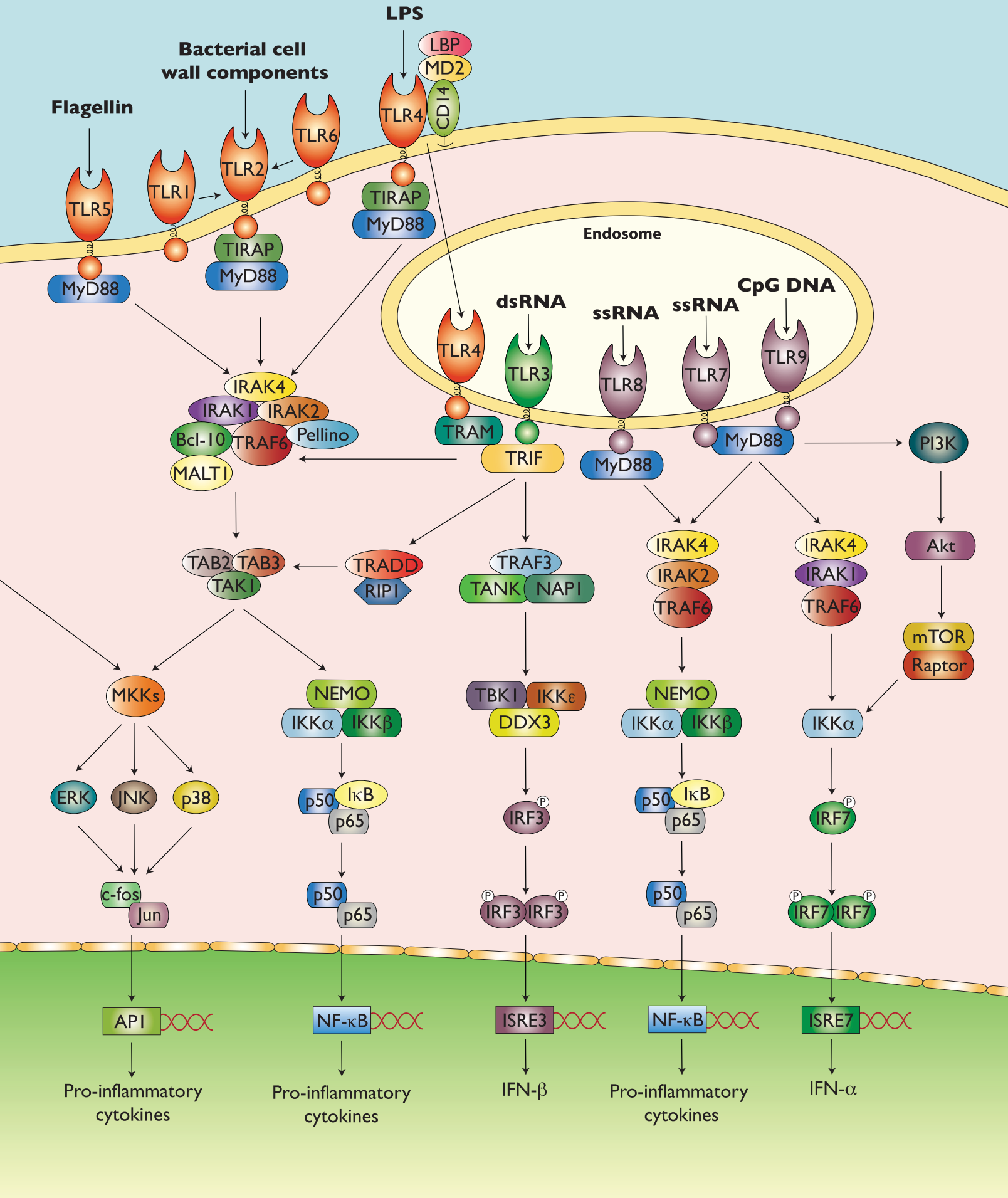
Inflammasomes are multiproteins caspase-1-activating complexes assembled by certain NLRs. Caspase-1 is activated by inflammasomes through autoproteolytic maturation, leading to the processing and secretion of the pro-inflammatory cytokines. Four inflammasomes have been identified and are defined by the NLR protein that they contain; the NLRP1/NALP1b inflammasome, the NLRC4/IPAF inflammasome, the NLRP3/NALP3 inflammasome, and the AIM2 containing inflammasome. Inflammasomes fulfill a central role in innate immunity by detecting and responding to bacterial components, danger signals and potentially dangerous cytoplasmic DNA.

Autophagy and Innate Immunity

Autophagy pathways function to eliminate unwanted constituents from the cells such as intracellular pathogens, damaged organelles and long-lived, aggregate-prone proteins, and to recycle cytoplasmic material to maintain cellular homeostasis. Autophagy has been shown to interact with PRRs, such as the TLRs, NLRs and RLRs to regulate inflammation. Recent advances indicate an essential role of autophagy in innate and adaptive immune responses.

PRR Signaling Pathways




LPS
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CD14
TLR4
Bacterial cell wall components
Flagellin
TLR6
TLR2
TLR1
TLR5
TIRAP
MyD88
TIRAP
MyD88
MyD88
Endosome
dsRNA
ssRNA
ssRNA
CpG DNA
TLR4
TLR3
TLR8
TLR7
TLR9
IRAK4
IRAK1
IRAK2
Bcl-10
TRAF6
Pellino
MALT1
TRAM
TRIF
MyD88
MyD88
PI3K
Akt
mTOR
Raptor
TAB2
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TAK1
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RIP1
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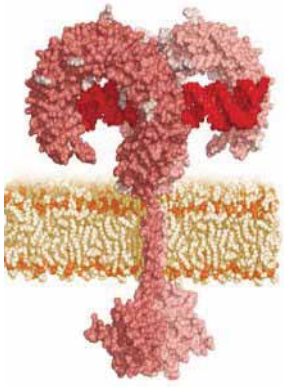
IRF7
c-fos
Jun
p50
p65
IRF3
IRF3
p50
p65
IRF7
IRF7
AP1
NF-κB
ISRE3
NF-κB
ISRE7
Pro-inflammatory cytokines
Pro-inflammatory cytokines
IFN-β
Pro-inflammatory cytokines
IFN-α

TOLL-LIKE RECEPTORS

Toll-Like Receptors (TLRs) play a critical role in the early innate immune response to invading pathogens by sensing microorganism and are involved in sensing endogenous danger signals. TLRs are evolutionarily conserved receptors are homologues of the Drosophila Toll protein, discovered to be important for defense against microbial infection[1]. TLRs recognize highly conserved structural motifs known as pathogen-associated microbial patterns (PAMPs), which are exclusively expressed by microbial pathogens, or danger-associated molecular patterns (DAMPs) that are endogenous molecules released from necrotic or dying cells. PAMPs include various bacterial cell wall components such as lipopolysaccharide (LPS), peptidoglycan (PGN) and lipopeptides, as well as flagellin, bacterial DNA and viral double-stranded RNA. DAMPs include intracellular proteins such as heat shock proteins as well as protein fragments from the extracellular matrix. Stimulation of TLRs by the corresponding PAMPs or DAMPs initiates signaling cascades leading to the activation of transcription factors, such as AP-1, NF-κB and interferon regulatory factors (IRFs). Signaling by TLRs result in a variety of cellular responses including the production of interferons (IFNs), pro-inflammatory cytokines and effector cytokines that direct the adaptive immune response.

The TLR Family

TLRs are type I transmembrane proteins characterized by an extracellular domain containing leucine-rich repeats (LRRs) and a cytoplasmic tail that contains a conserved region called the Toll/IL-1 receptor (TIR) domain. The structure of the extracellular domain of TLR3 was recently revealed by crystallography studies as a large horseshoe-shape[2]. TLRs are predominantly expressed in tissues involved in immune function, such as spleen and peripheral blood leukocytes, as well as those exposed to the external environment such as lung and the gastrointestinal tract. Their expression profiles vary among tissues and cell types. TLRs are located on the plasma membrane with the exception of TLR3, TLR7, TLR9 which are localized in the endosomal compartment[3].



Ten human and twelve murine TLRs have been characterized, TLR1 to TLR10 in humans, and TLR1 to TLR9, TLR11, TLR12 and TLR13 in mice, the homolog of TLR10 being a pseudogene. TLR2 is essential for the recognition of a variety of PAMPs from Gram-positive bacteria, including bacterial lipoproteins, lipomannans and lipoteichoic acids. TLR3 is implicated in virus-derived double-stranded RNA. TLR4 is predominantly activated by lipopolysaccharide. TLR5 detects bacterial flagellin and TLR9 is required for response to unmethylated CpG DNA. Finally, TLR7 and TLR8 recognize small synthetic antiviral molecules[4], and recently single-stranded RNA was reported to be their natural ligand[5]. TLR11(12) has been reported to recognize uropathogenic *E. coli*[6] and a profilin-like protein from *Toxoplasma gondii*[7].

The repertoire of specificities of the TLRs is apparently extended by the ability of TLRs to heterodimerize with one another. For example, dimers of TLR2 and TLR6 are required for responses to diacylated lipoproteins while TLR2 and TLR1 interact to recognize triacylated lipoproteins[8]. Specificities of the TLRs are also influenced by various adapter and accessory molecules, such as MD-2 and CD14 that form a complex with TLR4 in response to LPS[9].

TLR Signaling (see pathway previous page)

TLR signaling consists of at least two distinct pathways: a MyD88-dependent pathway that leads to the production of inflammatory cytokines, and a MyD88-independent pathway associated with the stimulation of IFN-β and the maturation of dendritic cells. The MyD88-dependent pathway is common to all TLRs, except TLR3[10]. Upon activation by PAMPs or DAMPs, TLRs hetero- or homodimerize inducing the recruitment of adaptor proteins via the cytoplasmic TIR domain. Adaptor proteins include the TIR-domain containing proteins, MyD88, TIRAP (TIR-associated protein), Mal (MyD88 adaptor-like protein), TRIF (TIR domain-containing adaptor protein-inducing IFN-β) and TRAM (TRIF-related adaptor molecule). Recruitment of MyD88 for instance, in turn recruits IRAK1 and IRAK4. IRAK4 subsequently activates IRAK1 by phosphorylation. Both IRAK1 and IRAK4 leave the MyD88-TLR complex and associate temporarily with TRAF6 leading to its ubiquitination. Bcl10 and MALT1 form oligomers that bind to TRAF6 promoting TRAF6 self-ubiquitination[11]. Recently, IRAK2 was shown to play a central role in TRAF6 ubiquitination[12]. Following ubiquitination, TRAF6 forms a complex with TAB2/TAB3/TAK1 inducing TAK1 activation[13]. TAK1 then couples to the IKK complex, which includes the scaffold protein NEMO, leading to the phosphorylation of IκB and the subsequent nuclear localization of NF-κB. Activation of NF-κB triggers the the production of pro-inflammatory cytokines such as TNF-α, IL-1 and IL-12.

Individual TLRs induce different signaling reponses by usage of the different adaptor molecules. TLR4 and TLR2 signaling requires the adaptor TIRAP/Mal, which is involved in the MyD88-dependent pathway[14]. TLR3 triggers the production of IFN-β in response to double-stranded RNA, in a MyD88-independent manner, through the adaptor TRIF/TICAM-1[15]. TRAM/TICAM-2 is another adaptor molecule involved in the MyD88-independent pathway[5] which function is restricted to the TLR4 pathway[16].

TLR3, TLR7, TLR8 and TLR9 recognize viral nucleic acids and induce type I IFNs. The signaling mechanisms leading to the induction of type I IFNs differ depending on the TLR activated. They involve the interferon regulatory factors, IRFs, a family of transcription factors known to play a critical role in antiviral defense, cell growth and immune regulation. Three IRFs (IRF3, IRF5 and IRF7) function as direct transducers of virus-mediated TLR signaling. TLR3 and TLR4 activate IRF3 and IRF7[17], while TLR7 and TLR8 activate IRF5 and IRF7[18]. Furthermore, type I IFN production stimulated by TLR9 ligand CpG-A has been shown to be mediated by PI(3)K and mTOR[19].

TLR-Targeted Therapeutics

Significant progress has been made over the past years in the understanding of TLR function[20]. TLRs are essential receptors in host defense against pathogens by activating the innate immune system, a prerequisite to the induction of adaptive immune responses. Although TLR-mediated signaling is paramount in eradicating microbial infections and promoting tissue repair, the regulation must be tight. TLRs are implicated in a number of inflammatory and immune disorders and play a role in cancer[21]. Many single nucleotide polymorphisms have been identified in various TLR genes and are associated with particular diseases. Several therapeutic agents targeting the TLRs are now under pre-clinical and clinical evaluation[22]. However, the complexity lies in that TLRs act as double-edged swords either promoting or inhibiting disease progression. Furthermore, therapeutic agents targeting the TLRs must be able to antagonize the harmful effects resulting without affecting host defense functions. Nonetheless, the potential of harnessing and directing the innate immune system with drugs targeting TLRs, to prevent or treat human inflammatory and autoimmune diseases as well as cancer, appears to be promising.

TLR	Immune Cell Expression	PAMPS	DAMPS	Signal Adaptor	Production
TLR1 + TLR2	Cell surface Mo, MΦ, DC, B	Triacylated lipoproteins (Pam3CSK4) Peptidoglycans, Lipopolysaccharides	(TLR2 DAMPs listed below)	TIRAP, MyD88, Mal	IC
TLR2 + TLR6	Cell surface Mo, MΦ, MC, B	Diacylated lipoproteins (FSL-1)	Heat Shock Proteins (HSP 60, 70, Gp96) High mobility group proteins (HMGB1) Proteoglycans (Versican, Hyaluronic Acid fragments)	TIRAP, MyD88, Mal	IC
TLR3	Endosomes B, T, NK, DC	dsRNA (poly (I:C)) tRNA, siRNA	mRNA tRNA	TRIF	IC, type I IFN
TLR4	Cell surface/ endosomes Mo, MΦ, DC, MC, IE	Lipopolysaccharides (LPS) Paclitaxel	Heat Shock Proteins (HSP22, 60, 70,72, Gp96) High mobility group proteins (HMGB1) Proteoglycans (Versican, Heparin sulfate, Hyaluronic Acid fragments) Fibronectin, Tenascin-C	TRAM, TRIF TIRAP, MyD88 Mal	IC, type I IFN
TLR5	Cell surface Mo, MΦ, DC, IE	Flagellin		MyD88	IC
TLR7	Endosomes Mo, MΦ, DC. B	ssRNA Imidazoquinolines (R848) Guanosine analogs (Loxoribine)	ssRNA	MyD88	IC, type I IFN
TLR8	Endosomes Mo, MΦ, DC, MC	ssRNA, Imidazoquinolines (R848)	ssRNA	MyD88	IC, type I IFN
TLR9	Endosomes Mo, MΦ, DC, B,T	CpG DNA CpG ODNs	Chromatin IgG complex	MyD88	IC, type I IFN
TLR11	Endosomes Mo, MΦ, DC	profilin-like proteins		MyD88	IC

Mo: monocytes, MΦ: marcophages, DC: dendritic cells, MC: Mast cells, B: B cells, T: T cells, IE: Intestinal epithelium, IC: Inflammatory cytokines

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TLR Product Line

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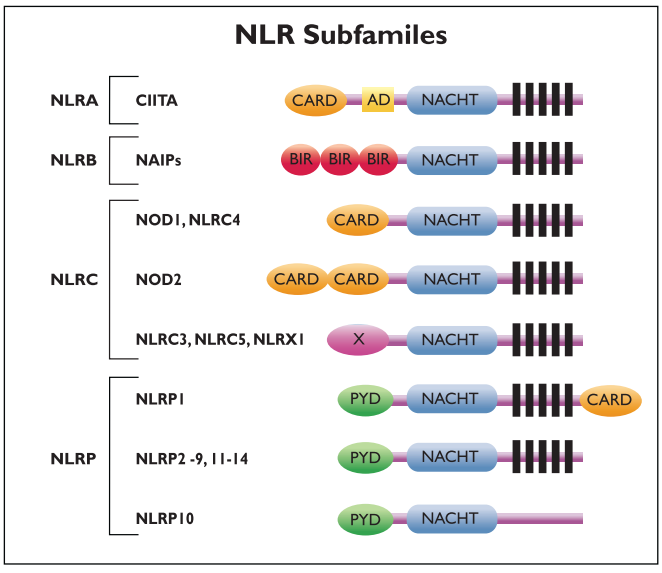
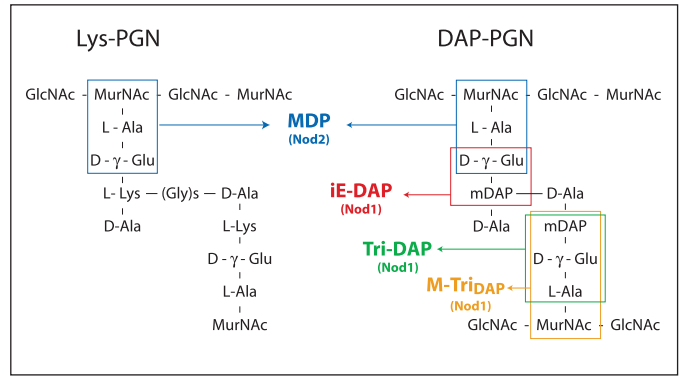
NOD-LIKE RECEPTORS

The cytosolic NOD-Like Receptors (NLRs, also known as CATERPILLERs, NODs or NALP/PAN/PYPAFs) are nucleotide-binding oligomerization domain containing receptors. To date, 22 NLRs have been identified in humans and constitute a major class of intracellular pattern recognition receptors (PRRs). The mammalian NLRs can be divided into four subfamilies, based on different N-terminal effector domains. The effector domains found in NLRs are CARDs, pyrin domains (PYDs), baculoviral inhibitor of apoptosis repeat (BIR) domains, or the transactivator domain (AD). The designated subfamilies are (based on the initial of the domain name): NLRC (formely known as NODs), NLRP (formerly known as NALPs), NLRB (formely known as NAIP or Birc) and NLRA. NLRs sense infection and stress through the recognition of cytoplasmic pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs), respectively. Subsequently, NLRs orchestrate an inflammatory response, autophagy or cell death. The physiological importance of these cytosolic sensors is underscored by a high incidence of genetic mutations that are associated with chronic inflammatory or autoimmune disorders.

NOD1 and NOD2

The founding NLRs members NOD1 (CARD4) and NOD2 (CARD15) fall into the NLRC subfamily and contain one and two N-terminal CARD domains, respectively. NOD1 and NOD2 recognize distinct motifs of peptidoglycan (PGN), an essential constituent of the bacterial cell wall. NOD1 senses the D-γ-glutamyl-meso-DAP dipeptide (iE-DAP), which is found in PGN of all Gram-negative and certain Gram-positive bacteria[1, 2] whereas NOD2 recognizes the muramyl dipeptide (MDP) structure found in almost all bacteria. Thus NOD2 acts as a general sensor of PGN and NOD1 is involved in the recognition of a specific subset of bacteria. Both iE-DAP and MDP must be delivered intracellularly either by bacteria that invade the cell or through other cellular uptake mechanisms. Ligand bound NOD1 and NOD2 oligomerize and signal via the serine/threonine RIP2 (RICK,CARDIAK) kinase through CARD-CARD homophilic interactions[3]. Once activated, RIP2 mediates ubiquitination of NEMO/IKKγ leading to the activation of NF-κB and the production of inflammatory cytokines. Furthermore, poly-ubiquitinated RIP2 recruits TAK1, which leads to IKK complex activation and the activation of MAPKs[4]. Signaling by NOD2 has been shown to involve the adapter protein CARD9, to mediate p38 and JNK signaling through RIP2[5]. Genetic mutations in NOD2 are associated with Crohn's disease, a chronic inflammatory bowel disease[6].





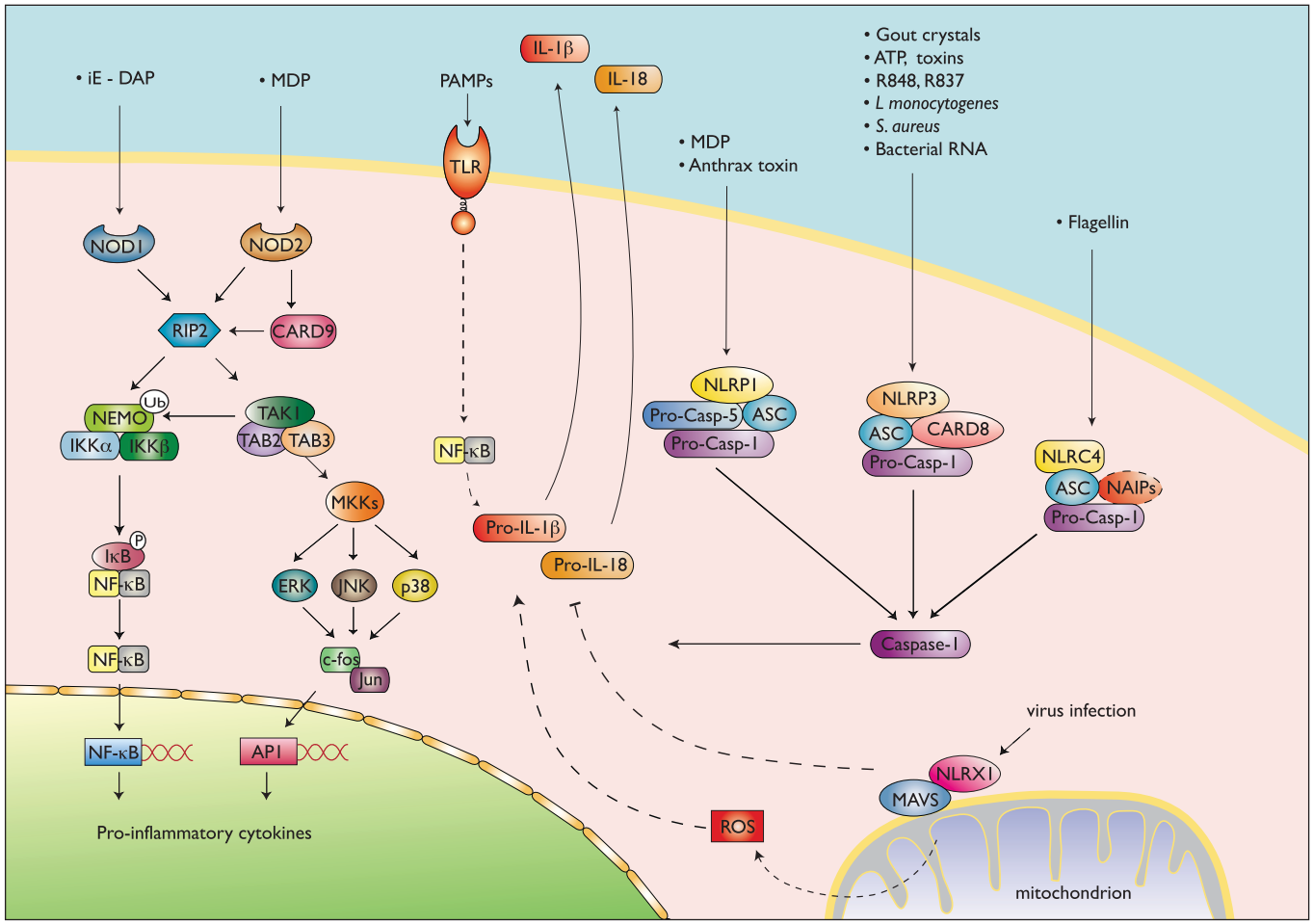
Schematic structure of Lys-PGN (found in Gram-positive bacteria) and DAP-PGN (found in Gram-negative bacteria)

NLRC4 and NLRX1

NLRC4 (IPAF, CLAN/CARD12) belongs to the NLRC subfamily. NLRC4 plays a key role in the regulation of caspase-1 by forming a multiprotein complex called the "inflammasome". Caspase-1 participates in the processing and subsequent release of proinflammatory cytokines, such as IL-1β and IL-18. Caspase-1 activation induced by cytosolic flagellin has been shown to be NLRC4-dependent, but TLR5-independent[7]. Thus, it appears that TLR5 and NLRC4 are distinct sensors that respond to extracellular and cytosolic flagellin, respectively[8]. Further, it has been been demonstrated that NAIPs (NLRB subfamily members) serve as NLRC4 inflammasome receptors for flagellin and a conserved type III secretion system (TTSS) rod component[9].
NLRX1 (NOD9) is the first NLR protein shown to be localized at the mitochondria[10]. NLRX1 negatively impacts antiviral inflammatory response via the RIG-I/IPS-I sensing pathway[11, 12] and LPS-elicited responses via the TRAF6-IKK signaling pathway[12, 13]. Conversely, it has been shown that NLRX1 positively controls NF-κB and JNK signaling pathway to activate reactive oxygen species (ROS) production in response to TNF-α, poly(I:C), and pathogens[14, 15]. Further studies are needed to clarify the role of NLRX1 in antiviral immunity.

NLRPs (NALPs)

The NLRP subfamily consists of 14 members characterized by their PYD effector domains. At least two types of NLRP inflammasomes have been identified: the NLRP1 inflammasome comprising NLRP1 (NALP1, CARD7), ASC, caspase-1 and caspase-5, and the NLRP3 inflammasome containing NLRP3 (NALP3, cryopyrin, CIAS1), ASC, Cardinal and caspase-1[16]. NLRP1 and NLRP3 recruit through their PYD domain the adaptor protein ASC, which in turn interacts with caspase-1 via a CARD-CARD interaction. NLRP1 also recruits caspase-5 via its additional CARD effector domain at the C terminus, whereas NLRP3, lacking such a CARD, interacts with the CARD-containing adaptor CARD8 (Cardinal) to recruit additional caspase-1.
Two molecules activate NLRP1: MDP and the anthrax toxin. NLRP1 oligomerization induced by MDP requires ATP. The anthrax toxin was shown to activate the murine variant of NLRP1 (NALP1b) suggesting NLRP1 inflammasome activation in the immune response to *Bacillus anthracis* infection[17]. NLRP3 mediates caspase-1 activation in response to a wide variety of stimuli: whole bacteria (*L. monocytogenes*, *S. aureus*), bacterial RNA, synthetic purine-like compounds (R848, R837), uric acid crystals, amyloid-b, extracellular ATP and pore-forming toxins (nigericin, maitotoxin)[18-20]. Mutations in NLRP3 are associated with inherited autoinflammatory diseases called cryopyrin-associated periodic syndromes (CAPS), such as familial cold autoinflammatory syndrome and Muckle-Wells syndrome[21].



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RIG-I-LIKE RECEPTORS & CYTOSOLIC DNA SENSORS

RIG-I-like receptors (RLRs) constitute a family of cytoplasmic RNA helicases that are critical for host antiviral responses. RIG-I (retinoic-acid-inducible protein 1, also known as Ddx58) and MDA-5 (melanoma-differentiation-associated gene 5, also known as Ifih1 or Helicard) sense double-stranded RNA (dsRNA), a replication intermediate for RNA viruses, leading to production of type I interferons (IFNs) in infected cells[1]. Viral dsRNA is also recognized by Toll-Like receptor 3 (TLR3) which is expressed on the cell surface membrane or endosomes. Recognition of dsRNA by RIG-I/MDA-5 or TLR3 is cell-type dependent. Studies of RIG-I- and MDA-5-deficient mice have revealed that conventional dendritic cells (DCs), macrophages and fibroblasts isolated from these mice have impaired IFN induction after RNA virus infection, while production of IFN is still observed in plasmacytoid DCs (pDCs)[2]. Thus in cDCs, macrophages and fibroblasts, RLRs are the major sensors for viral infection, while in pDCs, TLRs play a more important role.

RIG-I and MDA-5 contain a DExD/H box RNA helicase and two caspase recruiting domain (CARD)-like domains. The helicase domain interacts with dsRNA, whereas the CARD domains are required to relay the signal. Despite the overall structural similarity between these two sensors, they detect distinct viral species. RIG-I participates in the recognition of Paramyxoviruses (Newcastle disease virus (NDV), Sendai virus (SeV)), Rhabdoviruses (vesicular stomatitis virus (VSV)), Flaviviruses (hepatitis C (HCV)) and Orthomyxoviruses (Influenza), whereas MDA-5 is essential for the recognition of Picornaviruses (encephalo-myocarditis virus (EMCV)) and poly(I:C), a synthetic analog of viral dsRNA[3]. Notably, RIG-I binds specifically to single stranded RNA containing 5'-triphosphate such as viral RNA and *in vitro*-transcribed long dsRNA[4]. It has been shown that RIG-I binds preferentially to short dsRNA while MDA-5 recognizes preferentially long dsRNA[5]. Further cytosolic B DNA, such as transfected poly(dA:dT), can be transcribed by RNA polymerase III into a double-stranded RNA intermediate. This RNA intermediate contains a 5'-triphosphate moiety which is detected by RIG-I[6,7].

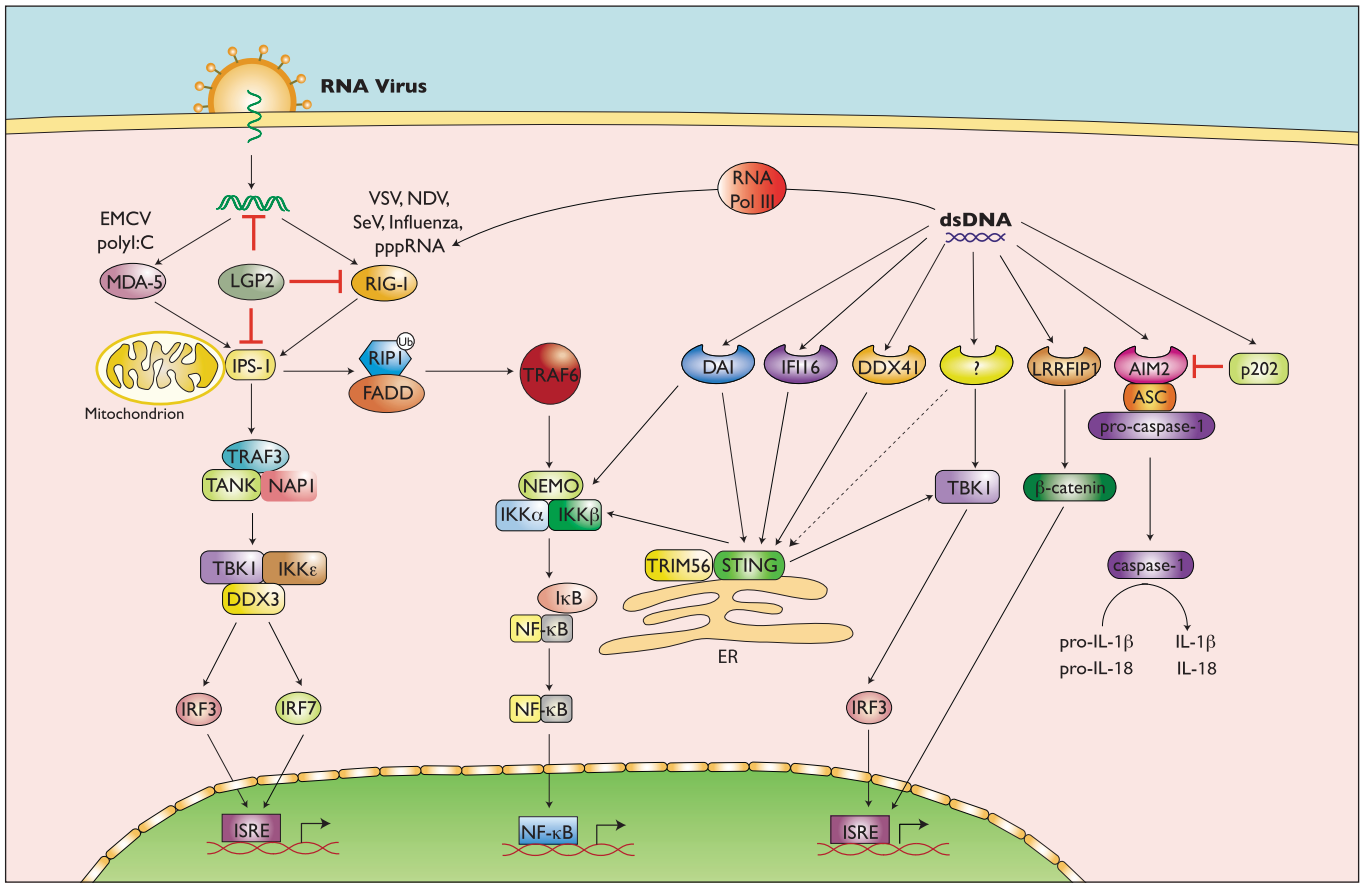
Nucleic Acid Sensor	Ligand	Ref.
AIM2	**Viruses:** Vaccinia, mouse cytomegalovirus **Bacteria:** *F. tularensis, L.monocytogenes* **Synthetic ligand:** AT-rich B DNA	19
DAI	**Viruses:** Human cytomegalovirus, Herpes simplex 1 **Bacteria:** *S. pneumoniae*	23
DDX41	**Synthetic ligand:** AT-rich B DNA	22
IFI16	**Viruses:** Herpes simplex 1 **Synthetic ligand:** dsDNA sequence-independent 70>>50 bp	23
LRRFIP1	**Viruses:** Vesicular stomatitis **Bacteria:** *L. monocytogenes,* **Synthetic ligand:** dsDNA, dsRNA, AT-rich B DNA, GC-rich Z-DNA	23
MDA-5	**Viruses:** Picornavirus, Encephalomyocarditis, Rabies, Sendai, Dengue, Rotavirus, murine hepaitis, murine norovirus 1 **Synthetic ligand:** Poly(I:C)	24
RIG-I	**Viruses:** Newcastle disease, Sendai, Influenza, Vesicular stomatitis, Japanese encephalitis, measles, Rabies, Hepatitis C, Dengue **Synthetic ligand:** 5' triphosphate double stranded RNA (5'ppp-dsRNA)	24
RNA pol III	**Viruses:** Adenovirus, Epstein Barr **Bacteria:** *L. pneumophila* **Synthetic ligand:** AT-rich B DNA	23

Although RIG-I and MDA-5 recognize different ligands, they share common signaling features. Upon recognition of dsRNA, they are recruited by the adaptor IPS-1 (also known as MAVS, CARDIF or VISA) to the outer membrane of the mitochondria leading to the activation of several transcription factors including IRF3, IRF7 and NF-κB[8]. IRF3 and IRF7 control the expression of type I IFNs, while NF-κB regulates the production of inflammatory cytokines. IRF3 and IRF7 activation involves TNF (tumor necrosis factor) receptor-associated factor 3 (TRAF3), NAK-associated protein 1 (NAP1), TANK and the protein kinase TANK-binding kinase 1 (TBK1) or IκB kinase epsilon (IKKε)[8-10]. DDX3, a DEAD box helicase, was shown to interact with TBK1/IKKε[11]. IPS-1 interacts also with Fas-associated-death-domain (FADD) and receptor interacting protein 1 (RIP1) which induces the activation of the NF-κB pathway[8-11].

A third RLR has been described: laboratory of genetics and physiology 2 (LGP2). LGP2 contains a RNA binding domain but lacks the CARD domains and thus acts as a negative feedback regulator of RIG-I and MDA-5. LGP2 appears to exert this activity at multiple levels by i) competitively sequestering dsRNA, ii) forming a protein complex with IPS-1, and/or iii) binding directly to RIG-I through a repressor domain[13-15]. Many other molecules seem to be involved in the negative control of RIG-I/MDA-5-induced IFN production. Dihydroxy-acetone kinase (DAK), A20, ring-finger protein 125 (RNF125), suppressor of IKKε (SIKE), and peptidyl-propyl isomerase 1 (Pin1), have been recently described as physiological suppressors of the RIG-I/MDA-5 signaling pathway.

While the recognition of cytosolic RNA by RLRs has been investigated for some time, more recently the recognition of cytosolic DNA has been under the spotlight. The first identified cytosolic DNA sensor, named DNA-dependent activator of IFN-regulatory factors (DAI), binds cytosolic dsDNA and leads to the production of type I IFNs[16]. DAI induces the production of type I IFNs through the TBK1/IRF3 pathway. The endoplasmic reticulum (ER)-resident transmembrane protein stimulator of IFN genes (STING) functions as an essential signalling adaptor, linking the cytosolic detection of DNA to the TBK1-IRF3 signalling axis[17]. STING is induced by an IFN-inducible ligase called TRIM56[18]. The DNA sensor IFI16 has been found to recruit STING to activate a TBK1-IRF3-dependent pathway to IFN-β induction. IFI16 is part of a larger protein family termed the pyrin and HIN domain (PYHIN) family.

Another member of the PYHIN family, AIM2 (absent in melanoma 2), is a cytosolic DNA receptor that forms an inflammasome with ASC triggering caspase 1 activation and the subsequent of production of IL-1β and IL-18. DNA of various origins, such as poly(dA:dT), plasmidic DNA and DNA from the bacterium *L. monocytogenes* have been shown to activate AIM2[19]. Upon activation, AIM2 interacts with ASC, a common adapter of the inflammasomes, leading to the cleavage of caspase-1 and the secretion of IL-1β and IL-18. p202 is another member of the PYHIN family shown to bind cytoplasmic dsDNA but, in contrast to AIM2, it represses caspase activation[20]. LRRFIP1 can recognize AT-rich B-form dsDNA as well as GC-rich Z-form dsDNA[21]. With the use of LRRFIP1-specific siRNA, Yang *et al.* demonstrated that LRRFIP1 triggers the production of IFN-β in a β-catenin-dependent manner. β-Catenin binds to the C-terminal domain of IRF3 inducing an increase in IFN-β expression. More recently, the helicase DDX41 has been identified as an additional DNA sensor that depends on STING to sense pathogenic DNA[22]. The recognition of cytosolic DNA is more complicated than first anticipated. Several sensors have been identified that trigger different signaling pathways in a cell type-specific manner. Still, the general consensus is that another unknown cytosolic DNA-recognition system, independent of the TLRs and RIG-I, may exist. Further studies to elucidate the complex mechanisms of cytosolic DNA recognition may help the development of new strategies to treat inflammatory diseases.



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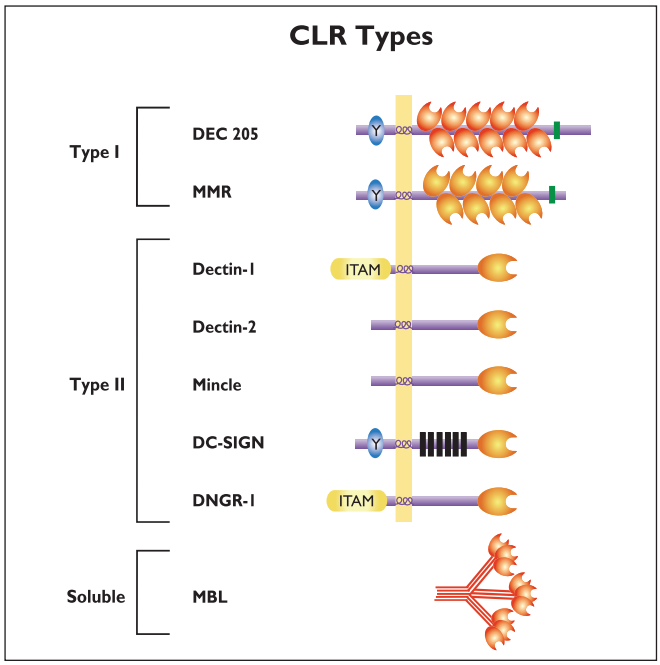
C-TYPE LECTIN RECEPTORS

C-type lectin receptors (CLRs) comprise a large family of receptors that bind to carbohydrates in a calcium-dependent manner. The lectin activity of these receptors is mediated by conserved carbohydrate-recognition domains (CRDs). On the basis of their molecular structure, two groups of membrane-bound CLRs can be distinguished and a group of soluble CLRs. DEC-205 and the macrophage mannose receptor (MMR), important in antigen uptake, are type I transmembrane proteins containing several CRDs or CRD-like domains. Type II transmembrane CLRs typically carry a single CRD domain and include Dectin-1, Dectin-2, macrophage-inducible C-type lectin (Mincle), the dendritic cell-specific ICAM3-grabbing nonintegrin (DC-SIGN), and DC NK lectin group receptor-1 (DNGR-1). These receptors are involved in fungal recognition and the modulation of the innate immune response. Soluble CLRs include MBL, an oligermeric protein that binds an array of carbohydrate patterns on pathogen surfaces. CLRs are expressed by most cell types including macrophages and dendritic cells (DCs), which phagocytoze various glycoproteins and microbes for the purposes of clearance and antigen presentation to T lymphocytes.

Dectin-1

Dectin-1 plays an important role in antifungal innate immunity. Dectin-1 is a specific receptor for β-glucans[1]. β-Glucans are glucose polymers found in the cell walls of fungi, including the yeasts *Saccharomyces cerevisiae* and *Candida albicans*. Dectin-1 has a CRD connected by a stalk to the transmembrane region, followed by a cytoplasmic tail containing an ITAM-like motif. Upon binding to its ligand, Dectin-1 triggers phagocytosis and activation of Src and Syk kinases, through its ITAM-like motif. Syk, in turn, induces the CARD9-Bcl10-Malt1 complex leading to the production of reactive oxygen species (ROS), activation of NF-κB and the subsequent secretion of proinflammatory cytokines[2, 3]. ROS have a direct microbicidal role in the phagosome but also can affect IL-1β secretion by activating the NLRP3 inflammasome, which in turn activates caspase-1 and permits processing of pro-IL-1β[4]. Dectin-1 signaling has been shown to collaborate with TLR2 signaling to enhance the responses triggered by each receptor[3, 5]. Furthermore, Dectin-1 can modulate cytokine expression by inducing NFAT through the Ca^{2+}-calcineurin-NFAT pathway[6].



Dectin-2

Dectin-2 is also important in antifungal innate immunity. Dectin-2 binds high mannose-type carbohydrates and was shown to be the functional receptor for α-mannans. Moreover, Dectin-2 has been implicated in anti-bacterial immunity and allergy[7]. Like Dectin-1, Dectin-2 belongs to the selective group of CLRs that link pathogen recognition to adaptive immunity. In fact, it has been demonstrated that Dectin-2 is the predominant receptor in response to fungal infection and the induction of Th17 immunity. Similar to Dectin-1, activation of Dectin-2 triggers ROS and potassium efflux, leading to NLRP3 inflammasome activation and processing of pro-IL-1β[8].

Mincle

Mincle is a member of the Dectin-2 family. Mincle recognizes a variety of exogenous and endogenous stimuli, such as mycobacteria, certain fungi and necrotic cells[9, 10]. Exogenous ligands for Mincle include fungal α-mannose, and the mycobacterial glycolipid, trehalose-6'6'-dimycolate (TDM), also known as cord factor the immunostimulatory component of *Mycobacterium tuberculosis*[11]. Furthermore, Mincle senses damaged cells by recognizing the endogenous damage-associated molecular patterns (DAMPs). One such DAMP identified is the spliceosome-associated protein 130 (SAP130), a soluble factor released by necrotic cells[12]. Mincle interacts with the Fc receptor common γ-chain (FcRγ), which triggers intracellular signaling through Syk leading to CARD9-dependent NF-κB activation. Syk induces also the mobilization of intracellular calcium (Ca^{2+}) and the activation of the calcineurin-NFAT pathway.

DC-SIGN

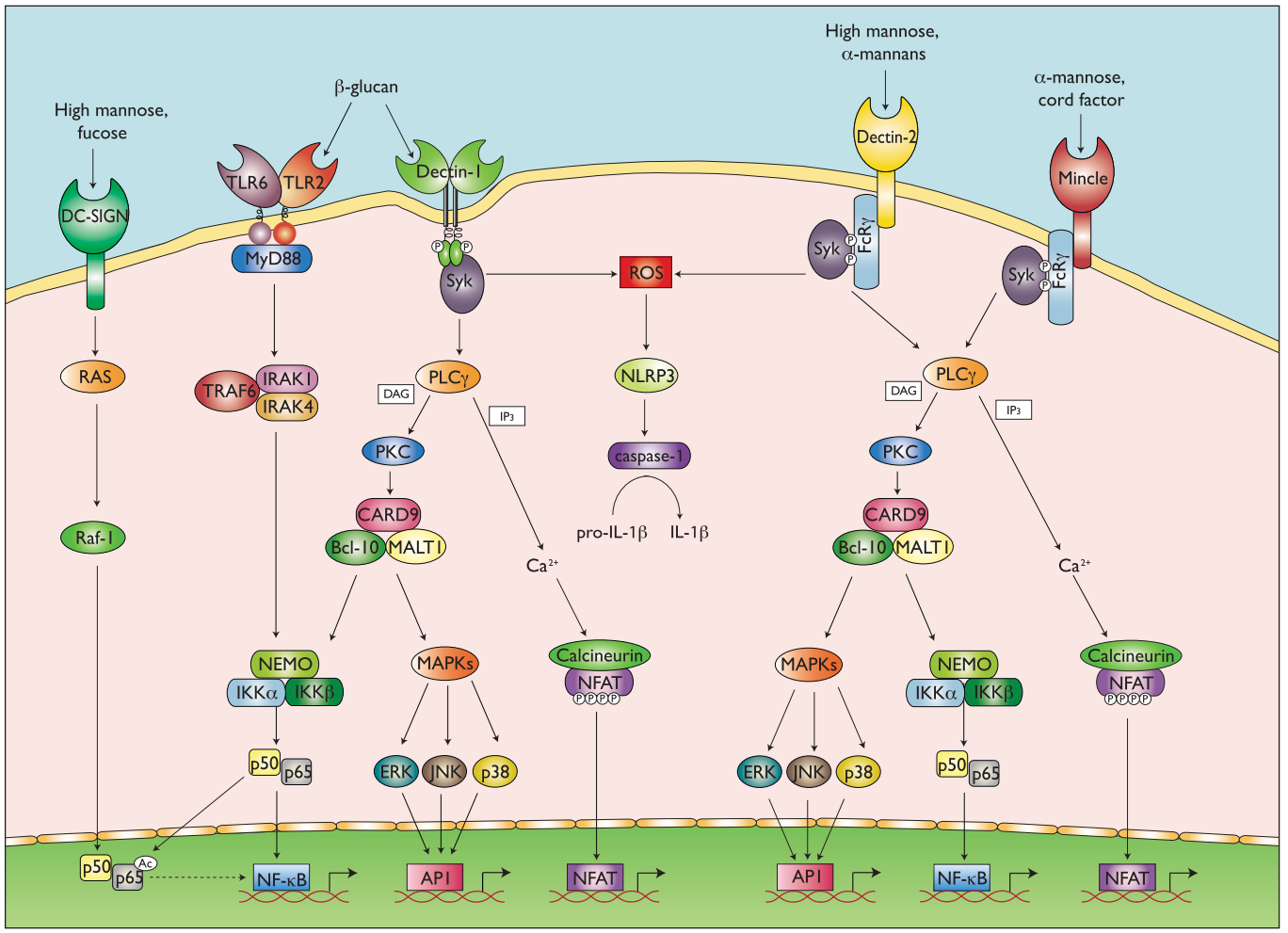
DC-SIGN is of interest due to its involvement in the recognition of several viruses (HIV-1, HCV, dengue virus, CMV, ebola virus) and other microbes of the *Leishmania* and *Candida* species. This type II transmembrane protein has a single C-type lectin domain and is expressed on immature monocyte-derived DCs. DC-SIGN modulates TLR signaling at the level of the transcription factor NF-κB, however, prior TLR activation of NF-κB is required. It has been demonstrated that pathogens trigger DC-SIGN on human DCs to activate the serine and threonine kinase Raf-1, which subsequently leads to acetylation of the NF-κB subunit p65. Acetylation of p65 both prolonged and increased IL-10 transcription to enhance the anti-inflammatory cytokine response[13]. To date, it has been shown that *M. tuberculosis, M. leprae, C. albicans*, measles virus, and HIV-1 interact with DC-SIGN to activate the Raf-1-acetylation-dependent signaling pathway and modulate TLR signaling[14]. Thus, this pathway is involved in regulation of adaptive immunity by DCs to bacterial, fungal, and viral pathogens.

DNGR-1

DNGR-1 (CLEC9A) is particularly interesting because of its restricted pattern of expression in DCs that may be exploited for cancer therapy. It has recently been revealed that DNGR-1 binds damaged or dead cells via exposed actin filaments[15,16]. DNGR-1 is therefore considered to be DAMPs receptor since no microbial ligand has yet been identified.

MBL

MBL (Mannose –binding lectin) is a soluble C-type lectin. MBL plays a crucial role in innate immunity against yeast by enhanced complement activation and enhanced uptake of polymorphonuclear cells[17]. MBL binds to repetitive mannose and/or N-acetylglucosamine residues on microorganisms, leading to opsonization and activation of the lectin complement pathway. MBL also interacts with carbohydrates on the glycoprotein (gp)120 of HIV-1. MBL may inhibit DC-SIGN-mediated uptake and spread of HIV[18].
Much remains to be understood about CLRs in general, their ligands and and cooperation with other molecules.



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CLR Product Line

Innate Immunity Genes	**p. 22**
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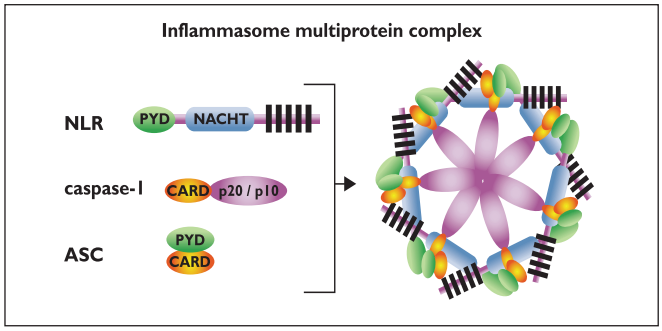
INFLAMMASOMES

The nucleotide-binding oligomerization domain-like receptor (NLR) family of proteins is involved in the regulation of innate immunity responses. These proteins sense pathogen-associated molecular patterns (PAMPs) in the cytosol as well as the host-derived signals known as damage-associated molecular patterns (DAMPs). Certain NLRs induce the assembly of large caspase-1-activating complexes called inflammasomes[1,2]. Activation of caspase-1 through autoproteolytic maturation leads to the processing and secretion of the pro-inflammatory cytokines interleukin-1β (IL-1β) and IL-18. So far, four inflammasomes have been identified and defined by the NLR protein that they contain; the NLRP1/NALP1b inflammasome[3]; the NLRC4/IPAF inflammasome[4,5]; the NLRP3/NALP3 inflammasome[6]; and the AIM2 (absent in melanoma 2) containing inflammasome[7,8].

IL-1β and IL-18 are related cytokines that cause a wide variety of biological efffects associated with infection, inflammation and autoimmune processes. IL-1β participates in the generation of systemic and local responses to infection and injury by generating fever, activating lymphocytes and by promoting leukocyte infiltration at sites of infection or injury. IL-18 induces IFN-γ production and contributes to T-helper 1 (Th1) cell polarization. Maturation of IL-1β and IL-18 by cleavage with caspase-1 is a prerequisite for inducing the immune responses. Caspase-1 itself is synthesized as an inactive 45 kDa zymogen (pro-caspase-1) that undergoes autocatalytic processing following an appropriate stimulus. The active form of the enzyme comprises the subunits p20 and p10[9]. Caspase-1 is activated within the inflammasome multiprotein complex through interaction with ASC (apoptosis-associated speck-like protein containing a carboxy-terminal CARD), a bipartite adapter protein that bridges NLRs and caspase-1[10].



It is now generally accepted that activation and release of IL-1β requires two distinct signals. The nature of these signals *in vivo* during infection or inflammation is not completely defined. However, *in vitro* studies indicate that the first signal can be triggered by various PAMPs following Toll-like receptor (TLR) activation which induces the synthesis of pro-IL-1β. The second signal is provided by the activation of the inflammasome and caspase-1 leading to IL-1β processing. The requirement for a second signal for IL-1β maturation might constitute a fail-safe mechanism to ensure that induction of potent inflammatory responses occurs only in the presence of a *bona fide* stimulus, such as pathogen infection and/or tissue injury.

NLRP1 Inflammasome

NLRP1 assembles a multimolecular complex inflammsome with caspase-1, caspase-5, ASC, and a triphosphate ribonucleotide[1,2,10]. NLRP1 directly binds to ASC, via its pyrin (PYD) domain and directly to caspase-1 via its CARD domain. Activity of the NLRP1 inflammasome is induced by muramyl dipeptide (MDP) and anthrax lethal toxin (mouse NLRP1b)[3]. Studies have indicated that NOD2 is needed for *in vitro* sensing of both MDP and anthrax lethal toxin. Activation of the NLRP1 inflammasome is tightly linked to the apoptotic pathway. The anti-apoptotic proteins Bcl-2 and Bcl-X(L) bind NLRP1 in resting conditions, suppressing caspase-1 activation and IL-1β secretion. Several NLRP1 gene variations have been associated with an increased risk of autoimmune disorders and vitiligo, an autoimmune condition that results in patchy changes in skin pigmentation. However, the precise role of the NLRP1 inflammasome in immune responses remains poorly understood.

NLRC4 Inflammasome

NLRC4 (also known as IPAF) is the only member of the NLRC family currently known to assemble an inflammasome[2,4,5]. NLRC4 associates with pro–caspase-1 with its CARD domain without the need of an adaptor protein, and interaction with ASC is required for robust IL-1β secretion. Oligomerization of NLRC4 is triggered by cytosolic flagellin from a variety of bacteria such as Salmonella typhimurium, Legionella pneumophila, Shigella flexneri, and Pseudomonas aeruginosa or other stimuli possibly delivered by a bacterial secretion system (type III or type IV). NAIP5, another member of the NLR family, appears to be involved in the recognition of the ligand under certain circumstances[11]. Flagellin is an interesting ligand triggering both TLR5 and the NLRC4 inflammasome[12]. As such, flagellin is likely to independently signal the production of cytokines and drive their maturation via caspase-1.

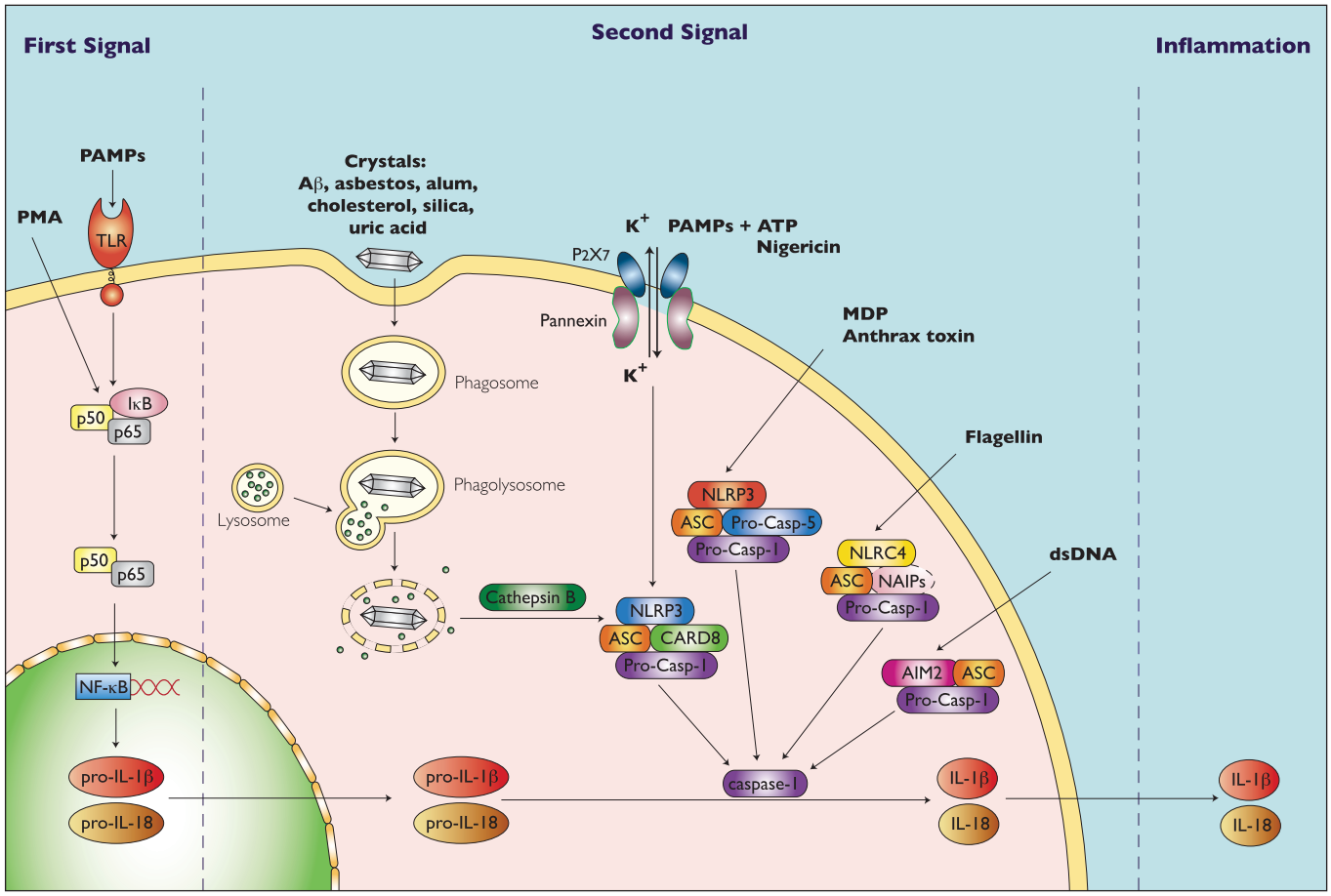
NLRP3 Inflammasome

Among the inflammasomes, the NLRP3 inflammasome is the most studied. Its activation in macrophages can be achieved with a plethora of PAMPs, such as liposaccharide, peptidoglycan, and bacterial nucleic acids, provided the cells are exposed to ATP. Indeed, in the absence of ATP, macrophages stimulated with LPS produce large quantities of pro-IL-1β, but release little mature cytokine into the medium. ATP and certain bacterial toxins, such as nigericin and maitotoxin, cause a change in the intracellular ion composition leading to the activation of the NLRP3 inflammasome. The effect of ATP is mediated by the purinergic $P2X_7$ receptor together with pannexin, which causes a rapid potassium efflux from the cytosol upon activation[13]. Crystals of monosodium urate (MSU) and calcium phosphate dihydrate (CPPD) are known to activate caspase-1 in a NLRP3-dependent manner[14]. Deposition of MSU and CPPD crystals in joints is responsible for the inflammatory conditions gout and pseudogout, respectively, implicating NLRP3 in their etiology. Uric acid in addition is released into the extracellular milieu by necrotic cells, suggesting an important role of NLRP3 in the detection of endogenous 'danger' signal. Crystalline silica and asbestos are known to activate the NLRP3 inflammasome, implicating its role in the pathogenesis of silicosis and asbestosis[15-17]. Aluminium salt (alum) crystals can also activate the NLRP3 inflammasome, albeit in the presence of PAMPs such as LPS[17-19]. Phagocytosis of crystals leads to lysosomal swelling and damage. The lysosomal perturbation together with the release of cathepsin B, a lysosomal cysteine protease, result in the activation of the NLRP3 inflammasome[17].

AIM2 Inflammasome

AIM2 (absent in melanoma 2), a receptor for cytoplasmic DNA, forms an inflammasome with its ligand and ASC to activate caspase-1[20-22]. AIM2 is an interferon-inducible HIN-200 family member that contains an amino-terminal pyrin domain and a carboxy-terminal oligonucleotide/oligosaccharide-binding domain. AIM2 senses cytoplasmic double-stranded DNA through its oligonucleotide/ oligosaccharide-binding domain and interacts with ASC via its pyrin domain to activate caspase-1. The interaction of AIM2 with ASC also leads to the formation of the ASC pyroptosome, which induces pyroptotic cell death in cells containing caspase-1. AIM2 is necessary and sufficient for inflammasome activation in reponse to cytoplasmic DNA.

Clearly, inflammasomes fulfill a central role in innate immunity. They detect and respond to bacterial components, 'danger signals' and potentially dangerous cytoplasmic DNA. Further understanding on how they are activated should provide new insights into the mechanism of host defense and the pathogenesis of autoimmune diseases.



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Inflammasome Product Line

Innate Immunity Genes	**p. 22**
• NLR Genes	p. 24
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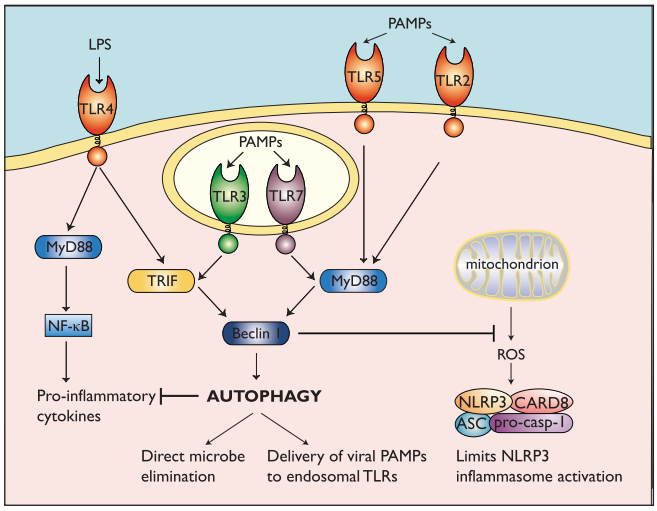
AUTOPHAGY AND INNATE IMMUNITY

Autophagy has recently been highlighted to play an important role in innate and adaptive immune responses. Autophagy pathways function to sequester cellular stress-constituents, such as intracellular pathogens, damaged organelles and long-lived, aggregate-prone proteins, in a double-membrane-bound autophagosome. Once formed, the outer membrane of the autophagosome fuses with a lysosome, where cellular stress constituents are degraded. The role of autophagy is to eliminate unwanted constituents from the cell and recycle cytoplasmic material allowing the cell to maintain macromolecular synthesis and energy homeostasis during starvation and other stressful conditions. Induction of autophagy therefore exerts anti-aging and oncosuppressive functions. The classical autophagy pathway proceeds through a series of stages. It starts with the nucleation of the autophagic vesicle followed by the elongation and closure of the autophagosome membrane to envelop cytoplasmic constituents. Then the autophagosome docks with the lysosome leading to the breakdown of the inner membrane and the subsequent exposure of the sequestered cytoplasmic material to lysosomal hydrolases[1,2].

The classic autophagy pathway requires the concerted action of evolutionarily conserved genes. Vesicle nucleation depends on a class III phosphatidylinositol-3-OH kinase (PI(3)K) complex formed by Beclin 1, Vps34 and other proteins. Atg7 participates in two ubiquitin-like conjugation pathways, conjugation of Atg5 to Atg12 and conversion of LC3 to its phosphatidylethanolamine (PE)-conjugated LC3-II form. The Atg5-Atg12 conjugate forms a large complex with the Atg16L1 protein. Both conjugation systems are required for the generation of the autophagosome.

Autophagy has implications in the prevention of aging, cancer and neurodegeneration in addition to its protective role against intracellular pathogens. Furthermore, autophagy genes are associated with inflammatory disorders including Crohn's disease (CD). Autophagy has been shown to interact with pattern recognition receptors (PRRs), such as the Toll-like receptors (TLRs), Nod-like receptors (NLRs) and RIG-I-like receptors (RLRs) to regulate inflammation. The autophagic machinery has both positive and negative relationships with these innate immunity receptors. The autophagic machinery for instance can deliver pathogen-associated molecular patterns (PAMPs) to endosomal TLRs[3], suggesting that autophagy enhances TLR recognition of PAMPs. Conversely, TLRs have been shown to promote autophagy. Several groups have reported the induction of autophagy by signaling through TLR4, TLR7, TLR3, TLR2 and TLR5[4-6]. TLR-induced autophagy appears to depend on MyD88 and TRIF. Both signaling adapters trigger autophagy through a direct interaction with Beclin 1[6]. More details on the mechanisms of autophagy regulation are starting to emerge[7]. Other receptors of the innate immune system have been described to work in concert with autophagy, and this is likely to be in a cell-type specific manner[8,9]. In macrophages, the NLRs, Nod1 and Nod2 interact with Atg16L1 and signal to induce autophagy[10]. The Atg16L1 protein plays a role in inhibiting production of the proinflammatory cytokines IL-1β and IL-18 after endotoxin stimulation of TLR[11]. RLRs can induce autophagy and in turn, signaling by RLRs has been shown to be inhibited by autophagy, suggesting a negative feedback to limit type I IFN production and signaling[11,12]. Autophagy has also been demonstrated to regulate inflammasome activation of different sorts[13,14,15,16]. One example is when the induction of autophagy limits NLRP3-inflammasome activation, serving as an anti-inflammatory defense mechanism[13,15]. Autophagic proteins that regulate the NLRP3-inflammasome were demonstrated to act through preserving mitochondrial integrity.

The interplay between autophagy and immune-related functions in different cell types is still in its infancy. Overall, the vast advances in the research field have underscored the immunological significance of autophagy in maintaining cellular homeostasis.



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Autophagy Product Line

Innate Immunity Genes	**p. 22**
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1 INNATE IMMUNITY GENES

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INNATE IMMUNITY GENES

Innate immunity genes is an expanding collection of genes encoding pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs), NOD-like receptors (NLRs) and RIG-I-like receptors (RLRs) as well as proteins involved in their signaling pathways. These genes are either native or modified by addition of a tag and/or deletion to generate dominant negative (DN) variants. They are provided as full-length, entirely sequenced open reading frames (ORFs) cloned into plasmidic expression vectors.

PRODUCT	GENE PRODUCT	GENE FAMILIES	DESCRIPTION
pUNO	**Native Genes**	- Toll-Like Receptors - NOD-Like Receptors - RIG-I-Like Receptors - Cytosolic DNA Sensors - C-type Lectin Receptors - Adaptors & Co-receptors - Signaling Effectors & Inhibitors - Cytokines - Autophagy Inducers - and more	- Native gene from ATG to Stop codon - Selectable with blasticidin
pUNO-TLR-HA	**HA-Tagged TLR Genes**	- Toll-Like Receptors	- Genes fused at their 3' end to the influenza hemagglutinine (HA) tag - Selectable with blasticidin
pUNO-TLR-GFP	**GFP-Tagged TLR Genes**	- Cell surface Toll-Like Receptors	- Genes fused at their C terminus to the GFP gene - Selectable with blasticidin
pDUO	**Gene Associations**	- TLR / TLR - TLR / Co-receptor - Co-receptor / Co-receptor	-Two transcription units - Selectable with blasticidin (or hygromycin)
pZERO-TLR **pDeNy**	**Dominant Negative Variants**	- Toll-Like Receptors - NOD-Like Receptors - RIG-I-Like Receptors - Adaptors - Signaling Effectors	- Dominant negative variants created by insertion of a mutation and/or deletion of a key region - Selectable with puromycin (pZERO-TLR) or Zeocin™ (pDeNy)
pZERO-TLR-HA	**HA-Tagged Dominant Negative Variants**	- Toll-Like Receptors	- HA-tagged TIR-deleted TLR genes - Selectable with blasticidin
pSELECT-GFP-LC3	**GFP-LC3 Fusion Protein**	- Autophagy	- LC3 gene fused at its 5' end to the GFP gene - Selectable with Zeocin™

Each plasmid is provided with 4 pouches of **Fast-Media®**. Fast-Media® are lyophilized *E. coli* selection media that allow the preparation of 200 ml of sterile liquid or agar medium. They contain different selective antibiotics, including blasticidin, puromycin and Zeocin™, at the appropriate concentration. They are also available with chromogenic substrates for the selection of blue/white colonies. Preparation of Fast-Media requires only 5 minutes in a microwave oven. Fast-Media® is extensively tested to guarantee sterility, antibiotic activity and *E. coli* growth.

For more information on Fast-Media®, go to
http://www.invivogen.com/fast-media

pUNO - Native Genes

Features and Benefits

Numerous genes encoding pattern recognition receptors (PRRs), co-receptors, adaptors, signaling effectors and signaling inhibitors are available in the pUNO plasmid. The genes are cloned from the ATG to the Stop codon, excluding introns and untranslated regions. All genes are fully sequenced, the sequences are available online at www.invivogen.com or can be emailed upon request.

PRR and related genes are cloned under the control of the strong and ubiquitous mammalian promoter, EF1α/HTLV. This composite promoter comprises the elongation factor 1 alpha (EF-1α) core promoter and the R-U5' of the human T cell leukemia virus (HTLV).

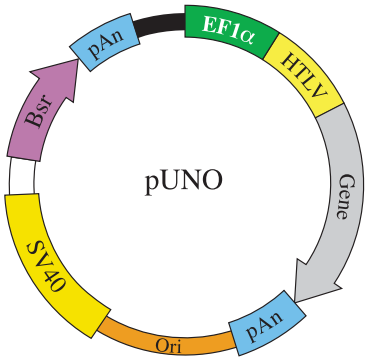
pUNO plasmids can be used directly for *in vitro* or *in vivo* transfection experiments. They are selectable with blasticidin in both *E. coli* and mammalian cells. To facilitate the excision and subcloning of the gene of interest into another vector, each gene is flanked by unique restriction sites that are compatible with many others.
Some genes are provided in the pUNO2 (Zeocin™-resistant) or pUNO3 (hygromycin-resistant) backbone.

Contents and Storage

Each pUNO 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 1 year when properly stored. Each plasmid is provided with 4 pouches of *E. coli* Fast-Media® Blas (2 TB and 2 Agar).

Related Products

Blasticidin, page 60
Hygromycin B, page 61
Zeocin™, page 61
Fast-Media® Blas, page 22
Fast-Media® Hygro, page 22
Fast-Media® Zeo, page 22



Toll-Like Receptors (TLRs)

TLR genes from different species are available:
- human
- mouse
- pig
- bovine

Human TLR genes are available in two different plasmid backbones:
- pUNO/pUNO1, selectable with blasticidin
- pUNO2, selectable with Zeocin™
- pUNO3, selectable with hygromycin

Cytokines

Some cytokine genes are available in the pORF plasmid.
More information at www.invivogen.com/gene.php

A selection of InvivoGen's innate immunity genes (more at www.invivogen.com)

GENE NAME	DESCRIPTION	CAT. CODE (human)	CAT. CODE (human, hygro)	CAT. CODE (mouse)	CAT. CODE (pig)	CAT. CODE (bovine)
Toll-Like Receptors (TLRs)						
TLR1	Toll-like Receptor 1	puno-htlr1	puno3-htlr1	puno-mtlr1	puno1-ptlr1	-
TLR2	Toll-like Receptor 2	puno-htlr2	puno3-htlr2	puno-mtlr2	puno1-ptlr2	puno1-btlr2
TLR3	Toll-like Receptor 3	puno-htlr3	puno3-htlr3	puno-mtlr3	-	-
TLR4	Toll-like Receptor 4	puno1-htlr4a	puno3-htlr4a	puno-mtlr4	-	-
TLR5	Toll-like Receptor 5	puno-htlr5	puno3-htlr5	puno-mtlr5	puno1-ptlr5	puno1-btlr5
TLR6	Toll-like Receptor 6	puno-htlr6	puno3-htlr6	puno-mtlr6	puno1-ptlr6	puno1-btlr6
TLR7	Toll-like Receptor 7	puno-htlr7	puno3-htlr7	puno-mtlr7	puno1-ptlr7	puno1-btlr7
TLR8	Toll-like Receptor 8	puno1-htlr8b	puno3-htlr8b	puno-mtlr8	-	-
TLR9	Toll-like Receptor 9	puno1-htlr9a	puno3-htlr9a	puno-mtlr9	puno1-ptlr9	puno1-btlr9
TLR10	Toll-like Receptor 10	puno-htlr10	puno3-htlr10	-	puno1-ptlr10	-
TLR11	Toll-like Receptor 11	-	-	puno-mtlr11t	-	-
TLR11/12	Toll-like Receptor 11/12	-	-	puno-mtlr11z	-	-
TLR13	Toll-like Receptor 13	-	-	puno-mtlr13	-	-

GENE NAME/ALIASES	DESCRIPTION	CAT. CODE (human)	CAT. CODE (mouse)
NOD-Like Receptors (NLRs)			
IPAF / CARD12	Flagellin receptor - Caspase-1-activating protein	puno-hcard12	-
NAIP5 / BIRC1E	Flagellin receptor - Caspase-1-activating protein	-	puno1-mnaip5
NALP1 / CARD7	Anthrax toxin receptor - Caspase-1-activating protein	puno-hnalp1a	-
NALP2	Caspase-1-activating protein	puno-hnalp2	-
NALP3 / NLRP3	Receptor of various ligands - Caspase-1-activating protein	puno-hnalp3a	-
NALP12 / Monarch-1	Negative regulator of inflammation	puno-hnalp12	-
NOD1 / CARD4	PGN receptor	puno-hnod1	puno-mnod1
NOD2 / CARD15	PGN receptor	puno-hnod2a	puno-mnod2a
NOD9 / NLRX1	Mitochondrial PRR modulator	puno1-hnod9a	puno1-mnod9
RIG-I-Like-Receptors (RLRs) and Cytosolic DNA Sensors (CDS)			
AIM2 / IFI210	Cytoplasmic DNA receptor - Caspase-1-activating protein	puno1-haim2	puno1-maim2
DAI / ZBP1	B-DNA binding protein	puno1-hzbp1	puno1-mzbp1a
IFI16	Cytosolic DNA sensor	puno1-hifi16	puno1-mifi16
LGP2	Negative regulator of RIG-I and MDA-5	puno1-hlgp2	puno1-mlgp2
MDA5 / IFIH1	dsRNA receptor	puno1-hmda5	puno1-mmda5
RIG-I / DDX58	dsRNA receptor	puno-hrigi	puno-mrigi
C-Type Lectin Receptors (CLRs)			
CLEC9A	C-type lectin-like receptor - Ligand unknown	puno1-hclec9a	puno1-mclec9a
DC-SIGN / CD209	Mannose-binding C-type lectin	puno-hdcsign1a	puno-mdcsign
Dectin-1	Beta-glucan receptor	puno-hdectin1b	puno-mdectin1
L-SIGN	Mannose-binding C-type lectin	puno-hdcsign2a	-
MBL1, 2	Mannose binding lectins	-	puno-mmbl1/2
Mincle / CLEC4E	Macrophage-inducible C-type lectin	puno1-hmincle	puno1-mmincle
PGPR-L, -S	Peptidoglycan recognition protein, long or short	puno-hpgrps	puno-mpgrpl/s
PGPR-1A	Peptidoglycan Recognition protein 1 α	puno-hpgrp1a	-
SIGNR1, 2, 3, 4	DC-SIGN related proteins 1, 2, 3, 4	-	puno-msignr1/2/3/4
Adaptors			
ASC / PYCARD / CARD5	NLR adaptor protein	puno-hasca	puno1-masc
Cardinal / CARD8	NALP3 adaptor protein	porf-hcard8	-
IPS1 / MAVS / VISA	RLR adaptor protein	puno-hips1	puno-mips1
MyD88	TLR/IL-1R adaptor protein	puno-hmyd88	puno-mmyd88
RAC1	Regulator of TLR2 and TLR4 signaling	-	puno-mrac1
SARM1	Specific inhibitor of TRIF-dependent TLR signaling	puno-hsarm1a	puno-msarm1b
TIRAP / Mal	Adaptor protein of MyD88-dependent TLR signaling	puno-htirap	puno-mtirap
TRAM / TICAM2	Adaptor protein of MyD88-independent TLR4 signaling	puno-htram	puno-mtram
TRIF / TICAM1	Adaptor protein of MyD88-independent TLR3/4 signaling	puno2-htrif	puno-mtrif
Co-receptors			
CD14	Membrane-associated co-receptor of TLR4	puno-hcd14	puno-mcd14
CD36	Scavenger receptor interacting with TLR2	puno3-hcd36	puno3-hcd36
LBP	LPS Binding Protein	puno-hlbp	puno-mlbp
MD2 / Ly96	Accessory molecule for LPS-induced TLR4 signaling	puno-hmd2	puno-mmd2
PRAT4A, 4B	Regulators of cell surface expression of TLR4	puno1-hprat4a/4b	puno1-mprat4a/4b
PRR Signaling Effectors			
BCL10 / CLAP	NF-κB activator	puno1-hbcl10	puno1-mbcl10
CARD9	Mediator of NOD2 & Dectin-1 signaling	puno-hcard9	puno-mcard9
DDX3 / DDX3X	Mediator of TBK1/IKKε signaling	-	puno-mddx3x
FADD / MORT1	Effector of Fas-mediated apoptosis	puno-hfadd	puno-mfadd
IKKα / IKBKA	Subunit alpha of IkappaB Kinase	puno-hikka	puno-mikka
IKKβ / IKBKB	Subunit beta of IkappaB Kinase	puno-hikkb	puno-mikkb
IKKε / IKBKE	Effector of RIG-I/MDA-5-induced activation of IRF	puno-hikke	puno-mikke
IRAK-1	Signal transduction mediator for TLR signaling	puno-hirak1	puno-mirak1

GENE NAME/ALIASES	DESCRIPTION	CAT. CODE (human)	CAT. CODE (mouse)
PRR Signaling Effectors			
IRAK-4	Signal transduction mediator for TLR signaling	puno-hirak4	puno-mirak4
ITCH	p38 and JNK activator and NF-κB inhibitor	puno1-hitch	puno1-mitch
MAPK1	p38 and JNK activator	puno1-hmapk1	-
MAPK2	p38 and JNK activator	puno1-hmapk2	puno1-mmapk2
NAP1 / AZI2	Regulatory subunit of TBK1/IKKε	puno-hnap1	puno-mnap1
NEMO / IKKγ	Regulatory subunit of IKK complex	puno-hnemo	puno-mnemo
Pellino1	Co-factor of IL-1-mediated signaling	puno-hpeli1	puno-mpeli1
Pellino2	Modulator of IL-1 and LPS signaling	puno-hpeli2	puno-mpeli2
Pellino3	Promoter of c-Jun and Elk-1 activation	puno-hpeli3a	-
PKR	Mediator in dsRNA-induced TLR3 and LPS-induced TLR4 signaling	puno-hpkr	puno-mpkr
PRKRA / PACT	Modulator of PKR activity	puno-hprkra	puno-mprkra
RIP1 / RIPK1	Mediator of TLR3-induced NF-κB activation	puno-hripk1	puno-mripk1
RIP2 / RIPK2 / RICK	Signal transducer for TLRs and NODs	puno-hrick	puno-mrick
RIP3 / RIPK3	Modulator of NF-κB activation	puno-hripk3	puno-mripk3
STING	Stimulator of interferon genes	puno1-hsting	puno1-msting
SUGT1	Regulator of NOD1 activation	puno1-hsugt1b	puno1-msugt1
Syk	Mitochondrial PRR modulator	puno1-hsyk	puno1-msyk
TAB1, 2, 3	TAK1 binding proteins 1, 2 ,3 - Mediators of NF-κB activation	puno-htab1/2a/3	-
TAK1 / MAP3K7	Modulator of TLR3/TLR4-mediated NF-κB and AP-1 activation	puno-hmap3k7b	puno-mmap3k7b
TANK	Mediator of RLR- and TLR-induced IFN production	puno1-htank	puno1-mtank
TBK1	Mediator of RLR- and TLR-induced IFN production	puno-htbk1	puno-mtbk1
TIFA	Mediator of TRAF6/IRAK1 complexes	puno-htifa	puno-mtifa
TRADD	TNF-R1-Associated via Death Domain	puno1-htradd	puno1-mtradd
TRAF3	Mediator of TBK1 signaling	puno-htraf3	puno-mtraf3
TRAF6	Key signal transducer of TLR pathways	puno-htraf6	puno-mtraf6
UNC93B1	Multitransmembrane endoplasmic reticulum protein	puno1-hunc93b1	puno1-munc93b1
PRR Signaling Inhibitors			
ABIN1, 2, 3	A20-associating protein inhibitor of NF-κB	puno1-htnip1a/2a/3	puno1-mtnip1a/2/3
ATF3	Negative regulator of TLR signaling	puno-hatf3	puno-matf3
AXL / UFO	Negative regulator of TLR signaling	puno1-haxl	puno1-maxl
BCL3	Inhibitor of NF-κB p50 ubiquitination	puno1-hbcl3	puno1-mbcl3
DAK	Negative regulator of MDA-5	puno1-hdak	puno1-mdak
DUBA / OTUD5	TRAF3 inhibitor	-	puno1-motud5
FLII / Fliih	TLR4/MyD88 signaling complex inhibitor	puno-hflii	puno-mflii
IRAK-M	Inhibitor of IRAK-1/TRAF6 complexes	puno-hirakm	puno-mirakm
MD1	RP105 co-receptor and inhibitor TLR4/MD2 complex	puno-hmd1	puno-mmd1
MFN2	IPS-1 inhibitor	puno1-hmfn2	puno1-mmfn2
MULAN/ Dublin	Inhibitor of RIG-I/MDA-5 signaling	puno1-hmul1	puno1-mmul1
PIN1 / DOB	IRF3 inhibitor	puno-hpin1	puno-mpin1
PPP3CA	MyD88 and TRIF inhibitor - Calcineurin A (NFAT pathway)	puno1-hppp3cab	puno1-mppp3ca
PPP3CB	MyD88 and TRIF inhibitor - Calcineurin B (NFAT pathway)	puno1-hppp3cb	puno1-mppp3cb
PPP3R1	MyD88 and TRIF inhibitor	puno1-hppp3r1	puno1-mppp3r1
RNF125 / TRAC-1	Inhibitor of RIG-I signaling	puno1-hrnf125	puno1-mrnf125
RP105	Inhibitor TLR4/MD2 complex	puno-hrp105	puno-mrp105
SHP-1 / PTPN6	Negative regulator of TLR signaling	puno1-hptpn6	puno1-mptpn6
SIGIRR / TIR8	Negative regulator of TLR/IL-1R signaling	puno-hsigirr	puno-msigirr
SIKE	Physiological repressor of IKKε and TBK1	puno1-hsike	puno1-msike
T1 / ST2 / IL1RL1	Negative regulator of IL-1R and TLR2, 4, 9 signaling	puno-hil1rl1b	-
TNFAIP3 / A20	Inhibitor of TLR and RLR-induced NF-κB activation	puno-htnfaip3	puno-mtnfaip3
Tollip / IL-1RAcPIP	Inhibitor of NF-κB activation induced by IL-1R, TLR2 and TLR4	puno-htollip	puno-mtollip
TRAFD1 / FLN29	Inhibitor of LPS-induced TRAF6 function	puno-htrafd1	puno1-mtrafd1
TRIAD3A	Negative regulator of TIRAP, TRIF and RIP1	puno-htriad3a	puno-mtriad3a
Tyro3 / BYK	Negative regulator of TLR signaling	puno1-htyro3	puno1-mtyro3

GENE NAME/ALIASES	DESCRIPTION	CAT. CODE (human)	CAT. CODE (mouse)
Cytokines			
CD40L	CD40 ligand	porf-hcd40l	porf-mcd40l
FasL / CD95L	Fas ligand	porf-hfasl	porf-mfasl
GM-CSF	Granulocyte macrophage colony-stimulating factor	porf-hgmcsf	porf-mgmcsf
IFNA2	Interferon alpha 2, allele b	puno1-hifna2	-
IFNB1	Interferon beta 1	puno1-hifnb	puno1-mifnb
IFNG	Interferon gamma	puno1-hifng	puno1-mifng
IL-1β	Interleukin-1 beta	porf-hil1b	porf-mil1b
IL-2	Interleukin-2	puno1-hil2	puno1-mil2
IL-4	Interleukin-4	puno1-hil4	porf-mil4
IL-5	Interleukin-5	porf-hil5	porf-mil5
IL-6	Interleukin-6	porf-hil6	porf-mil6
IL-8 / CXCL8	Interleukin-8	porf-hil8	-
IL-10	Interleukin-10	puno1-hil10	puno1-mil10
IL-12elasti (p40::p35)	Interleukin-12	porf-hil12g2	porf-mil12
IL-13	Interleukin-13	porf-hil13	porf-mil13
IL-17	Interleukin-17, isoform A	puno1-hil17a	puno1-mil17a
IL-23elasti (p40::p19)	Interleukin-23	porf-hil23	porf-mil23
IL-28 / IFNL2	Interleukin-28A / Interferon lambda 2	puno1-hil28a	-
IL-29 / IFNL1	Interleukin-29 / Interferon lambda 1	puno1-hil29	-
IL-33	Interleukin-33	porf-hil33	porf-mil33
MIP1-α / CCL3	Macrophage inflammatory protein 1 alpha	porf-hmip1a	porf-mmip1a
RANTES / CCL5	T cell-specific protein	porf-hrantes	porf-mrantes
TGFB1	Transforming Growth Factor beta-1	porf-htgfb1	porf-mtgfb1
TNF-α	Tumor necrosis factor alpha	porf-htnfa	porf-mtnfa
TRAIL / CD253	TNF-related apoptosis-inducing ligand	porf-htrail	porf-mtrail
TRANCE	TNF-related activation-induced cytokine	porf-htrancea	puno1-mtrancea
Autophagy Inducers			
ATG3	Autophagy-related protein 3	puno1-hatg3	puno1-matg3
ATG4A	Autophagy-related protein 4A	puno1-hatg4a	puno1-matg4a
ATG4B	Autophagy-related protein 4B	puno1-hatg4b	puno1-matg4b
ATG5	Autophagy-related protein 5	puno1-hatg5	puno1-matg5
ATG7	Autophagy-related protein 7	puno1-hatg7	puno1-matg7
ATG10	Autophagy-related protein 10	puno1-hatg10	puno1-matg10
ATG16L1	Autophagy-related protein 16-like 1, isoform 1 (longest)	puno1-hatg16l1	puno1-matg16l1
BECLIN-1	Coiled-coil myosin-like BCL2-interacting protein	puno1-hbecn1	puno1-mbecn1
LC3A	Microtubule-associated protein 1 light chain 3 alpha	puno1-hlc3a	puno1-mlc3a
LC3B	Microtubule-associated protein 1 light chain 3 beta	puno1-hlc3b	puno1-mlc3b

Recent articles using InvivoGen's pUNO plasmids

pUNO-hMD2 - Matsunaga N. et al., 2011. TAK-242 (Resatorvid), a Small-Molecule Inhibitor of Toll-Like Receptor (TLR) 4 Signaling, Binds Selectively to TLR4 and Interferes with Interactions between TLR4 and Its Adaptor Molecules. Mol. Pharmacol., 79: 34 - 41.

pUNO-hNOD1 & pUNO-hNOD2 - Okugawa T. *et al.,* 2010. NOD1 and NOD2 Mediate Sensing of Periodontal Pathogens. Journal of Dental Research, 89: 186 - 191.

pUNO-hRIG-I & pUNO-hMDA5 - Subba-Reddy CV. *et al.,* 2011. VPg-Primed RNA Synthesis of Norovirus RNA-Dependent RNA Polymerases by Using a Novel Cell-Based Assay. J. Virol., 85: 13027 - 13037.

pUNO-mRIG-I - Luke JM. *et al.,* 2011. Coexpressed RIG-I Agonist Enhances Humoral Immune Response to Influenza Virus DNA Vaccine. J. Virol., 85(3): 1370 - 1383.

pUNO-hSIGIRR - Khan MA. *et al.,* 2010. The Single IgG IL-1–Related Receptor Controls TLR Responses in Differentiated Human Intestinal Epithelial Cells. J. Immunol., 184: 2305 - 2313.

pUNO-hTLR3 & pUNO-hTLR4 - Salaun B. et al., 2011. TLR3 as a Biomarker for the Therapeutic Efficacy of Double-stranded RNA in Breast Cancer. Cancer Res., 71: 1607 - 1614.

pUNO-mTLR7 & pUNO-mTLR8 - Martinez J. *et al.,* 2010. Toll-like receptor 8-mediated activation of murine plasmacytoid dendritic cells by vaccinia viral DNA. PNAS, 107: 6442 - 6447.

pUNO-hTNFAIP3 - Billmann-Born S. et al., 2011. Genome-Wide Expression Profiling Identifies an Impairment of Negative Feedback Signals in the Crohn's Disease-Associated NOD2 Variant L1007fsinsC. J. Immunol., 186: 4027 - 4038.

pDUO - Gene Associations

Features and Benefits

pDUO is a mammalian expression vector containing two transcription units allowing the co-expression of two TLR or TLR-related genes.

pDUO plasmids feature two strong composite promoters derived from the ferritin light chain (FerL) and heavy chain (FerH) core promoters. Both promoters work concomitantly to express Ferritin, a ubiquitous protein, therefore eliminating potential transcriptional interferences.

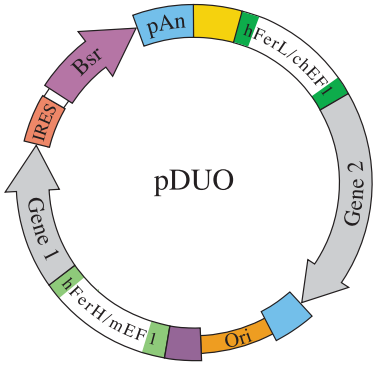
Each pDUO can be used for transient or stable transfection experiments. Most pDUO plasmids are selectable with blasticidin in both *E. coli* and mammalian cells. Some are selectable with hygromycin (pDUO2).

Contents and Storage

Each pDUO 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 1 year when properly stored.
Each pDUO plasmid is provided with 4 pouches of the appropriate *E.coli* Fast-Media® (2 TB and 2 Agar).

Related Products

Blasticidin, page 60
Hygromycin B, page 61
Fast-Media® Blas, page 22
Fast-Media® Hygro, page 22



TLR Associations Available in pDUO Plasmids

TLR/TLR Associations
- TLR1/TLR2
- TLR6/TLR2

TLR/Co-receptor Associations
- CD14/TLR2
- CD14/TLR4
- MD2/TLR4

Co-receptor/Co-receptor Associations
- MD2/CD14 (available in pDUO2)
- RP105/MD1

GENES	PLASMID	DESCRIPTION	QTY	CAT. CODE (human)	CAT. CODE (mouse)
CD14/TLR2	pDUO-CD14-TLR2	CD14 with Toll-like receptor 2	*E. coli* disk	pduo-hcd14tlr2	pduo2-mcd14tlr2
CD14/TLR4	pDUO-CD14-TLR4	CD14 with Toll-like receptor 4	*E. coli* disk	pduo-hcd14tlr4	pduo-mcd14tlr4
MD2/CD14	pDUO2-MD2-CD14 (hygro)	MD2 with CD14	*E. coli* disk	pduo2-hmd2cd14	pduo2-mmd2cd14
MD2/TLR4	pDUO-MD2-TLR4	MD2 with Toll-like receptor 4	*E. coli* disk	pduo-hmd2tlr4	pduo-mmd2tlr4
RP105/MD1	pDUO-RP105/MD1	RP105 (CD180) with MD1	*E. coli* disk	pduo-hrp105md1	pduo-mrp105md1
TLR1/TLR2	pDUO-TLR1/TLR2	Toll-like receptors 1 and 2	*E. coli* disk	pduo-htlr12	pduo-mtlr12
TLR6/TLR2	pDUO-TLR6/TLR2	Toll-like receptors 6 and 2	*E. coli* disk	pduo-htlr62	pduo-mtlr62

Recent articles using InvivoGen's pDUO, pUNO-HA or pUNO-TLR-GFP plasmids

pDUO-mTLR1/TLR2 - Geng D. *et al.,* 2010. Amplifying TLR-MyD88 Signals within Tumor-Specific T Cells Enhances Antitumor Activity to Suboptimal Levels of Weakly Immunogenic Tumor Antigens. Cancer Res., 70: 7442 - 7454.

pDUO-hMD2/TLR4 - Smythies LE. *et al.*, 2010. Inflammation Anergy in Human Intestinal Macrophages Is Due to Smad-induced I{kappa}B{alpha} Expression and NF-{kappa}B Inactivation. J. Biol. Chem., 285: 19593 - 19604.

pUNO-hTLR9-HA - Michiel van Gent M. *et al.,* 2011. EBV Lytic-Phase Protein BGLF5 Contributes to TLR9 Downregulation during Productive Infection. J. Immunol., Feb 2011; 186: 1694 - 1702.

pUNO-hTLR3-HA, pUNO-hTLR7-HA & pUNO-hTLR8-HA
Kuznik A. *et al.,* 2011. Mechanism of Endosomal TLR Inhibition by Antimalarial Drugs and Imidazoquinolines. J. Immunol., 186: 4794 - 4804.

pUNO-hTLR3-HA, pUNO-hTLR5-HA & pUNO-hTLR9-HA
Avbelj M. *et al.*, 2011. The Role of Intermediary Domain of MyD88 in Cell Activation and Therapeutic Inhibition of TLRs. J. Immunol., 187: 2394 - 2404.

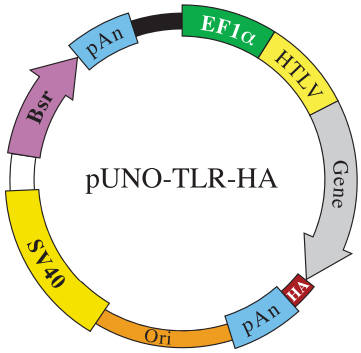
pUNO-hTLR3-GFP - Shiose S. et al., 2011. Toll-like Receptor 3 Is Required for Development of Retinopathy Caused by Impaired All-trans-retinal Clearance in Mice. J. Biol. Chem., 286: 15543 - 15555.

pUNO-TLR-HA - TLR-HA Tagged Genes

Features and Benefits

pUNO-TLR-HA is a family of mammalian expression vectors featuring HA-tagged TLR genes. The genes have been fused at the 3'end to the influenza hemagglutinine (HA) tag. This short sequence (YPYDVPDYA) encodes a peptide, which is the epitope of a very efficient and specific monoclonal antibody. The use of HA-tagged TLR genes provides a simple and convenient method to detect the expression of the TLR genes by Western blot. All TLR genes can be detected using the same primary antibody, the HA tag monoclonal antibody (see page 98).

pUNO-TLR-HA plasmids are selectable with blasticidin in both *E. coli* and mammalian cells.



PLASMID	QTY	CAT. CODE (human)	CAT. CODE (mouse)
pUNO-TLR1-HA	*E. coli* disk	punoha-htlr1	punoha-mtlr1
pUNO-TLR2-HA	*E. coli* disk	punoha-htlr2	punoha-mtlr2
pUNO-TLR3-HA	*E. coli* disk	punoha-htlr3	punoha-mtlr3
pUNO-TLR4-HA	*E. coli* disk	punoha-htlr4a	punoha-mtlr4
pUNO-TLR5-HA	*E. coli* disk	punoha-htlr5	punoha-mtlr5
pUNO-TLR6-HA	*E. coli* disk	punoha-htlr6	punoha-mtlr6
pUNO-TLR7-HA	*E. coli* disk	punoha-htlr7	punoha-mtlr7
pUNO-TLR8-HA	*E. coli* disk	punoha-htlr8a	punoha-mtlr8
pUNO-TLR8-HA	*E. coli* disk	punoha-htlr9a	punoha-mtlr9
pUNO-TLR10-HA	*E. coli* disk	punoha-htlr10	-
pUNO-TLR11-HA	*E. coli* disk	-	punoha-mtlr11

Contents and Storage

Each pUNO-TLR-HA 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 1 year when properly stored.
Each plasmid is provided with 4 pouches of *E.coli* Fast-Media® Blas (2 TB and 2 Agar). For more information about Fast-Media®, see page 22.

Related Products

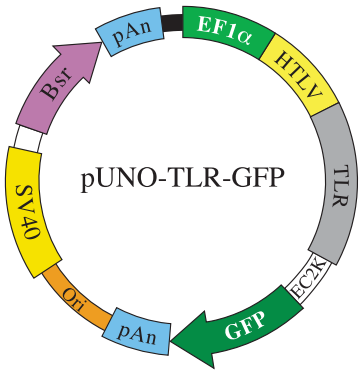
Blasticidin, page 60

Fast-Media® Blas, page 22

pUNO-TLR-GFP - TLR-GFP Fusion Genes

Features and Benefits

TLR-GFP fusion proteins can be used to study the localization of the TLRs. Transfected cells can be analyzed for GFP expression by flow cytometry and by Western-blotting using GFP antibodies.
TLR-GFP fusion genes were generated by fusing the GFP gene to the C terminus of various human TLR genes (TLR1 to TLR6). A synthetic intron was added between both moieties to increase the activity of GFP.
The TLR-GFP fusion genes are available in the pUNO plasmid selectable with blasticidin in both *E. coli* and mammalian cells.
All TLR::GFP fusion genes have been fully sequenced, their fluorescence confirmed and their function tested in HEK293 cells coexpressing an NF-κB reporter plasmid and stimulated with the appropriate ligand.



PLASMID	QTY	CAT. CODE
pUNO-hTLR1-GFP	*E. coli* disk	phtlr1-gfp
pUNO-hTLR2-GFP	*E. coli* disk	phtlr2-gfp
pUNO-hTLR3-GFP	*E. coli* disk	phtlr3-gfp
pUNO-hTLR4-GFP	*E. coli* disk	phtlr4-gfp
pUNO-hTLR5-GFP	*E. coli* disk	phtlr5-gfp
pUNO-hTLR6-GFP	*E. coli* disk	phtlr6-gfp

Contents and Storage

Each pUNO-TLR-GFP 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 1 year when properly stored.
Each plasmid is provided with 4 pouches of *E. coli* Fast-Media® Blas (2 TB and 2 Agar, see page 22).

pZERO-TLR - TLR-ΔTIR Genes

Features and Benefits

TLR-ΔTIR Genes

TLR-ΔTIR genes were generated by deleting a fragment of approximately 500 bp located at the 3' end of each TLR gene corresponding to the TIR domain. These truncated genes are still able to recognize their ligands but are unable to induce the signaling cascade. TLR-ΔTIR genes act as dominant negative mutants.

TLR-ΔTIR genes are provided in the pZERO plasmid. They are cloned downstream of the strong and ubiquitous mammalian promoter EF1α/HTLV. pZERO plasmids are selectable with puromycin in both *E. coli* and mammalian cells. Therefore, they can be cotransfected with a pUNO or pDUO plasmid (see pages 23 and 27, respectively)

HA-Tagged TLR-ΔTIR Genes

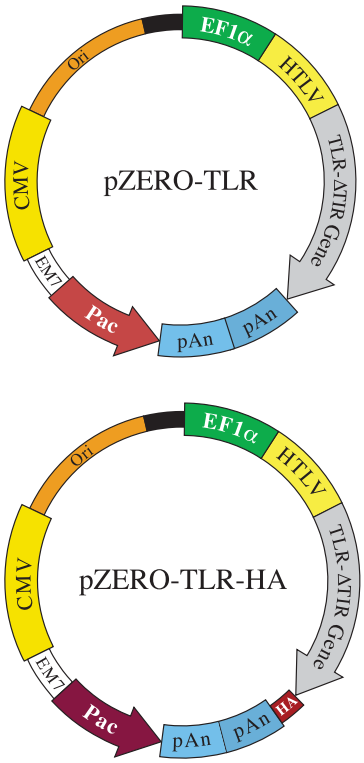
TLR-ΔTIR genes are available with the influenza hemagglutinine (HA) tag fused at their 3' end. The addition of the HA tag facilitates their detection by Western blot. All TLR-ΔTIR-HA proteins can be detected using the same antibody (anti-HAtag antibody, see page 98).

TLR-ΔTIR-HA genes are also provided in the pZERO plasmid.

PLASMID	QTY	CAT. CODE (human)	CAT. CODE (mouse)
DN TLR Genes			
pZERO-TLR1	*E. coli* disk	pzero-htlr1	pzero-mtlr1
pZERO-TLR2	*E. coli* disk	pzero-htlr2	pzero-mtlr2
pZERO-TLR3	*E. coli* disk	pzero-htlr3	pzero-mtlr3
pZERO-TLR4	*E. coli* disk	pzero-htlr4	pzero-mtlr4
pZERO-TLR5	*E. coli* disk	pzero-htlr5	pzero-mtlr5
pZERO-TLR6	*E. coli* disk	pzero-htlr6	pzero-mtlr6
pZERO-TLR7	*E. coli* disk	pzero-htlr7	pzero-mtlr7
pZERO-TLR8	*E. coli* disk	pzero-htlr8	pzero-mtlr8
pZERO-TLR9	*E. coli* disk	pzero-htlr9	pzero-mtlr9
pZERO-TLR10	*E. coli* disk	pzero-htlr10	-
HA-Tagged DN TLR Genes			
pZERO-TLR1-HA	*E. coli* disk	pzero-htlr1-ha	pzero-mtlr1-ha
pZERO-TLR2-HA	*E. coli* disk	pzero-htlr2-ha	pzero-mtlr2-ha
pZERO-TLR3-HA	*E. coli* disk	pzero-htlr3-ha	pzero-mtlr3-ha
pZERO-TLR4-HA	*E. coli* disk	pzero-htlr4-ha	pzero-mtlr4-ha
pZERO-TLR5-HA	*E. coli* disk	pzero-htlr5-ha	pzero-mtlr5-ha
pZERO-TLR6-HA	*E. coli* disk	pzero-htlr6-ha	pzero-mtlr6-ha
pZERO-TLR7-HA	*E. coli* disk	pzero-htlr7-ha	pzero-mtlr7-ha
pZERO-TLR8-HA	*E. coli* disk	pzero-htlr8-ha	pzero-mtlr8-ha
pZERO-TLR9-HA	*E. coli* disk	pzero-htlr9-ha	pzero-mtlr9-ha
pZERO-TLR10-HA	*E. coli* disk	pzero-htlr10-ha	-

Related Products

Puromycin, page 61
Fast-Media® Puro, page 22
Anti-HAtag antibody, page 98



Contents and Storage

pZERO-TLR and pZERO-TLR-HA plasmids are provided as lyophilized transformed *E. coli* strains on 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 1 year when properly stored.

Each plasmid is provided with 4 pouches of *E.coli* Fast-Media® Puro (2 TB and 2 Agar). For more information about Fast-Media®, see page 22.

Recent articles using pZERO or pDeNy

pZERO-hTLR3 - Galli R.. *et al.*, 2010. TLR Stimulation of Prostate Tumor Cells Induces Chemokine-Mediated Recruitment of Specific Immune Cell Types. J. Immunol., 184: 6658 - 6669.

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

pDeNy-mIRAK1 & pDeNy-mTRAF6 - Zivkovic A. *et al.*, 2011. TLR 2 and CD14 Mediate Innate Immunity and Lung Inflammation to Staphylococcal Panton–Valentine Leukocidin In Vivo. J. Immunol., 186: 1608 - 1617.

pDeNy-hTIRAP & pDeNy-hTRAM - Zughaier SM., 2011. Neisseria meningitidis capsular polysaccharides induce inflammatory responses via TLR2 and TLR4-MD-2. J. Leukoc. Biol., 89: 469 - 480.

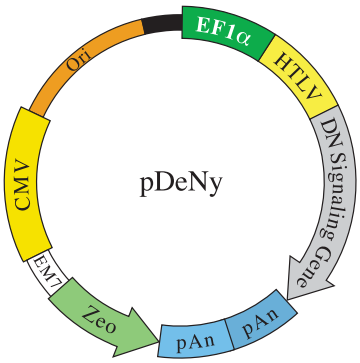
pDeNy-hMyD88 - Keestra AM. *et al.*, 2011. A Salmonella Virulence Factor Activates the NOD1/NOD2 Signaling Pathway. mBio, 2: e00266-11.

pDeNy-hTRIF - Choi YJ. *et al.*, 2010. TRIF Modulates TLR5-dependent Responses by Inducing Proteolytic Degradation of TLR5. J. Biol. Chem., 285: 21382 - 21390.

pDeNy - Dominant Negative Variants

Features and Benefits

Dominant negative (DN) forms of TLR signaling genes were created by inserting a mutation within the gene and/or deleting a region of the gene. These modifications have been described elsewhere (see table) and shown to block the wild-type form of these genes.
To facilitate the excision and subcloning of DN genes from pDeNy to another plasmid, each gene is flanked by unique restriction sites that are compatible with many others.
Each pDeNy plasmid can be used for transient or stable transfection experiments. pDeNy plasmids are selectable with Zeocin™ in both *E. coli* and mammalian cells. Therefore, they can be cotransfected with a pUNO plasmid, and selection of stable clones expressing both plasmids achieved with addition of Zeocin™ and blasticidin in the culture medium.



PLASMID	SPECIES	MUTATION/ DELETION	CAT. CODE
pDeNy-hIRAK	human	aa1-211	pdn-hirak
pDeNy-hMyD88	human	aa161-296	pdn-hmyd88
pDeNy-mMyD88	mouse	aa161-296	pdn-mmyd88
pDeNy-hNOD1	human	aa127-518	pdn-hnod1
pDeNy-hNOD2	human	L145P	pdn-hnod2
pDeNy-hPKR	human	aa361-366	pdn-hpkr
pDeNy-hRIG-I	human	aa1-217	pdn-hrigi
pDeNy-hTIRAP	human	P125H	pdn-htirap
pDeNy-hTRAM	human	C117H	pdn-htram
pDeNy-hTRAF6	human	aa289-522	pdn-traf6
pDeNy-hTRIF	human	aa387-566 + P434H	pdn-htrif

Contents and Storage

Each pDeNy 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 1 year when properly stored.
Each plasmid is provided with 4 pouches of *E.coli* Fast-Media® Zeo (2 TB and 2 Agar). For more information about Fast-Media®, see page 22.

Related Products

Zeocin™, page 61 **Fast-Media® Zeo ,** page 22

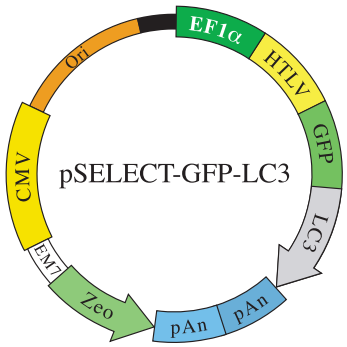
pSELECT-GFP-LC3 - Autophagy Reporter Plasmid

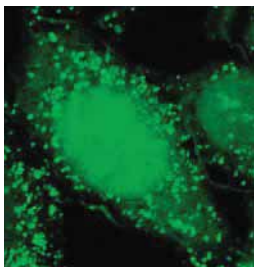
Description

pSELECT-GFP-LC3 is a mammalian expression vector containing the human LC3B gene fused at its 5' end to the green fluorescent protein (GFP) gene. The same plasmid is available with the GFP gene alone as a control. This control plasmid is called pSELECT-NGFP-zeo. Both plasmids are selectable in bacteria and mammalian cells with Zeocin™.

Application

Expression of the GFP-LC3 fusion gene allows to visualize autophagosome formation in real time in live cells. During autophagosome formation, GFP-LC3 is processed and recruited to the autophagosome membrane, where it can be imaged as cytoplasmic puncta by fluorescence microscopy (see picture). The percentage of GFP-LC3 positive cells can be determined and is indicative of autophagosome formation.





Green puncta representing autophagosome formation in cells expressing a GFP-LC3 fusion.

PRODUCT	QUANTITY	CAT. CODE
pSELECT-GFP-LC3	20 µg	psetz-gfplc3
pSELECT-NGFP-zeo	20 µg	psetz-ngfp

Contents and Storage

pSELECT-GFP-LC3 and pSELECT-NGFP-zeo are provided as 20 µg of lyophilized DNA. The plasmids can be purchased individually or together. They are shipped at room temperature and should be stored at -20°C.
Both plasmids are provided with 4 pouches of *E. coli* Fast-Media® Zeo (2 TB and 2 Agar). For more information about Fast-Media®, see page 22.

2 REPORTER CELL LINES

PRR Reporter Cells 32

Inflammasome Reporter Cells 42

Cytokine Reporter Cells 44

Reporter Cells Related Products 52

PRR REPORTER CELLS

Engineered HEK293 Cells

Cells that constitutively express a given functional TLR gene are valuable tools for many applications, such as the study of the mechanisms involved in TLR recognition or signaling, and the development of new potential therapeutic drugs. InvivoGen provides HEK293 cells stably expressing TLR or NOD genes specifically designed for monitoring the activity of these PRRs using ELISA analysis or NF-κB-inducible SEAP (secreted embryonic alkaline phosphatase) reporter systems.

- **293/TLR & 293/NOD Clones**
- **HEK-Blue™ TLR & HEK-Blue™ NOD Cells**

Immune Reporter Cells

Cells of the immune system, including lymphocytes, monocytes and macrophages, express a large repertoire of pattern recognition receptors (PRRs). InvivoGen has developed stable reporter cells in several well-established immune cell models, such as the human monocytic THP-1 cell line, the murine RAW 264.7 macrophages and the human Ramos B cells. These immune reporter cells stably express the SEAP and/or Lucia™ (see page 53) reporter genes under the control of specific inducible promoters allowing to efficiently and conveniently monitor the activity of PRR ligands.

- **THP1-XBlue™ Cells**
- **THP1-Blue™ NF-κB & THP1-Blue™ ISG Cells**
- **THP1-Lucia™ NF-κB & THP1-Dual™ (NF-κB-ISG) Cells**
- **RAW-Blue™ Cells**
- **Jurkat-Dual™ (ISG-NF-κB) Cells**
- **Ramos-Blue™ Cells**

MEF Reporter Cell Lines

InvivoGen provides murine embryonic fibroblasts (MEFs) isolated from various mouse strains and immortalized with the SV40 large antigen. They express a large repertoire of pattern recognition receptors. InvivoGen's MEFs express an NF-κB-inducible reporter (SEAP) system allowing the convenient monitoring of NF-κB activation following TLR or RLR stimulation.

- **C3H/TLR4mut & C3H/WT MEFs**
- **C57/WT MEFs**

293/TLR & 293/NOD Clones

293/TLR Clones

293/TLR clones are HEK293 cells stably transfected with a pUNO-TLR or pDUO-TLR plasmid. They can be used to determine TLR activation upon ligand stimulation by assessing IL-8 production or NF-κB activation. The latter can be evaluated by transfecting transiently or stably 293/TLR clones with pNiFty, a family of NF-κB inducible reporter plasmids expressing either the secreted alkaline phosphatase or luciferase reporter gene (see page 87).

293/TLR-HA Clones

293/hTLR-HA clones were obtained by stably transfecting HEK293 cells with a pUNO-TLR-HA plasmid. pUNO-TLR-HA plasmids express TLR genes that have been fused at the 3'end to the influenza hemaglutinine (HA) tag. Addition of this tag has no deleterious effect on the expression and function of the TLR genes. The HA tag is the epitope of a very efficient and specific monoclonal antibody (see page 98).

293/NOD Clones

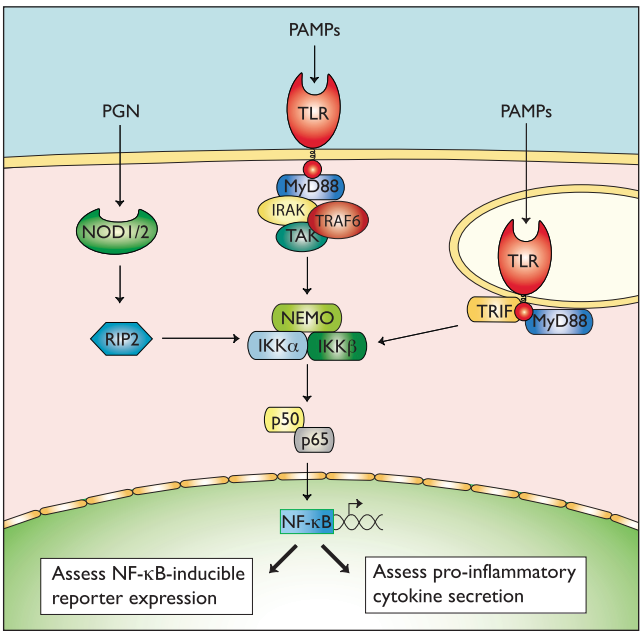
293/NOD clones are transfected HEK293 cells that express stably the NOD1 or NOD2 genes. These clones can be used to study NOD1 and NOD2 activation pathways following stimulation with their cognate ligand by assessing IL-8 production or NF-κB activation. NF-κB activation can be assessed using an NF-κB-inducible reporter system such as pNiFty.

293/Control Clones

293/Control clones were generated by stably transfecting HEK293 cells with a pUNO control plasmid expressing either the lacZ reporter gene (293/LacZ) or a multiple cloning site (293/null).

Contents

All 293 clones are grown in standard DMEM medium with 10% FBS, 2mM L-glutamine supplemented with blasticidin (10 μg/ml). Cells are provided frozen in a cryotube containing 5-7 x 10^6 cells and supplied with 100 μl of blasticidin at 10 mg/ml. Cells are shipped on dry ice. The cells are guaranteed mycoplasma-free.



CELL LINE	CAT. CODE (human)	CAT. CODE (mouse)
293/TLR Clones		
293/MD2-CD14	293-hmd2cd14	-
293/TLR1	-	293-mtlr1
293/TLR1/2	-	293-mtlr1/2
293/TLR2	293-htlr2	293-mtlr2
293/TLR2-CD14	293-htlr2cd14	-
293/TLR2/6	293-htlr2/6	293-mtlr2/6
293/TLR3	293-htlr3	293-mtlr3
293/TLR4	293-htlr4a	293-mtlr4
293/TLR4-MD2-CD14	293-htlr4md2cd14	293-mtlr4md2cd14
293/TLR5	293-htlr5	293-mtlr5
293/TLR5-CD14	293-htlr5cd14	-
293/TLR6	-	293-mtlr6
293XL/TLR7*	293xl-htlr7	293xl-mtlr7
293XL/TLR8A*	293xl-htlr8	-
293/TLR9	-	293-mtlr9
293XL/TLR9A*	293xl-htlr9	-
293/hTLR-HA Clones		
293/hTLR1-HA	293-htlr1ha	-
293/hTLR2-HA	293-htlr2ha	-
293/hTLR3-HA	293-htlr3ha	-
293/hTLR4-HA	293-htlr4ha	-
293/hTLR5-HA	293-htlr5ha	-
293/hTLR6-HA	293-htlr6ha	-
293XL/hTLR7-HA*	293xl-htlr7ha	-
293XL/hTLR8-HA*	293xl-htlr8ha	-
293XL/hTLR9-HA*	293xl-htlr9ha	-
293/hTLR10-HA	293-htlr10ha	-
293/NOD Clones		
293/NOD1	293-hnod1	293-mnod1
293/NOD2	293-hnod2	293-mnod2
293/Control Clones		
293/LacZ	293-lacz	-
293/null	293-null	-
293XL/null*	293xl-null	-

**293XL clones express the human anti-apoptotic Bcl-XL gene.*

Related Products

Blasticidin, page 60
TLR Ligands, pages 64-67
pNiFty, page 87
NOD Ligands, page 67-68

HEK-Blue™ TLR & HEK-Blue™ NOD Cells

InvivoGen introduces HEK-Blue™ TLR and HEK-Blue™ NOD cells, a collection of engineered cell lines designed to provide a rapid, sensitive and reliable method to screen and validate TLR and NOD agonists or antagonists. They express an NF-κB-inducible secreted embryonic alkaline phosphatase (SEAP) reporter gene that can be conveniently monitored using the SEAP detection media HEK-Blue™ Detection or QUANTI-Blue™.

HEK-Blue™ TLR cells

HEK-Blue™ TLR cells are engineered HEK293 cells that stably co-express a human or murine TLR gene and an NF-κB-inducible SEAP (secreted embryonic alkaline phosphatase) reporter gene. To increase the sensitivity to their cognate agonists, HEK-Blue™ TLR2 and HEK-Blue™ TLR4 cells were further transfected with the co-receptors CD14 and MD2/CD14, respectively.
HEK-Blue™ TLR cells are resistant to the selective antibiotics blasticidin and Zeocin™. HEK-Blue™ TLR2 and HEK-Blue™ TLR4 cells are additionally resistant to hygromycin.

HEK-Blue™ NOD cells

HEK-Blue™ NOD cells are engineered HEK293 cells that stably co-express the human and murine NOD1 or NOD2 gene and an NF-κB-inducible SEAP reporter gene.
HEK-Blue™ NOD cells are resistant to blasticidin and Zeocin™.

HEK-Blue™ Null cells

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

HEK-Blue™ Null1-derived cells:
- HEK-Blue™ hTLR2
- HEK-Blue™ hTLR3
- HEK-Blue™ hTLR5
- HEK-Blue™ hTLR8
- HEK-Blue™ hTLR9
- HEK-Blue™ mTLR9
- HEK-Blue™ hNOD1
- HEK-Blue™ mNOD1

HEK-Blue™ Null1-k-derived cells:
- HEK-Blue™ hTLR7
- HEK-Blue™ mTLR3

HEK-Blue™ Null1-v-derived cells:
- HEK-Blue™ mTLR4
- HEK-Blue™ mTLR5
- HEK-Blue™ mTLR8

HEK-Blue™ Null2-derived cells:
- HEK-Blue™ hTLR4
- HEK-Blue™ mTLR2
- HEK-Blue™ hNOD2
- HEK-Blue™ mNOD2

HEK-Blue™ Null2-k-derived cells:
- HEK-Blue™ mTLR7

Principle

Recognition of a TLR or NOD agonist by its cognate receptor triggers a signaling cascade leading to the activation of NF-κB and the production of SEAP. Reporter levels can be determined spectrophotometrically using HEK-Blue Detection or QUANTI-Blue™, both are SEAP detection media that turn purple/blue in the presence of alkaline phosphatase.

PRODUCT	CAT. CODE (human)	CAT. CODE (mouse)
HEK-Blue™ TLR Cells		
HEK-Blue™ MD2-CD14 Cells	hkb-hmdcd	-
HEK-Blue™ TLR2 Cells	hkb-htlr2	hkb-mtlr2
HEK-Blue™ TLR3 Cells	hkb-htlr3	hkb-mtlr3
HEK-Blue™ TLR4 Cells	hkb-htlr4	hkb-mtlr4
HEK-Blue™ TLR5 Cells	hkb-htlr5	hkb-mtlr5
HEK-Blue™ TLR7 Cells	hkb-htlr7	hkb-mtlr7
HEK-Blue™ TLR8 Cells	hkb-htlr8	hkb-mtlr8
HEK-Blue™ TLR9 Cells	hkb-htlr9	hkb-mtlr9
HEK-Blue™ NOD Cells		
HEK-Blue™ NOD1 Cells	hkb-hnod1	hkb-mnod1
HEK-Blue™ NOD2 Cells	hkb-hnod2	hkb-mnod2
HEK-Blue™ Null Cells		
HEK-Blue™ Null1 Cells	hkb-null1	-
HEK-Blue™ Null1-k Cells	hkb-null1k	-
HEK-Blue™ Null1-v Cells	hkb-null1v	-
HEK-Blue™ Null2 Cells	hkb-null2	-
HEK-Blue™ Null2-k Cells	hkb-null2k	-

Quality Control

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

Note: HEK-Blue™ TLR, HEK-Blue™ NOD and HEK-Blue™ Null cells express endogenous levels of TLR3, TLR5 and NOD1.

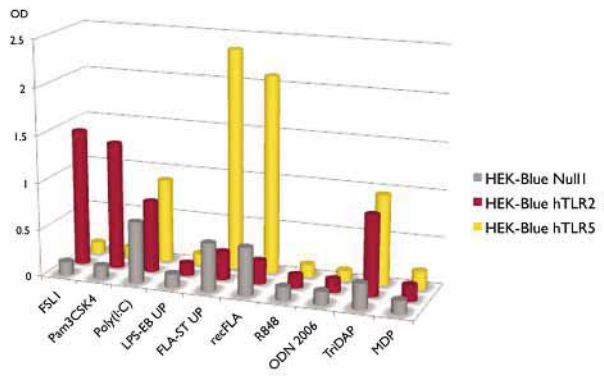
Contents and Storage

HEK-Blue™ TLR, HEK-Blue™ NOD and HEK-Blue™ Null cells are grown in DMEM medium, 2mM L-glutamine, 10% FBS and supplemented with 100 µg/ml Zeocin™, 30 µg/ml blasticidin and/or 200 µg/ml HygroGold™ (ultra-pure hygromycin) depending on the cell line. Cells are provided frozen in a cryotube containing 5-7 x 10^6 cells and supplied with the corresponding selective antibiotic(s), 1 ml Normocin™ (50 mg/ml) and 1 pouch of HEK-Blue™ Detection. Cells are shipped on dry ice.

Related Products

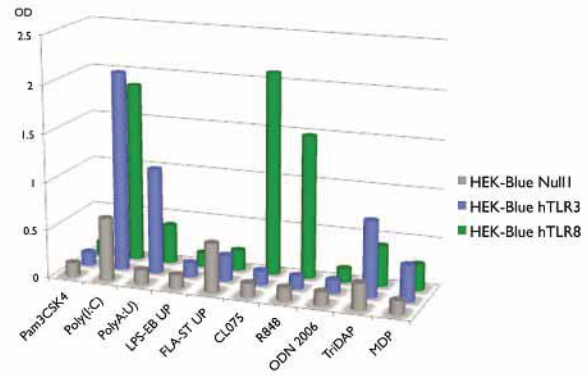
HEK-Blue™ Detection, page 56
QUANTI-Blue™, page 56
Blasticidin, page 60
HygroGold™, page 61
TLR Ligands, pages 64-67
NOD Ligands, pages 67-68
Zeocin™, page 61
Normocin™, page 59

HEK-Blue™ hTLR2 & HEK-Blue™ hTLR5 cells



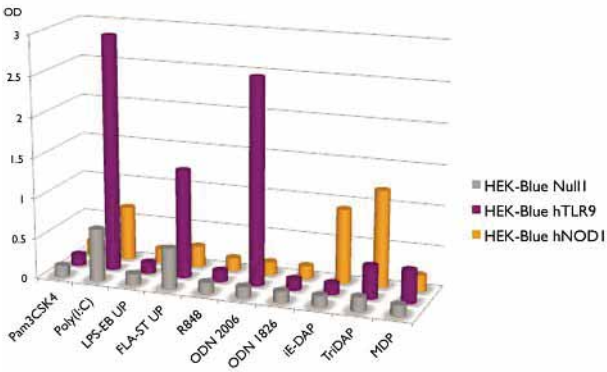
Response of HEK-Blue™ hTLR2 and HEK-Blue™ hTLR5 cells to a set of TLR/NOD agonists. Cells were incubated in HEK-Blue™ Detection medium and stimulated with 0.1 ng/ml FSL-1 (TLR2/6), 0.1 ng/ml Pam3CSK4 (TLR1/2), 100 ng/ml poly(I:C) (TLR3), 1 µg/ml LPS-EB UP (TLR4), 100 ng/ml FLA-ST UP (TLR5), 100 ng/ml recFLA (TLR5), 10 µg/ml R848 (TLR7/8), 10 µg/ml ODN 2006 (TLR9), 10 µg/ml TriDAP (NOD1) or 10 µg/ml MDP (NOD2). After 24h incubation, the levels of NF-κB-induced SEAP were determined by reading the OD at 655 nm.

HEK-Blue™ hTLR3 & HEK-Blue™ hTLR8 cells



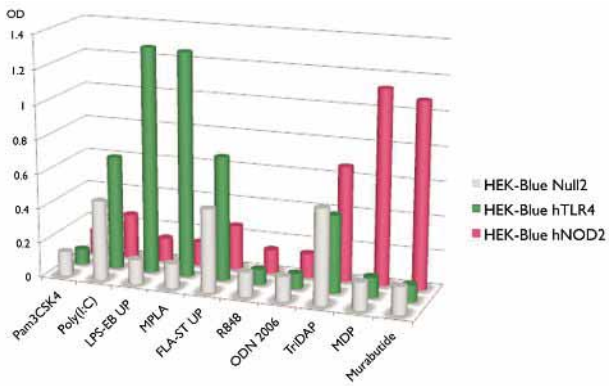
Response of HEK-Blue™ hTLR3 and HEK-Blue™ hTLR8 cells to a set of TLR/NOD agonists. Cells were incubated in HEK-Blue™ Detection medium and stimulated with 1 µg/ml Pam3CSK4 (TLR1/2), 100 ng/ml poly(I:C) (TLR3), 1 µg/ml poly(A:U) (TLR3), 1 µg/ml LPS-EB UP (TLR4), 100 ng/ml FLA-ST UP (TLR5), 1 µg/ml CL075 (TLR7/8), 1 µg/ml R848 (TLR7/8), 10 µg/ml ODN 2006 (TLR9), 10 µg/ml TriDAP (NOD1) or 10 µg/ml MDP (NOD2). After 24h incubation, the levels of NF-κB-induced SEAP were determined by reading the OD at 655 nm.

HEK-Blue™ hTLR9 & HEK-Blue™ hNOD1 cells



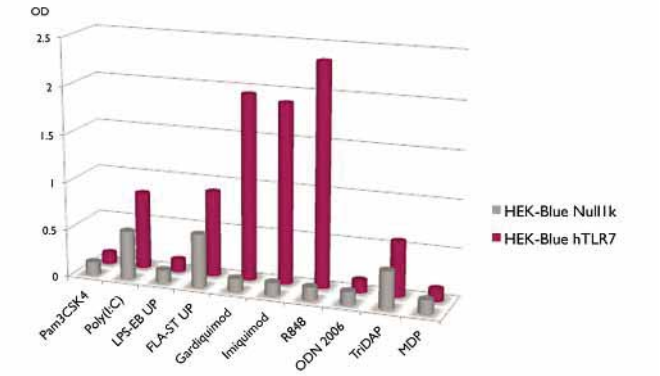
Response of HEK-Blue™ hTLR9 and HEK-Blue™ hNOD1 cells to a set of TLR/NOD agonists. Cells were incubated in HEK-Blue™ Detection medium and stimulated with 1 µg/ml Pam3CSK4 (TLR1/2), 100 ng/ml poly(I:C) (TLR3), 1 µg/ml LPS-EB UP (TLR4), 100 ng/ml FLA-ST UP (TLR5), 10 µg/ml R848 (TLR7/8), 10 µg/ml ODN 2006 (hTLR9), 10 µg/ml ODN 1826 (mTLR9), 10 µg/ml iE-DAP (NOD1), 1 µg/ml TriDAP (NOD1) or 10 µg/ml MDP (NOD2). After 24h incubation, the levels of NF-κB-induced SEAP were determined by reading the OD at 655 nm.

HEK-Blue™ hTLR4 & HEK-Blue™ hNOD2 cells



Response of HEK-Blue™ hTLR4 and HEK-Blue™ hNOD2 cells to a set of TLR/NOD agonists. Cells were incubated in HEK-Blue™ Detection medium and stimulated with 1 µg/ml Pam3CSK4 (TLR1/2), 100 ng/ml poly(I:C) (TLR3), 100 ng/ml LPS-EB UP (TLR4), 100 ng/ml MPLA (TLR4), 1 µg/ml FLA-ST UP (TLR5), 1 µg/ml R848 (TLR7/8), 10 µg/ml ODN 2006 (TLR9), 10 µg/ml TriDAP (NOD1), 10 µg/ml MDP (NOD2) or 10 µg/ml Murabutide (NOD2). After 24h incubation, the levels of NF-κB-induced SEAP were determined by reading the OD at 655 nm.

HEK-Blue™ hTLR7 cells



Response of HEK-Blue™ hTLR7 cells to a set of TLR/NOD agonists. Cells were incubated in HEK-Blue™ Detection medium and stimulated with 1 µg/ml Pam3CSK4 (TLR1/2), 100 ng/ml poly(I:C) (TLR3), 1 µg/ml LPS-EB UP (TLR4), 100 ng/ml FLA-ST UP (TLR5), 5 µg/ml Gardiquimod (TLR7), 5 µg/ml Imiquimod (TLR7), 100 ng/ml R848 (TLR7/8), 10 µg/ml ODN 2006 (TLR9), 10 µg/ml TriDAP (NOD1) or 10 µg/ml MDP (NOD2). After 24h incubation, the levels of NF-κB-induced SEAP were determined by reading the OD at 655 nm.

Recent articles using HEK-Blue™ Cells

HEK-Blue™ hTLR2 & HEK-Blue™ hTLR4 cells

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

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.

Jun 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.

HEK-Blue™ hTLR2 cells

O'Connell CM. *et al.*, 2011. Toll-Like Receptor 2 Activation by Chlamydia trachomatis Is Plasmid Dependent, and Plasmid-Responsive Chromosomal Loci Are Coordinately Regulated in Response to Glucose Limitation by C. trachomatis but Not by C. muridarum. Infect. Immun., 79: 1044 - 1056.

Konieczna P. *et al.*, 2012. Bifidobacterium infantis 35624 administration induces Foxp3 T regulatory cells in human peripheral blood: potential role for myeloid and plasmacytoid dendritic cells. Gut, 61: 354 - 366.

THP1-XBlue™ Cells - NF-κB/AP-1 SEAP Reporter Monocytes

THP1-XBlue™ cells are derived from THP-1, a human immune cell line that naturally expresses most TLRs. They stably express an NF-κB/AP-1-inducible reporter (SEAP) system to facilitate the monitoring of TLR-induced NF-κB/AP-1 activation. Three cell lines are available: THP1-XBlue™, THP1-XBlue™-MD2-CD14, which overexpresses the co-receptors MD2 and CD14 for enhanced sensitivity, and THP1-XBlue™-defMyD characterized by a deficient MyD88 activity.

THP1-XBlue™

THP1-XBlue™ cells were obtained by stable transfection of THP-1 cells with a reporter construct expressing a secreted embryonic alkaline phosphatase (SEAP) gene under the control of a promoter inducible by the transcription factors NF-κB and AP-1. Upon TLR stimulation, THP1-XBlue™ cells induce the activation of NF-κB and AP-1 and subsequently the secretion of SEAP. The reporter protein is easily detectable and measurable when using QUANTI-Blue™, a medium that turns purple/blue in the presence of SEAP (see page 56). THP1-XBlue™ cells are resistant to the selectable marker Zeocin™.

THP1-XBlue™-MD2-CD14

THP1-XBlue™-MD2-CD14 cells derive from the THP1-XBlue™ cell line by cotranfection of the MD2 and CD14 genes. MD2 is an accessory molecule essential for LPS-induced TLR4 response. CD14 interacts with several TLRs, including TLR4 and TLR2. Its overexpression was found to increase the reponse to the majority of TLR ligands (Figure 1). THP1-XBlue™-MD2-CD14 cells are resistant to the antibiotics Zeocin™ and G418.

THP1-XBlue™-defMyD

THP1-XBlue™-defMyD cells are THP1-XBlue™ cells deficient in MyD88 activity. They are unable to respond to the activation of receptors that dependent on MyD88 signaling, such as TLR2, TLR4 and IL-1Rs. They remain responsive to MyD88-independent receptors, such as NOD1 and TNFR (Figure 2).
MyD88 deficiency in THP1-XBlue™-defMyD cells was confirmed by qRT-PCR.

Contents

THP1-XBlue™, THP1-XBlue™-MD2-CD14 and THP1-XBlue™-defMyD cells are grown in RPMI medium, 2mM L-glutamine, 10% FBS supplemented with the appropriate selective antibiotic(s): 200 µg/ml Zeocin™ for THP1-XBlue™; 200 µg/ml Zeocin™ and 250 µg/ml G418 for THP1-XBlue™-MD2-CD14; 200 µg/ml Zeocin™ and 100 µg/ml HygroGold™ for THP1-XBlue™-defMyD. Cells are provided in a vial containing 5-7 x 10^6 cells and supplied with 10 mg Zeocin™ (and 10 mg G418 or 10 mg HygroGold™) and 1 pouch of QUANTI-Blue™. Cells are shipped on dry ice. They are guaranteed mycoplasma-free.

Related Products

Zeocin™, page 61
HygroGold™, page 61
TLR Ligands, pages 64-67
QUANTI-Blue™, page 56
G418, page 60
Normocin™, page 59
NOD Ligands, pages 67-68

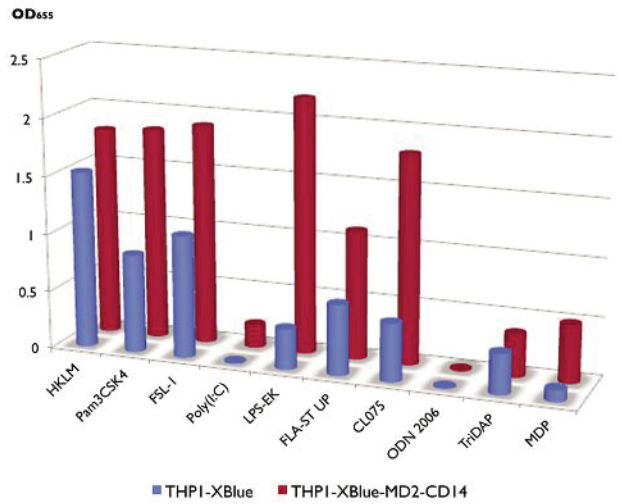


Figure 1: TLR and NOD stimulation profile in THP1-XBlue™ and THP1-XBlue™-MD2-CD14. Cells were incubated with 10^7 cells/ml HKLM (TLR2), 1 ng/ml Pam3CSK4 (TLR1/2), 1 ng/ml FSL-1 (TLR2/6), 10 µg/ml poly(I:C) (TLR3), 1 ng/ml LPS-EK (TLR4), 100 ng/ml FLA-ST (TLR5), 1 µg/ml CL075 (TLR8), 10 µg/ml ODN2006 (TLR9), 10 µg/ml Tri-DAP (NOD1) or 1 µg/ml MDP (NOD2). After 24h incubation, TLR/NOD stimulation was assessed by measuring the levels of SEAP in the supernatant by using QUANTI-Blue™.

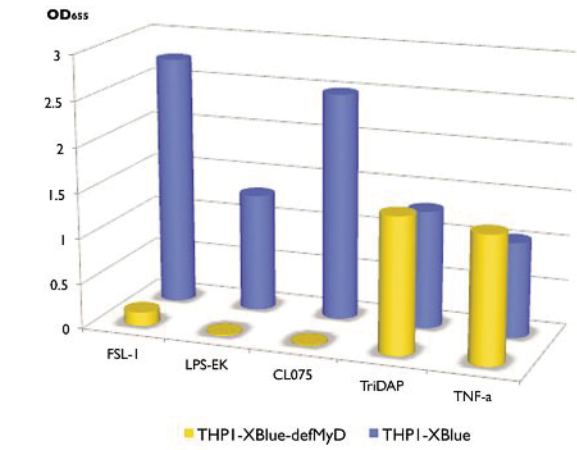


Figure 2: MyD88-dependent and -independent responses in THP1-XBlue™ and THP1-XBlue™-defMyD. Cells were incubated with 1 µg/ml FSL-1 (TLR2/6), 1 µg/ml LPS-EK (TLR4), 5 µg/ml CL075 (TLR8), 10 µg/ml Tri-DAP (NOD1) and 50 ng/ml TNF-α. After 24h incubation, NF-κB activation was determined using QUANTI-Blue™.

PRODUCT	QUANTITY	CAT. CODE
THP1-XBlue™ Cells	5-7 x 10^6 cells	thpx-sp
THP1-XBlue™-MD2-CD14 Cells	5-7 x 10^6 cells	thpx-mdcdsp
THP1-XBlue™-defMyD	5-7 x 10^6 cells	thpx-dmyd

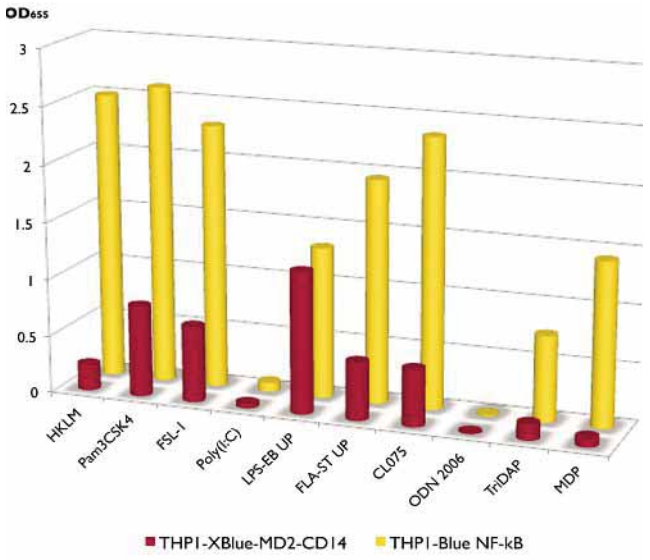
THP1-Blue™ NF-κB Cells - NF-κB SEAP Reporter Monocytes NEW

THP1-Blue™ NF-κB cells were specifically designed for monitoring the NF-κB signal transduction pathway in a physiologically relevant cell line. THP1-Blue™ were derived from the human THP-1 monocyte cell line by stable integration of an NF-κB-inducible SEAP reporter construct. THP1-Blue™ NF-κB cells express a secreted embryonic alkaline phosphatase (SEAP) reporter gene driven by an IFN-β minimal promoter fused to five copies of the NF-κB consensus transcriptional response element and three copies of the c-Rel binding site. As a result, THP1-Blue™ NF-κB cells allow the monitoring of NF-κB activation by determining the activity of SEAP. The levels of NF-κB-induced SEAP in the cell culture supernatant are readily assessed with QUANTI-Blue™, a SEAP detection reagent.
THP1-Blue™ NF-κB cells are highly responsive to PRR agonists that trigger the NF-κB pathway (see figure). THP1-Blue™ NF-κB cells are resistant to blasticidin.

Contents

THP1-Blue™ NF-κB cells are grown in RPMI medium, 2mM L-glutamine, 10% FBS supplemented with 10 µg/ml blasticidin. Cells are provided in a vial containing 5-7 x 10^6 cells and supplied with 1 mg blasticidin and 1 pouch of QUANTI-Blue™. Cells are shipped on dry ice. They are guaranteed mycoplasma-free.

PRODUCT	QUANTITY	CAT. CODE
THP1-Blue™ NF-κB Cells	5-7 x 10^6 cells	thp-nfkb



NF-κB response of THP1-Blue™ NF-κB cells. Cells were incubated with 10^7 cells/ml HKLM (TLR2), 10 ng/ml Pam3CSK4 (TLR1/2), 1 ng/ml FSL-1 (TLR2/6), 10 µg/ml poly(I:C) (TLR3), 100 ng/ml LPS-EB UP (TLR4), 100 ng/ml FLA-ST UP (TLR5), 3 µg/ml CL075 (TLR8), 10 µg/ml ODN2006 (TLR9), 10 µg/ml Tri-DAP (NOD1) or 10 µg/ml MDP (NOD2). After 24h incubation, the levels of NF-κB-induced SEAP were assessed from the cell culture supernatant using QUANTI-Blue™.

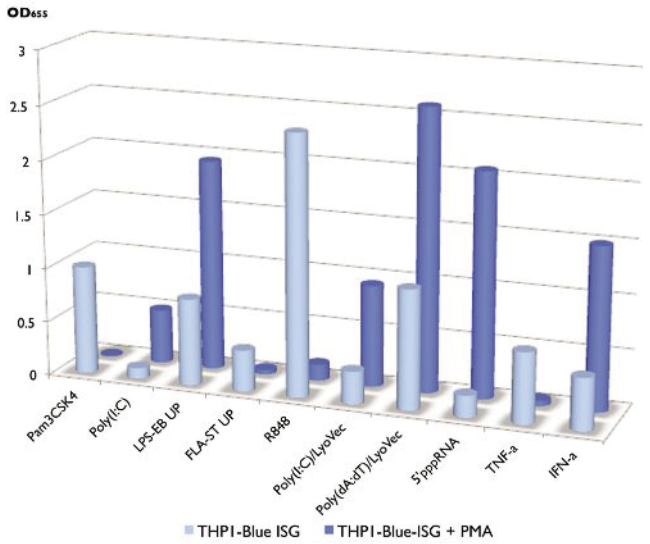
THP1-Blue™ ISG Cells - IRF SEAP Reporter Monocytes NEW

THP1-Blue™ ISG cells were specifically designed for monitoring the interferon signaling pathway in a physiologically relevant cell line. THP1-Blue™ were derived from the human THP-1 monocyte cell line by stable integration of an interferon regulatory factor (IRF)-inducible SEAP reporter construct. THP1-Blue™ ISG cells express a secreted embryonic alkaline phosphatase (SEAP) reporter gene under the control of an ISG54 minimal promoter in conjunction with five IFN-stimulated response elements. As a result, THP1-Blue™ ISG cells allow the monitoring of IRF activation by determining the activity of SEAP. The levels of IRF-induced SEAP in the cell culture supernatant are readily assessed with QUANTI-Blue™, a SEAP detection reagent.
THP1-Blue™ ISG cells are highly responsive to PRR agonists that trigger the IFN signaling pathway, such as LPS and transfected double-stranded nucleic acid (see figure). THP1-Blue™ ISG cells are resistant to Zeocin™.

Contents

THP1-Blue™ ISG cells are grown in RPMI medium, 2mM L-glutamine, 10% FBS supplemented with 100 µg/ml Zeocin™. Cells are provided in a vial containing 5-7 x 10^6 cells and supplied with 10 mg Zeocin™ and 1 pouch of QUANTI-Blue™. Cells are shipped on dry ice. They are guaranteed mycoplasma-free.

PRODUCT	QUANTITY	CAT. CODE
THP1-Blue™ ISG Cells	5-7 x 10^6 cells	thp-isg



IRF response of THP1-Blue™ ISG cells. Cells were incubated with 10 ng/ml Pam3CSK4 (TLR1/2), 10 µg/ml poly(I:C) (TLR3), 100 ng/ml LPS-EB UP (TLR4), 1 µg/ml FLA-ST UP (TLR5), 10 µg/ml R848 (TLR7/8), 100 ng/ml poly(I:C)/LyoVec (RLR), 10 ng/ml poly(dA:dT)/LyoVec (RLR), 100 ng/ml 5'pppRNA (RLR), 10 ng/ml TNF-α or 10 IU/ml IFN-α. After 24h incubation, the levels of NF-κB-induced SEAP were assessed from the cell culture supernatant using QUANTI-Blue™.

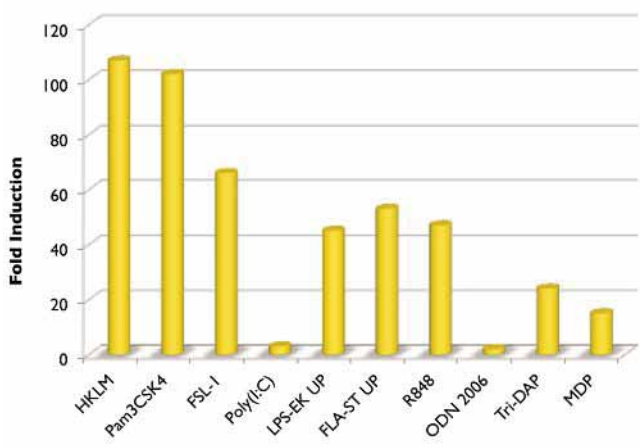
THP1-Lucia™ NF-κB Cells - NF-κB Luc Reporter Monocytes NEW

THP1-Lucia™ cells were specifically designed for monitoring the NF-κB signal transduction pathway in a physiologically relevant cell line. THP1-Lucia™ were derived from the human THP-1 monocyte cell line by stable integration of an NF-κB-inducible Luc reporter construct. THP1-Lucia™ cells feature the Lucia™ gene, a new secreted luciferase reporter gene, driven by an IFN-β minimal promoter fused to five copies of the NF-κB consensus transcriptional response element and three copies of the c-Rel binding site. As a result, THP1-Lucia™ cells allow the monitoring of NF-κB activation by determining the activity of Lucia™. The levels of NF-κB-induced Lucia™ in the cell culture supernatant are readily assessed with QUANTI-Luc™, a Lucia™ detection reagent (see page 55).
THP1-Lucia™ cells induce the activation of NF-κB in response to various PRR ligands, (see figure). THP1-Lucia™ cells are resistant to Zeocin™.

Contents

THP1-Lucia™ cells are grown in RPMI medium, 2mM L-glutamine, 10% FBS supplemented with 100 µg/ml Zeocin™. Cells are provided in a vial containing 5-7 x 10^6 cells and supplied with 10 mg Zeocin™ and 1 pouch of QUANTI-Luc™. Cells are shipped on dry ice. They are guaranteed mycoplasma-free.

PRODUCT	QUANTITY	CAT. CODE
THP1-Lucia™ Cells	5-7 x 10^6 cells	thpl-nfkb



NF-κB response of THP1-Lucia™ NF-κB cells. Cells were incubated with 10^7 cells/ml HKLM (TLR2), 1 ng/ml Pam3CSK4(TLR1/2), 0.1 ng/ml FSL-1 (TLR2/6), 10 µg/ml poly(I:C) (TLR3), 1 µg/ml LPS-EK UP (TLR4), 100 ng/ml FLA-ST UP (TLR5), 1 µg/ml R848 (TLR7/8), 10 µg/ml ODN2006 (TLR9), 10 µg/ml Tri-DAP (NOD1) or 10 µg/ml MDP (NOD2). After 24h incubation, the levels of NF-κB-induced Lucia were assessed from the cell culture supernatant using QUANTI-Luc™.

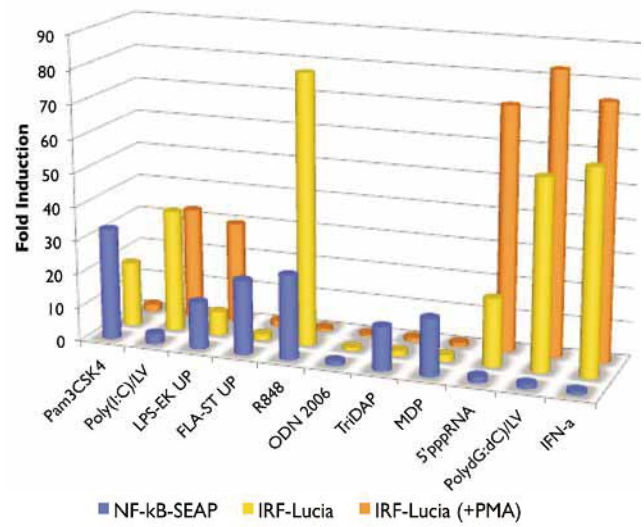
THP1-Dual™ (NF-κB - ISG) Cells NEW

NF-κB-SEAP IRF-Luc Reporter Monocytes

THP1-Dual™ cells were derived from the human THP-1 monocyte cell line by stable integration of two inducible reporter constructs. THP1-Dual™ cells feature the Lucia™ gene, a new secreted luciferase reporter gene, under the control of an ISG54 minimal promoter in conjunction with five IFN-stimulated response elements. THP1-Dual™ cells also express a secreted embryonic alkaline phosphatase (SEAP) reporter gene driven by an IFN-β minimal promoter fused to five copies of the NF-κB consensus transcriptional response element and three copies of the c-Rel binding site. As a result, THP1-Dual™ cells allow the simultaneous study of the NF-κB pathway, by monitoring the activity of SEAP, and the IRF pathway, by assessing the activity of Lucia™.
Both reporter proteins are readily measurable in the cell culture supernatant when using QUANTI-Blue™, a SEAP detection reagent, and QUANTI-Luc™, a Lucia™ detection reagent (see pages 55 and 56).
THP1-Dual™ cells induce the activation of NF-κB in response to certain TLR agonists, such as Pam3CSK4 and flagellin. They trigger the IRF pathway upon stimulation with type I IFNs and RLR or CDS agonists (see page 68), such as transfected dsRNA. THP1-Dual™ cells are resistant to the selectable markers Zeocin™ and blasticidin.

Contents

THP1-Dual™ cells are grown in RPMI medium, 2mM L-glutamine, 10% FBS supplemented with 100 µg/ml Zeocin™ and 10 µg/ml blasticidin. Cells are provided in a vial containing 5-7 x 10^6 cells and supplied with 10 mg Zeocin™, 1 mg blasticidin, 1 pouch of QUANTI-Luc™ and 1 pouch of QUANTI-Blue™. Cells are shipped on dry ice. They are guaranteed mycoplasma-free.



NF-κB/IRF dual Response of THP1-Dual™ cells. Cells were pretreated or not with PMA and incubated with 1 µg/ml Pam3CSK4, 1 µg/ml poly(I:C)/LyoVec, 1 µg/ml LPS-EK UP, 1 µg/ml FLA-ST UP, 10 µg/ml R848, 10 µg/ml ODN2006, 10 µg/ml Tri-DAP, 10 µg/ml MDP, 1 µg/ml 5'pppRNA, 1 µg/ml poly(dG:dC)/LyoVec or 10^4 U/ml IFN-α. After 24h incubation, levels of SEAP and Lucia™ were assessed using QUANTI-Blue™ and QUANTI-Luc™, respectively.

PRODUCT	QUANTITY	CAT. CODE
THP1-Dual™ Cells	5-7 x 10^6 cells	thpd-nfis

RAW-Blue™ Cells - NF-κB/AP-1 Reporter Macrophages

RAW-Blue™ Cells are derived from RAW 264.7 macrophages with chromosomal integration of a secreted embryonic alkaline phosphatase (SEAP) reporter construct inducible by NF-κB and AP-1.
RAW-Blue™ Cells are resistant to the selectable marker Zeocin™.

➢ TLR & NOD Reporter Macrophages

RAW-Blue™ Cells express all TLRs (with the exception of TLR5) as well as RIG-I, MDA-5, NOD1 and NOD2; expression of TLR3 and NOD1 being very low. The presence of specific agonists of these PRRs induces signaling pathways leading to the activation of NF-κB and AP-1 and subsequently to the secretion of SEAP, which can be easily monitored using QUANTI-Blue™ (figure 1).

➢ Dectin-1 Reporter Macrophages

RAW 264.7 cells express low endogenous levels of Dectin-1[1], while RAW-Blue™ cells express high levels of endogenous Dectin-1. Therefore RAW-Blue™ cells can be used as a Dectin-1 reporter cell line in particular when combined with a neutralizing anti-Dectin-1 antibody. Stimulation of RAW-Blue™ cells with zymosan or heat-killed preparations of yeast induces the activation of NF-κB in a Dectin-1-dependent manner (figure 2).

➢ Mincle Reporter Macrophages

RAW 264.7 cells express the macrophage-inducible C-type lectin, Mincle[2]. RAW-Blue cells are responsive to trehalose-6,6-dibehenate (TDB), a synthetic analog of Mycobacterium tuberculosis chord factor that is specifically recognized by Mincle. Stimulation with TDB induces the CARD9/Bcl-10/MALT1 signalosome leading to NF-κB activation. The response of RAW-Blue™ cells to TDB (and other Mincle ligands) can be determined by measuring the levels of NF-κB-induced SEAP in the cell supernatant using QUANTI-Blue™ (figure 2).

1. Brown GD. *et al.*, 2003, Dectin-1 Mediates the Biological Effects of ß-Glucans. J. Exp. Med., 197: 1119.
2. Matsumoto M. et al., 1999. A novel LPS-inducible C-type lectin is a transcriptional target of NF-IL6 in macrophages. J Immunol. 163(9):5039-48.

Contents

RAW-Blue™ Cells are grown in DMEM medium, 2mM L-glutamine, 10% FBS supplemented with 200 µg/ml Zeocin™. Each vial contains 5-7 x 10^6 cells and is supplied with 10 mg Zeocin™. Cells are shipped on dry ice. They are guaranteed mycoplasma-free.

PRODUCT	QUANTITY	CAT. CODE
RAW-Blue™ Cells	5-7 x 10^6 cells	raw-sp

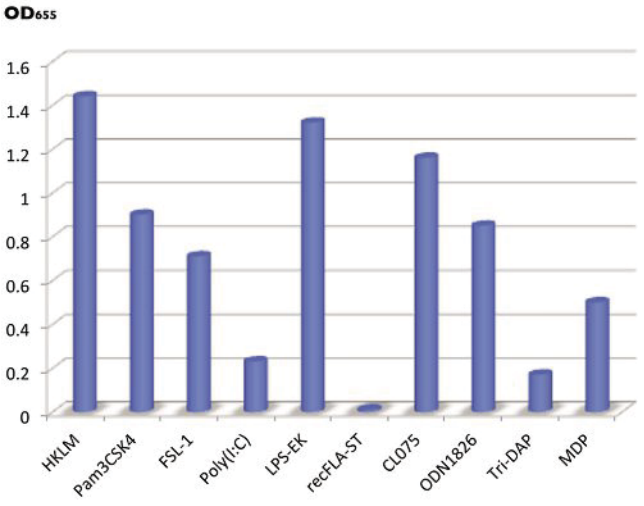


Figure 1. TLR and NOD stimulation profile in RAW-Blue™ Cells. RAW-Blue™ Cells were incubated with TLR or NOD agonists: 10^8 cells/ml HKLM (TLR2), 100 ng/ml Pam3CSK4 (TLR1/2), 100 ng/ml FSL-1 (TLR2/6), 100 ng/ml poly(I:C) (TLR3), 100 ng/ml LPS-EK (TLR4), 1 µg/ml RecFLA-ST (TLR5), 1 µg/ml CL075 (TLR7/8), 1 µg/ml ODN1826 (TLR9), 10 µg/ml Tri-DAP (NOD1), 1 µg/ml MDP (NOD2). After 24h incubation, TLR and NOD stimulation was assessed by measuring the levels of SEAP using QUANTI-Blue™.

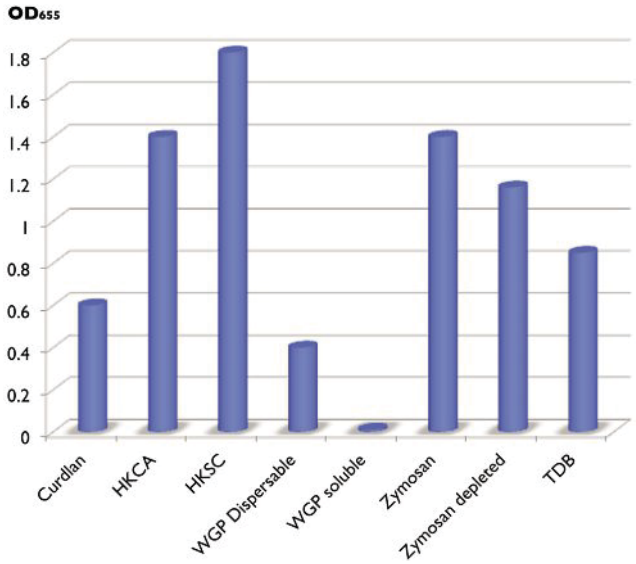


Figure 2. Response of RAW-Blue™ cells to Dectin-1 and Mincle agonists. RAW-Blue™ cells were stimulated with curdlan (100 µg/ml), HKCA (10^8 cells/ml), HKSC (10^8 cells/ml), WGP Dispersable (100 µg/ml), WGP Soluble (100 µg/ml), zymosan (10 µg/ml), zymosan depleted (100 µg/ml), or TDB (10 µg/ml). After 24h incubation, NF-κB activation was assessed by measuring the levels of SEAP in the supernatant by using QUANTI-Blue™.

Recent articles using RAW-Blue™ cells

Yang FL. *et al.*, 2011. Structure and immunological characterization of the capsular polysaccharide of a pyrogenic liver abscess caused by Klebsiella pneumoniae: activation of macrophages through Toll-like receptor 4. J. Biol. Chem. 286: 21041 - 21051.

Sintes J. *et al.*, 2010. Mouse CD84 is a pan-leukocyte cell-surface molecule that modulates LPS-induced cytokine secretion by macrophages. J. Leukoc. Biol. 88: 687 - 697.

Related Products

Zeocin™, page 61
PRR Ligands, pages 64-68
RAW-Blue™ ISG, page 46
QUANTI-Blue™, page 56
MAb mDectin-1, page 98

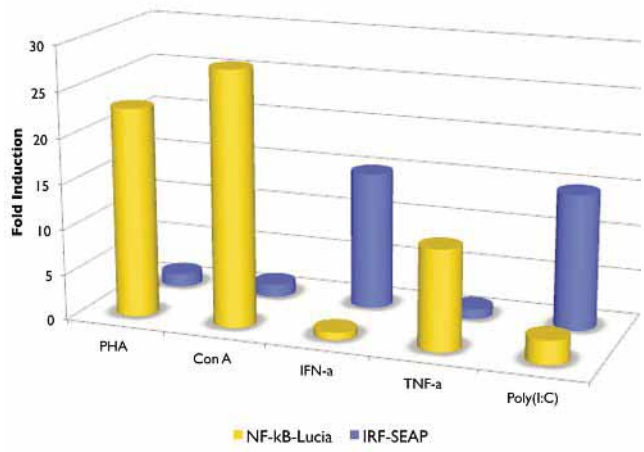
Jurkat-Dual™ (ISG - NF-κB) Cells NEW

IRF-SEAP and NF-κB-Luc Reporter T Lymphocytes

Jurkat-Dual™ cells were derived from the human T lymphocyte-based Jurkat cell line by stable integration of two inducible reporter constructs. Jurkat-Dual™ cells feature the Lucia™ gene, a new secreted luciferase reporter gene, driven by an IFN-β minimal promoter fused to five copies of the NF-κB consensus transcriptional response element and three copies of the c-Rel binding site. Jurkat-Dual™ cells also express a secreted embryonic alkaline phosphatase (SEAP) reporter gene under the control of an ISG54 minimal promoter in conjunction with five IFN-stimulated response elements. As a result, Jurkat-Dual™ cells allow the simultaneous study of the NF-κB pathway, by monitoring the activity of Lucia™, and the IRF pathway, by assessing the activity of SEAP.
Both reporter proteins are readily measurable in the cell culture supernatant when using QUANTI-Luc™, a Lucia™ detection reagent, and QUANTI-Blue™, a SEAP detection reagent (see pages 55 and 56). Jurkat-Dual™ cells are resistant to the selectable markers Zeocin™ and blasticidin. Jurkat-Dual™ cells induce the activation of NF-κB in response to TNF-α and T-lymphocyte mitogens, such as phytohemagglutinin and concanavalin A, They trigger the IRF pathway upon stimulation with type I IFNs and poly(I:C) (see figure).

Contents

Jurkat-Dual™ cells are grown in RPMI medium, 2mM L-glutamine, 10% FBS supplemented with 100 µg/ml Zeocin™ and 10 µg/ml blasticidin. Cells are provided in a vial containing 5-7 x 10^6 cells and supplied with 10 mg Zeocin™, 1 mg blasticidin, 1 pouch of QUANTI-Luc™ and 1 pouch of QUANTI-Blue™. Cells are shipped on dry ice. They are guaranteed mycoplasma-free.



NF-κB/IRF dual response of Jurkat-Dual™ cells. Cells were incubated with 50 µg/ml phytohemagglutinin (PHA), 50 µg/ml concanavalin A (Con A), 10^4 IU/ml IFN-α, 10 ng/ml TNF-α or 10 µg/ml poly(I:C). After 24h incubation, the levels of NF-κB-induced Lucia and IRF-induced SEAP were assessed from the cell culture supernatant using QUANTI-Luc™ or QUANTI-Blue™, respectively.

PRODUCT	QUANTITY	CAT. CODE
Jurkat-Dual™ Cells	5-7 x 10^6 cells	jktd-isnf

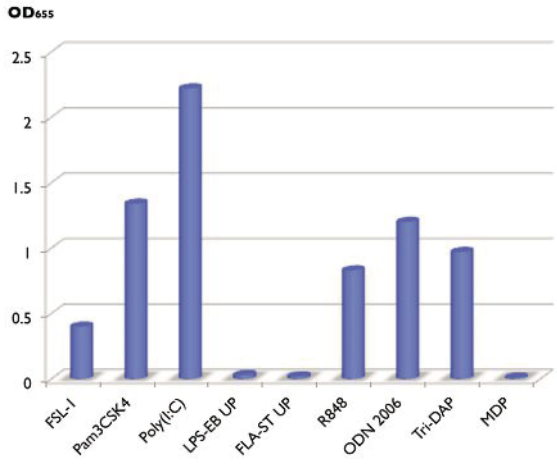
Ramos-Blue™ Cells - NF-κB/AP-1-SEAP Reporter B Lymphocytes

Ramos-Blue™ cells are B lymphocytes that stably expresses an NF-κB/AP-1-inducible SEAP (secreted embryonic alkaline phosphatase) reporter gene. Ramos-Blue™ cells derive from a human Burkitt's lymphoma which is negative for Epstein Barr virus. They have the characteristics of B lymphocytes and are routinely used as a model of B lymphocytes and for apoptosis studies. The Ramos-Blue™ cell line was isolated for its ability to respond to CpG ODNs (TLR9 ligands).
Ramos-Blue™ cells are responsive to various PRR agonists that activate the NF-κB pathway. When stimulated, they produce SEAP in the supernatant that can be readily monitored using QUANTI-Blue™, a SEAP detection medium (see page 56). Ramos-Blue™ cells are resistant to Zeocin™.

Contents

Ramos-Blue™ cells are grown in IMDM medium, 2 mM L-glutamine, 10% FBS supplemented with 100 µg/ml Zeocin™. Each vial contains 5-7 x 10^6 cells and is supplied with 10 mg Zeocin™. Cells are shipped on dry ice. They are guaranteed mycoplasma-free.

PRODUCT	QUANTITY	CAT. CODE
Ramos-Blue™ Cells	5-7 x 10^6 cells	rms-sp



Response of Ramos-Blue™ cells to PRR agonists. Cells were incubated with 10 µg/ml each of FSL-1 (TLR2/6), Pam3CSK4 (TLR1/2), poly(I:C) (TLR3), LPS-EB (TLR4), FLA-ST UP (TLR5), R848 (TLR7/8), ODN2006 (TLR9), TriDAP (NOD1) and MDP (NOD2). After 24h incubation, NF-κB/AP-1 activation was assessed by measuring the levels of SEAP in the supernatant using QUANTI-Blue™.

MEF-Blue™ Cells

NF-κB/IRF-SEAP Reporter Murine Embryonic Fibroblasts

C3H/TLR4mut & C3H/WT MEF cells

TLR4 Reporter Murine Embryonic Fibroblasts

C3H/TLR4mut and C3H/WT MEF cell lines were isolated from C3H/HeJ (TLR4-deficient) and C3H/HeN (wild-type) mouse embryos respectively. They stably express an NF-κB-inducible SEAP reporter construct that allows to monitor in a simple and convenient manner the activation of NF-κB.
C3H/WT MEFs express high levels of TLR2 and TLR4 and low levels of TLR3 and TLR5. The presence of TLR2, TLR3, TLR4, or TLR5 agonists triggers a signaling cascade in C3H/WT MEFs leading to the activation of NF-κB and the subsequent induction of SEAP. The amount of SEAP secreted in the supernatant can be readily detected when using QUANTI-Blue™, a SEAP detection medium. In C3H/TLR4mut MEFs, TLR4 agonists do not induce the activation of NF-κB and the production of SEAP in contrast to TLR2, TLR3 and TLR5 ligands. Thus these two cell lines provide a useful tool to determine whether a given compound is a specific TLR4 agonist. They can be used to assess the purity of a given LPS by detecting the presence of contaminants that stimulate TLR2 (see figure 1, LPS-EB standard versus LPS-EB ultrapure).

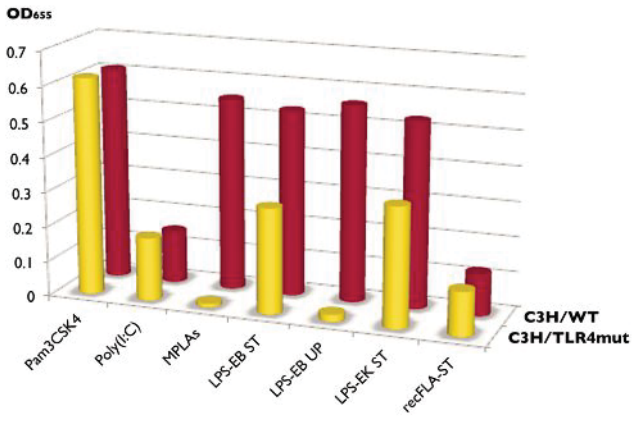


Figure 1. TLR stimulation profile of C3H/TLR4mut and C3H/WTMEF cells. Cells were incubated with TLR agonists: Pam3CSK4, 100 ng/ml (TLR2), poly(I:C), 10 μg/ml (TLR3), MPLAs, 10 μg/ml (TLR4), LPS-EB standard, 10 μg/ml (TLR4, TLR2), LPS-EB ultrapure, 10 μg/ml (TLR4), LPS-EK standard, 10 μg/ml (TLR2/4), recombinant flagellin, 1 μg/ml (TLR5). After 24h incubation, TLR stimulation was assessed by measuring the levels of SEAP in the supernatant by using QUANTI-Blue™.

C57/WT MEF cells

RLR Reporter Murine Embryonic Fibroblasts

MEFs produce IFN-β in response to viral infection in a RLR-dependent manner[1]. Thus, these cells are commonly used to study the RLR pathway. C57/WT MEFs were isolated from embryos under C57BL/6 background and immortalized with the SV40 large antigen. They stably express a SEAP reporter gene inducible by NF-κB and IRF3/7 providing a convenient method to monitor the activation of these transcription factors upon stimulation with a RIG-I/MDA-5 ligand.
C57/WT MEFs express both RIG-I and MDA-5. Stimulation of C57/WT cells with poly(I:C)/LyoVec complexes induces the secretion of SEAP in a dose-dependent manner (see figure 2). In contrast, stimulation with naked poly(I:C) has no effect on SEAP secretion although the cells express also TLR3. These data confirm that C57/WT MEFs respond to viral dsRNA primarily through the RLR pathway[1]. Both RIG-I and MDA-5 appear to respond to transfected poly(I:C) in MEFs[2].

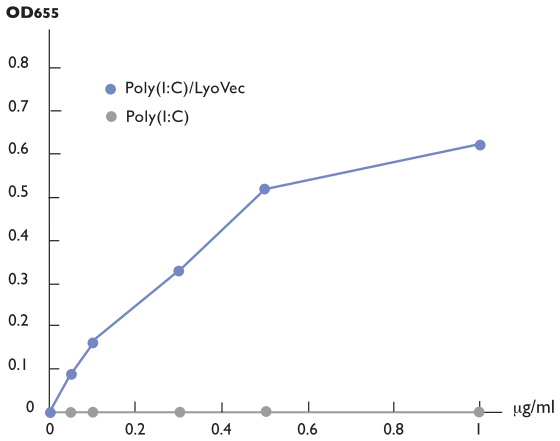


Figure 2. RLR stimulation in C57/WT MEFs. C57/MEFs were incubated with increasing concentrations of poly(I:C) or poly(I:C)/LyoVec™ complexes prepared extemporaneously at a 1:12 ratio. After 24h incubation, RLR stimulation was assessed by measuring the levels of SEAP secreted in the supernatant by using QUANTI-Blue™.

1. Kato H. *et al.*, 2005. Cell type-specific involvement of RIG-I in antiviral response. Immunity 23:19-28. **2. Venkataraman T. *et al.*, 2007.** Loss of DExD/H box RNA helicase LGP2 manifests disparate antiviral responses. J Immunol. 178:6444-6455.

Contents

MEF-Blue™ cells are grown in DMEM medium with 2mM L-glutamine, 10% FBS supplemented with 10 μg/ml blasticidin (C3H/TLR4mut and C3H/WT MEF cells) or 100 μg/ml Zeocin™ and 3 μg/ml blasticidin (C57/WT MEF cells). MEF-Blue™ cells are provided frozen in a cryotube containing 5-7 x 10^6 cells and shipped on dry ice. MEF-Blue™ cells are guaranteed mycoplasma-free.

PRODUCT	QUANTITY	CAT. CODE
C3H/TLR4mut MEFs	5-7 x 10^6 cells	mef-c3h4m
C3H/WT MEFs	5-7 x 10^6 cells	mef-c3hwt
C57/WT MEFs	5-7 x 10^6 cells	mef-c57wt

Related Products

Blasticidin, page 60
QUANTI-Blue™, page 56
Zeocin™, page 61
PRR Ligands, pages 64-68

INFLAMMASOME REPORTER CELLS

The inflammasome is a multiprotein complex that activates caspase 1, leading to the processing and secretion of the pro-inflammatory cytokines interleukin-1β (IL-1β) and IL-18. Each inflammasome includes a member of the nucleotide oligomerization domain-like receptor (NLR) family of proteins. Diverse pathogen-associated molecular patterns (PAMPs) and non-microbial danger-associated molecular patterns (DAMPs) are sensed intracellularly by NLRs. The subsequent oligomerization of NLRs results in NLR interaction with ASC (apoptosis-associated speck-like protein containing a caspase recruitment domain), a central adaptor protein of inflammasome. ASC then interacts with pro-caspase-1 yielding cleavage and activation of caspase-1, which leads to the maturation of pro-IL-1β to active IL-1β. A number of inflammasomes have been described, including the NLRP1, NLRP3 and NLRC4 inflammasomes. The NLRP3 inflammasome is the most extensively studied and also the most versatile due to its broad recognition of a wide range of PAMPs and DAMPs. It is however still unclear how these highly diverse signals can be detected by a single inflammasome. To foster your research on the inflammasome, InvivoGen has developed a variety of cell-based tools.

Inflammasome Test Cells

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

THP1-defASC cells

THP-1 cells were engineered to knock-down the expression of ASC. The resulting cell line, THP1-defASC, expresses negligible levels of ASC but express native levels of NLRP3 and pro-caspase-1. THP1-defASC cells are unable to respond to inducers of ASC-dependent inflammasomes, such as inducers of the NLRP3 inflammasome.
Application: To determine if a given signal is an inflammasome inducer.

THP1-defNLRP3 cells

THP-1 cells were engineered to knock-down the expression of NLRP3. The resulting cell line, THP1-defNLRP3, is deficient for NLRP3 activity but proficient for ASC and caspase-1 activities. THP1-defNLRP3 cells are unable to respond to inducers of the NLRP3 inflammasome, such as ATP and MSU (see figure). However, they may respond to signals that activate other ASC-dependent inflammasomes such as NLRP1 and NLRC4 inflammasomes.
Application: To study the involvement of NRLP3 in response to a given signal.

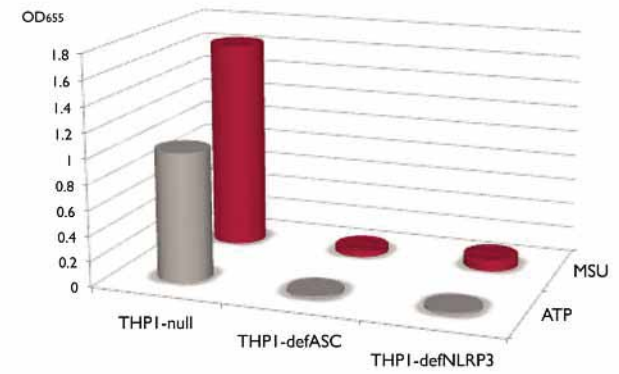
THP1-Null cells

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

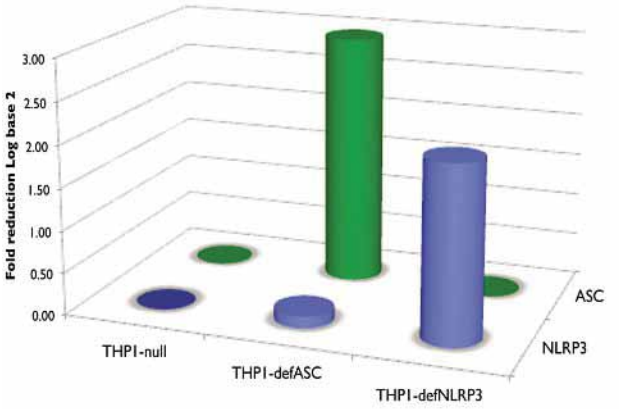
All three cells lines are resistant to hygromycin.

Contents and Storage

THP1-defASC, THP1-defNLRP3 and THP1-Null cells are grown in RPMI medium with 10% FBS, 2mM L-glutamine, 100 µg/ml Normocin™ and 200 µg/ml HygroGold™ (ultrapure Hygromycin). Each vial contains 5-7 x 10^6 cells and is supplied with 50 mg Normocin™ and 10 mg HygroGold™. Cells are shipped on dry ice. They are guaranteed mycoplasma-free.



IL-1β production in THP1-null, THP1-defASC and THP1-defNLRP3 cells following their stimulation with ATP and MSU. Cells primed with LPS (1 µg/ml) were stimulated with ATP (5 mM) or MSU (100 µg/ml). After 24h incubation, the supernatants were added to HEK-Blue™ IL-1β cells. IL-1β-induced activation of NF-κB was assessed by measuring the levels of SEAP in the supernatant of HEK-Blue™ IL-1β cells using QUANTI-Blue™.



Quantitative RT-PCR analysis showing the fold reduction of NLRP3 and ASC genes in THP1-defASC and THP1-defNLRP3 cells compared to THP1-null cells.

PRODUCT	QTY	CAT. CODE
THP1-Null	5-7 x 10^6 cells	thp-null
THP1-defASC	5-7 x 10^6 cells	thp-dasc
THP1-defNLRP3	5-7 x 10^6 cells	thp-dnlp

IL-1β Reporter Cells - IL-1β Sensor Cells for Inflammasome Studies

Many studies on the inflammasome use the human monocytic THP-1 cell line and Western blot or ELISA for the detection of mature IL-1β. InvivoGen has developed a new method to detect bioactive IL-1β that is simple, rapid and cost-effective. This method is based on HEK293 cells specifically engineered to selectively respond to IL-1β, named HEK-Blue IL-1β.

Description

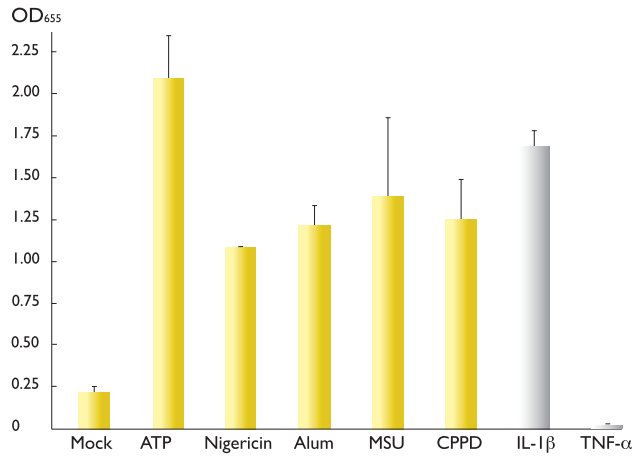
HEK-Blue™ IL-1β cells provide a convenient read-out to determine the amount of IL-1β secreted by THP-1 cells following stimulation by NLRP3 inflammasome inducers.
HEK-Blue™ IL-1β cells feature the SEAP (secreted embryonic alkaline phosphotase) reporter gene under the control of an NF-κB-inducible promoter. They naturally express the IL-1β receptor (IL-1R), and all the proteins involved in the MyD88-dependent IL-1R signaling pathway that leads to NF-κB activation. Thus upon IL-1β binding to IL-1R, a signaling cascade is initiated triggering NF-κB activation and the subsequent production of SEAP. Detection of SEAP in the supernatant of HEK-Blue™ IL-1β cells can be readily assessed using QUANTI-Blue™, a SEAP detection medium. QUANTI-Blue™ turns blue in the presence of SEAP which can be easily quantified using a spectrophotometer.

➢ **Detection range for human IL-1β: 100 pg - 100 ng/ml**

The specificity of the HEK-Blue™ IL-1β cells for the detection of IL-1β can be confirmed using a neutralizing antibody against IL-1β, such as anti-hIL-1β-IgA (see page 99).
HEK-Blue™ IL-1β cells are resistant to the selective antibiotics Zeocin™ and hygromycin B.

Contents and Storage

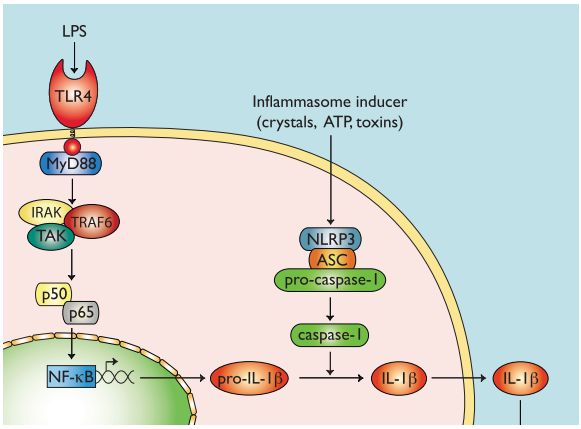
HEK-Blue™ IL-1β cells are grown in DMEM medium with 10% FBS, 2mM L-glutamine, 100 µg/ml Zeocin™ and 200 µg/ml HygroGold™ (ultrapure Hygromycin). Each vial contains 5-7 x 10^6 cells and is supplied with 10 mg Zeocin™, 10 mg HygroGold™ and 1 pouch of QUANTI-Blue™. Cells are shipped on dry ice. They are guaranteed mycoplasma-free.



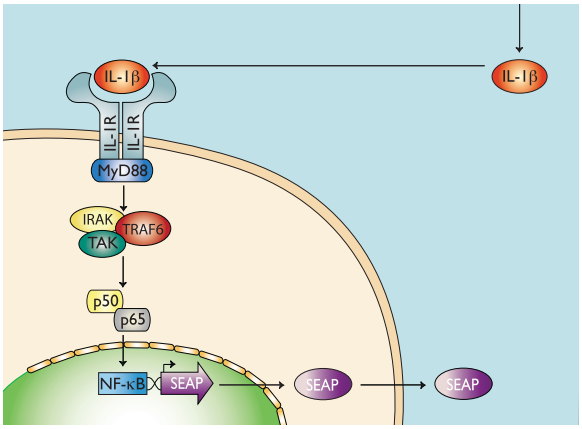
IL-1β detection using HEK-Blue™ IL-1β cells. THP1 cells pretreated with PMA and primed with LPS (1 µg/ml) were stimulated with ATP (5 mM), nigericin (1 µM), alum (200 µg/ml), MSU (200 µg/ml) or CPPD (200 µg/ml). After 24h incubation, THP-1 supernatants or recombinant IL-1β (0.1 ng/ml) or TNF-α (10 ng/ml) were added to HEK-Blue™ IL-1β cells. IL-1β-induced NF-κB activation was assessed by measuring the levels of SEAP in the supernatant of HEK-Blue™ IL-1β cells using the QUANTI-Blue™ assay.

THP-1/HEK-Blue™ IL-1β Assay

1- Production of IL-1β by THP-1 cells



2- Detection of IL-1β by HEK-Blue IL-1β cells



1- Production of IL-1β by THP-1 cells: Typically, THP-1 cells are pretreated with phorbol 12-myristate acetate (PMA) to become more susceptible to inflammasome activators, then are primed with lipopolysaccharide (LPS). These treatments induce the production of pro-IL-1β, the immature form of IL-1β. Subsequent stimulation with inflammasome inducers, such as crystals or ATP, leads to NRLP3 and caspase-1 activation resulting in IL-1β maturation and secretion.

2- Detection of IL-1β by HEK-Blue™ IL-1β cells: IL-1β-containing THP-1 supernatants are added to HEK-Blue™ IL-1β cells leading to NF-κB activation and the subsequent production of SEAP. The presence of SEAP in HEK-Blue™ IL-1β supernatants is assessed using QUANTI-Blue™, a SEAP detection medium.

PRODUCT	QUANTITY	CAT. CODE
HEK-Blue™ IL-1β cells	5-7 x 10^6 cells	hkb-il1b

CYTOKINE REPORTER CELLS

InvivoGen's cytokine reporter cells comprise an expanding family of engineered cell lines designed to provide a simple, rapid and reliable method to monitor the activation of signaling pathways induced by key cytokines. Cytokine reporter cells allow to detect these biologically active cytokines and can also be used to screen for compounds exhibiting agonist and antagonist activities. The cytokine reporter cells are derived from different cell types, including the human embryonic kidney 293 and murine B16 melanoma cell lines. They express an inducible secreted embryonic alkaline phosphatase (SEAP) reporter that can be quantitatively detected using QUANTI-Blue™, a SEAP colorimetric detection medium. InvivoGen introduces new cytokine reporter cells that feature the Lucia™ gene which encodes a novel secreted luciferase that can be readily measured using QUANTI-Luc™. The cytokine reporter cells express the SEAP or Lucia™ reporter genes under the control of specific promoters that are selectively activated by the cytokines or other immune modulators known to induce the pathway of interest. Therefore, in the presence of the cytokine, agonist or antagonist compound, the pathway is activated or inhibited modulating the reporter activity.

REPORTER CELL LINE	CYTOKINE(S) DETECTED	PATHWAY ACTIVATED	DETECTION RANGE	REPORTER GENE	PAGE
IFN-α/β Reporter Cells					
B16-Blue™ IFN-α/IFN-β	Murine IFN-α/β	JAK / ISGF3 / IRF	mIFN-α : 5×10^{1} - 10^{4} IU/ml mIFN-β : 5×10^{1} - 10^{4} IU/ml	SEAP	46
HEK-Blue™ IFN-α/IFN-β	Human IFN-α/β	JAK / ISGF3 / IRF	hIFN-α : 5 - 10^{4} IU/ml hIFN-β : 20 - 10^{4} IU/ml	SEAP	45
RAW-Blue™ ISG NEW	Murine IFN-α/β	JAK / ISGF3 / IRF	mIFN-α : 5×10^{2} - 5×10^{4} IU/ml mIFN-β : 10^{1} - 10^{3} IU/ml	SEAP	46
IFN-γ Reporter Cells					
HEK-Blue™ IFN-γ NEW	Human IFN-γ	JAK / STAT1 / IRF	hIFN-γ : 5 - 100 IU/ml	SEAP	47
HEK-Dual™ IFN-γ NEW	Human IFN-α	JAK / STAT1 / IRF	hIFN-γ : 1 - 10^{3} IU/ml (Lucia™) hIFN-γ : 1 - 10^{3} IU/ml (SEAP)	Lucia™ & SEAP	47
IL-1 & TNF Reporter Cells					
HEK-Blue™ TNF-α/IL-1β	Human & murine TNF-α Human & murine IL-1β	NF-κB / AP-1	hTNF-α : 0.5 - 1000 ng/ml hIL-1β : 0.2 - 100 ng/ml	SEAP	49
HEK-Blue™ IL-1β	Human & murine IL-1β	NF-κB / AP-1	hIL-1β : 10^{-3} - 10^{1} ng/ml mIL-1β : 10 - 1000 ng/ml	SEAP	49
HEK-Blue™ IL-18/IL-1β	Human IL-18 Human & murine IL-1β	NF-κB / AP-1	hIL-18 : 0.5 - 100 ng/ml hIL-1β : 0.5 - 100 ng/ml	SEAP	50
HEK-Blue™ IL-33/IL-1β	Human IL-33 Human & murine IL-1β	NF-κB / AP-1	hIL-33 : 0.5 - 100 ng/ml hIL-1β : 0.5 - 100 ng/ml	SEAP	50
HEK-Dual™ TNF-α NEW	Human & murine TNF-α	NF-κB	hTNF-α : 10^{-3} - 10^{1} ng/ml (Lucia™) hTNF-α : 5×10^{-3} - 1 ng/ml (SEAP)	Lucia™ & SEAP	48
Th2 Cytokine Reporter Cells					
HEK-Blue™ IL-4/IL-13	human IL-4 human & murine IL-13	JAK / STAT6	hIL-4 : 0.5 - 100 ng/ml hIL-13 : 5 - 1000 ng/ml	SEAP	51
HEK-Blue™ IL-6	human IL-6	JAK / STAT3	hIL6 : 0.5 - 50 ng/ml	SEAP	51

IFN-α/β Reporter Cells

- **HEK-Blue™ IFN-α/β -** Human IFN-α and IFN-β SEAP-reporter cells
- **B16-Blue™ IFN-α/β -** Murine IFN-α and IFN-β SEAP-reporter cells
- **RAW-Blue™ ISG -** Murine IFN-α and IFN-β SEAP-reporter cells NEW

Background

Type I interferons, in particular interferon alpha (IFN-α) and interferon beta (IFN-β), play a vital role in host resistance to viral infections. They signal mainly through the JAK-STAT pathway. Following their production, IFN-α and IFN-β bind to a common receptor (IFNAR) and recruit the Janus kinases (JAK1 and TyK2). JAKs phosphorylate STAT1 and STAT2, which then dimerize and interact with IFN regulatory factor 9 (IRF9), forming a complex named ISGF3. ISGF3 binds to IFN-stimulated response elements (ISRE) in the promoters of IFN-stimulated genes (ISG) to regulate their expression (figure 1).

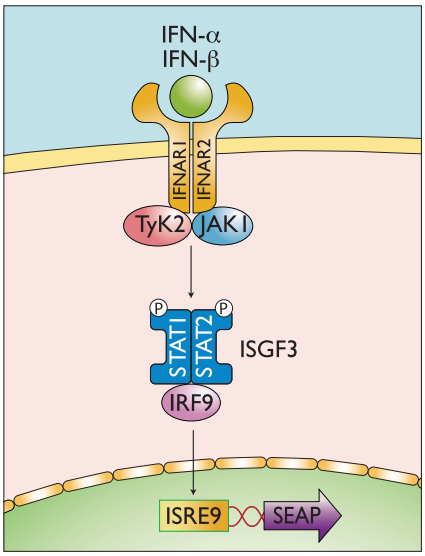


Figure 1: JAK-STAT pathway induced by type I IFNs.

HEK-Blue™ IFN-α/β

HEK-Blue™ IFN-α/β cells allow the detection of bioactive human type I IFNs by monitoring the activation of the ISGF3 pathway. These cells were generated by stable transfection of HEK293 cells with the human STAT2 and IRF9 genes to obtain a fully active type I IFN signaling pathway. The other genes of the pathway are naturally expressed in sufficient amounts. The cells were further transfected with a SEAP reporter gene under the control of the IFN-α/β-inducible ISG54 promoter.
Stimulation of HEK-Blue™ IFN-α/β cells with human IFN-α or IFN-β activates the JAK/STAT/ISGF3 pathway and subsequently induces the production of SEAP (figure 2).
Stimulation of HEK-Blue™ IFN-α/β cells with recombinant human IFN-α can be blocked by anti-hIFN-α-IgA, a neutralizing monoclonal antibody of the IgA isotype (see page 99).

➢ **Detection range for hIFN-α:** 5 - 10^4 IU/ml
➢ **Detection range for hIFN-β:** 20 - 10^4 IU/ml

HEK-Blue™ IFN-α/β cells are resistant to blasticidin and Zeocin™.

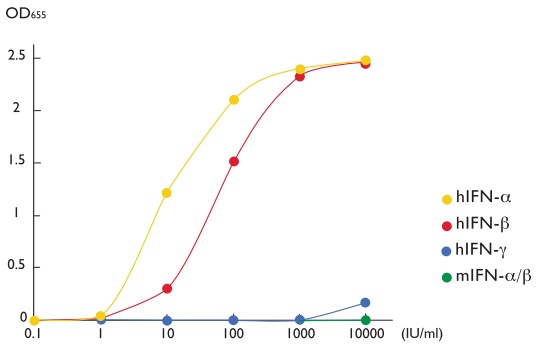


Figure 2: Responses of HEK-Blue™ IFN-α/β cells to IFNs. HEK-Blue™ IFN-α/β cells were incubated with increasing concentrations of recombinant human IFN-α, IFN-β or IFN-γ, or recombinant mouse IFN-α or IFN-β. After 24h incubation the levels of ISGF3-induced SEAP were determined using QUANTI-Blue™.

Contents and Storage

HEK-Blue™ IFN-α/β cells are grown in standard DMEM medium, 2mM L-glutamine, 10% FBS supplemented with 100 µg/ml Normocin, 30 µg/ml blasticidin and 100 µg/ml Zeocin™. Cells are provided frozen in a cryotube containing 5-7 x 10^6 cells and supplied with 100 µl of blasticidin at 10 mg/ml, 100 µl of Zeocin™ at 100 mg/ml, 1 ml Normocin™ at 50 mg/ml, and 1 pouch of QUANTI-Blue™. Cells are shipped on dry ice.

PRODUCT	QUANTITY	CAT. CODE
HEK-Blue™ IFN-α/β cells	5-7x 10^6 cells	hkb-ifnab

Related Products

Blasticidin, page 60
Zeocin™, page 61
Anti-hIFN-α-IgA , page 99
QUANTI-Blue™ page 56

Recent article using HEK-Blue™ IFN-α/β cells

Alain T. *et al.*, 2010. Vesicular stomatitis virus oncolysis is potentiated by impairing mTORC1-dependent type I IFN production. PNAS, 107: 1576 - 1581.

Neerincx A. *et al.*, 2010. A Role for the Human Nucleotide-binding Domain, Leucine-rich Repeat-containing Family Member NLRC5 in Antiviral Responses. J. Biol. Chem., 285: 26223 - 26232.

Bego MG. *et al.*, 2012. Virus-Activated Interferon Regulatory Factor 7 Upregulates Expression of the Interferon-Regulated BST2 Gene Independently of Interferon Signaling. J.Virol., 86: 3513 - 3527.

B16-Blue™ IFN-α/β

B16-Blue™ IFN-α/β cells allow the detection of bioactive murine type I IFNs by monitoring the activation of the JAK/STAT/ISGF3 pathway. They derive from the murine B16 melanoma cell line of C57B1/6 origin after stable transfection with a SEAP reporter gene under the control of the IFN-α/β-inducible ISG54 promoter.
Stimulation of B16-Blue™ IFN-α/β cells with murine IFN-α or IFN-β, but not human IFN-α or IFN-β, activates the JAK/STAT/ISGF3 pathway and triggers the subsequent production of SEAP (figure 2A). B16-Blue™ IFN-α/β cells also respond to pathogen associated molecular patterns (PAMPs) that induce the production of IFN-α and IFN-β, such as transfected poly(dA:dT) or poly(dG:dC) (figure 3A).

- **Detection range for mIFN-α:** 0.5×10^2 - 10^4 IU/ml
- **Detection range for mIFN-β:** 0.5×10^2 - 10^4 IU/ml

B16-Blue™ IFN-α/β cells are resistant to Zeocin™.

RAW-Blue™ ISG NEW

RAW-Blue™ ISG cells were designed for the detection of bioactive murine type I IFNs in a physiologically relevant cell line. RAW-Blue™ ISG cells were derived from the murine RAW 264.7 macrophage cell line by stable integration of an interferon regulatory factor (IRF)-inducible secreted embryonic alkaline phosphatase (SEAP) reporter construct. RAW-Blue™ ISG cells express a SEAP reporter gene under the control of an ISG54 minimal promoter in conjunction with five IFN-stimulated response elements. As a result, RAW-Blue™ ISG cells allow the monitoring of IRF activation by determining the activity of SEAP. The levels of IRF-induced SEAP in the cell culture supernatant are readily assessed with QUANTI-Blue™, a SEAP detection reagent.
RAW-Blue™ ISG cells are responsive to murine IFN-α and IFN-β but do not respond to their human counterparts (figure 2B). They are also responsive to PRR ligands that trigger the IFN signaling pathway, such as transfected double-stranded nucleic acid (see figure 3B). RAW-Blue™ ISG cells are resistant to Zeocin™.

- **Detection range for mIFN-α:** 5×10^2 - 5×10^4 IU/ml
- **Detection range for mIFN-β:** 10^1 - 10^3 IU/ml

RAW-Blue™ ISG cells are resistant to Zeocin™

Contents and Storage

B16-Blue™ IFN-α/β cells are grown in RPMI medium, 2 mM L-glutamine, 10% FBS supplemented with 100 µg/ml Normocin™ and 100 µg/ml Zeocin™.

RAW-Blue™ ISG cells are grown in DMEM medium, 2 mM L-glutamine, 10% FBS supplemented with 100 µg/ml Normocin™ and 200 µg/ml Zeocin™.

B16-Blue™ IFN-α/β and RAW-Blue™ ISG cells are provided frozen in a cryotube containing 5-7 x 10^6 cells and supplied with 50 mg of Normocin™, 10 mg Zeocin™ and 1 pouch of QUANTI-Blue™. Cells are shipped on dry ice. They are guaranteed mycoplasma-free.

PRODUCT	QUANTITY	CAT. CODE
B16-Blue™ IFN-α/β cells	5-7 x 10^6 cells	bb-ifnab
RAW-Blue™ ISG cells	5-7 x 10^6 cells	raw-isg

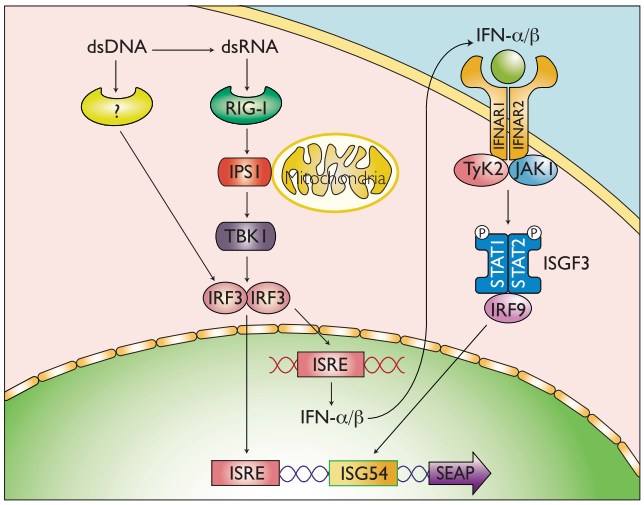
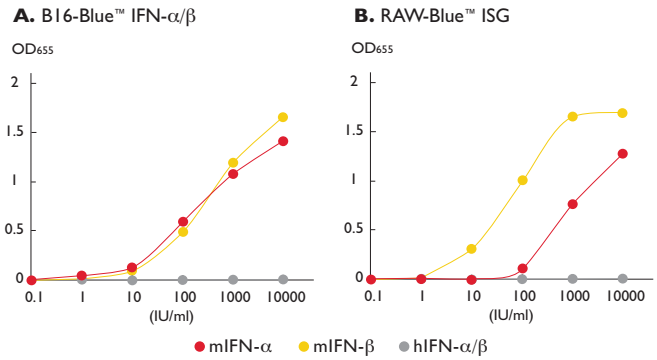
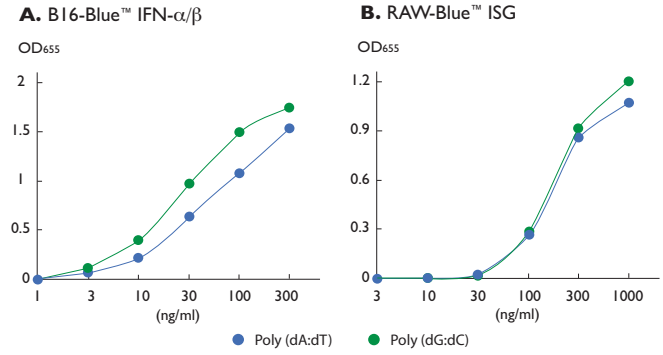


Figure 1: IFN-α/β induction and signaling pathways. Interferon-alpha (IFN-α) and interferon beta (IFN-b), play an important role in viral infections. They bind to an IFN receptor complex consisting of two alpha chains (IFNAR1 and IFNAR2) and recruit JAK1 and TyK2. These kinases phosphorylate STAT1 and STAT2 leading to the formation of the ISGF3 complex. ISGF3 binds to IFN-stimulated response elements (ISRE) in the promoters of IFN-stimulated genes (ISG) to regulate their expression.



Figures 2A & 2B: Responses of B16-Blue™IFN-α/β cells and RAW-Blue™ ISG cells to type I IFNs. B16-Blue™ IFN-α/β cells (A) and RAW-Blue™ cells (B) were incubated with increasing concentrations of mouse or human recombinant IFN-α or IFN-β. After 24h incubation the levels of IRF-induced SEAP were determined using QUANTI-Blue™.



Figures 3A & 3B: Responses of B16-Blue™IFN-α/β cells and RAW-Blue™ ISG cells to transfected double-stranded nucleic acid. B16-Blue™ IFN-α/β cells (A) and RAW-Blue™ cells (B) were incubated with increasing concentrations of poly(dA:dT)/LyoVec or poly(dG:dC)/LyoVec. After 24h incubation the levels of IRF-induced SEAP were determined using QUANTI-Blue™.

IFN-γ Reporter Cells NEW

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

HEK-Dual™ IFN-γ cells - Human IFN-γ SEAP/Lucia™-reporter cells

HEK-Blue™ IFN-γ cells

HEK-Blue™ IFN-γ cells allow the detection of bioactive human IFN-γ by monitoring the activation of the JAK/STAT-1 pathway. HEK-Blue™ IFN-γ cells were generated by stable transfection of HEK293 cells with the human STAT1 gene to obtain a fully active STAT1 pathway. The other genes of the pathway are naturally expressed in sufficient amounts. The cells were further transfected with a SEAP reporter gene under the control of an ISG54 promoter fused to four interferon-gamma-activated sites (GAS). HEK-Blue™ IFN-γ cells produce SEAP in response to IFN-γ stimulation only. They are unresponsive to type I IFNs (Figure 2).

➢ **Detection range for hIFN-γ :** 5 - 100 IU/ml

HEK-Blue™ IFN-γ cells are resistant to blasticidin and Zeocin™.

HEK-Dual™ IFN-γ cells

HEK-Dual™ IFN-γ cells were derived from the HEK-Blue™ IFN-γ cell line by stable integration of an additional IFN-γ inducible reporter construct. HEK-Dual™ IFN-γ cells feature the Lucia™ gene, a new secreted luciferase reporter gene, under the control of the same inducible promoter that drives the SEAP reporter gene, that is an ISG54 promoter fused to four interferon-gamma-activated sites (GAS). As a result, HEK-Dual™ IFN-γ cells allow to study the activation of JAK/STAT-1 pathway, by monitoring the activity of two different reporters, SEAP or Lucia™.
Both reporter proteins are readily measurable in the cell culture supernatant when using QUANTI-Blue™, a SEAP detection reagent, and QUANTI-Luc™, a Lucia™ detection reagent (see pages 55 and 56).

➢ **Detection range for hIFN-γ using QUANTI-Luc™:** 1 - 10^3 IU/ml
➢ **Detection range for hIFN-γ using QUANTI-Blue™:** 5 - 100 IU/ml

HEK-Dual™ IFN-γ cells are resistant to blasticidin, Zeocin™ and puromycin.

Contents and Storage

HEK-Blue™ IFN-γ and HEK-Dual™ IFN-γ cells are grown in standard DMEM medium, 2mM L-glutamine, 10% FBS supplemented with 100 µg/ml Normocin and the appropriate selective antibiotics. Cells are provided frozen in a cryotube containing 5-7 x 10^6 cells.
HEK-Blue™ IFN-γ cells are cultivated with 30 µg/ml blasticidin and 100 µg/ml Zeocin™. They are supplied with 50 mg of Normocin™, 1 mg of blasticidin, 10 mg of Zeocin™ and 1 pouch of QUANTI-Blue™.
HEK-Dual™ IFN-γ cells are cultivated with 30 µg/ml blasticidin, 100 µg/ml Zeocin™ and 1 µg/ml puromycin. They are supplied with 50 mg of Normocin™, 1 mg of blasticidin, 10 mg of Zeocin™, 1 mg of puromycin, 1 pouch of QUANTI-Blue™ and 1 pouch of QUANTI-Luc™.
Cells are shipped on dry ice. They are guaranteed mycoplasma-free.

PRODUCT	QUANTITY	CAT. CODE
HEK-Blue™ IFN-γ cells	5-7 x 10^6 cells	hkb-ifng
HEK-Dual™ IFN-γ cells	5-7 x 10^6 cells	hkd-ifng

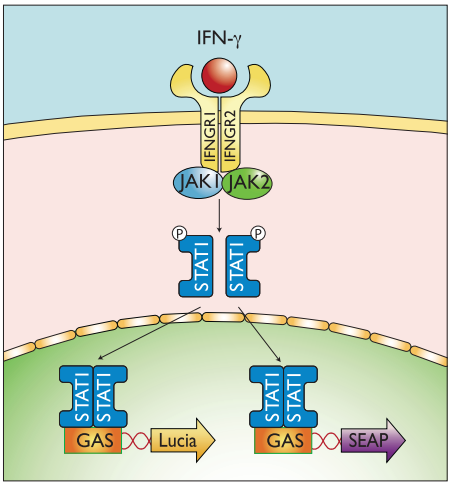
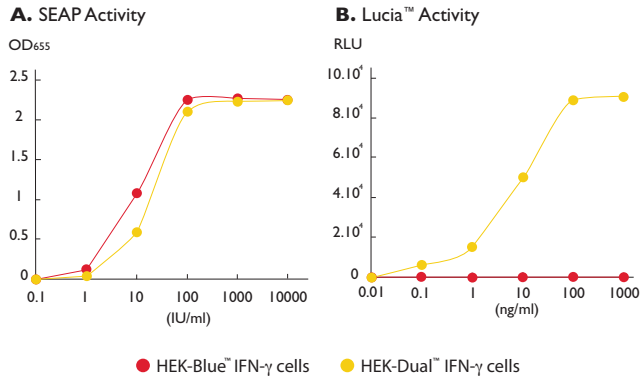


Figure 1: Simplified JAK/STAT signaling pathway induced by IFN-γ. IFN-γ exerts its action by first binding to a heterodimeric receptor consisting of two chains, IFNGR1 and IFNGR2, causing its dimerization and the activation of specific Janus family kinases (JAK1 and JAK2). Two STAT1 molecules then associate with this ligand-activated receptor complex and are activated by phosphorylation. Activated STAT1 form homodimers and are translocated to the nucleus where they bind interferon-gamma-activated sites (GAS) in the promoter of IFN-γ inducible genes.



Figures 2A & 2B: SEAP and Lucia™ Responses of HEK-Blue™ IFN-γ cells and HEK-Dual™ IFN-γ cells to IFN-γ. HEK-Blue™ IFN-γ cells and HEK-Dual™ IFN-γ cells were incubated with increasing concentrations of recombinant human IFN-γ. After 24h incubation the levels of STAT1-induced SEAP (A) and STAT1-induced Lucia™ (B) were determined using QUANTI-Blue™ and QUANTI-Luc™, respectively. SEAP activity was assessed by measuring the OD at 655 while Lucia™ activity was determined by measuring Relative Light Units (RLU).

Related Products

Normocin™, page 59
Puromycin, page 61
QUANTI-Blue™, page 56
Recombinant human IFN-γ, page 96
Blasticidin, page 60
Zeocin™, page 61
QUANTI-Luc™, page 55

IL-1 and TNF Reporter Cells

- **HEK-Dual TNF-α -** Human and Murine TNF-α SEAP/Lucia™ Reporter Cells **NEW**
- **HEK-Blue TNF-α/IL-1β -** Human and Murine TNF-α and IL-1β SEAP-reporter cells
- **HEK-Blue IL-1β -** Human and Murine IL-1β SEAP-reporter cells
- **HEK-Blue IL-18/IL-1β -** Human IL-18 and human/murine IL-1β SEAP-reporter cells
- **HEK-Blue IL-33/IL-1β -** Human IL-33 and human/murine IL-1β SEAP-reporter cells

Background

Tumor necrosis factor alpha (TNF-α) and interleukin 1 beta (IL-1β) are proinflammatory cytokines produced in response to microbial infection. The common inflammatory responses of TNF-α and IL-1β are mediated by interactions of these cytokines with distinct receptors: TNF-α binds TNFR1 and TNFR2, IL-1β binds IL-1RI. Binding of these cytokines to their receptors induces intracellular signaling. TNF-α signaling involves TRADD, TRAF2 and RIP, while IL-1β signaling is mediated by MyD88, IRAK-1, TRAF-6, and Rac1. Both signalings lead to the activation of the transcription factors NF-κB and AP-1 (figure 1).

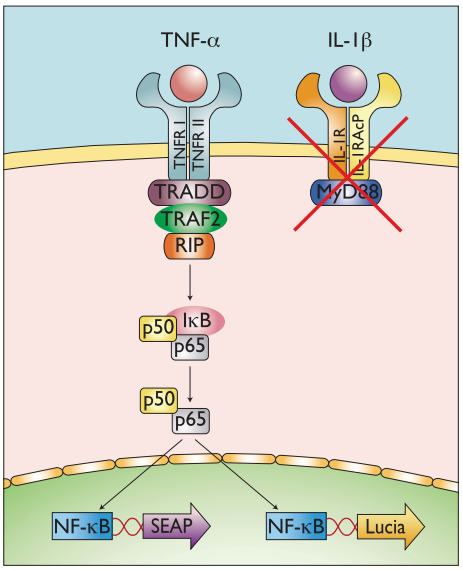


Figure 1: Simplified NF-κB signaling pathway induced by TNF-α in HEK-Dual™ TNF-α cells.

HEK-Dual™ TNF-α NEW

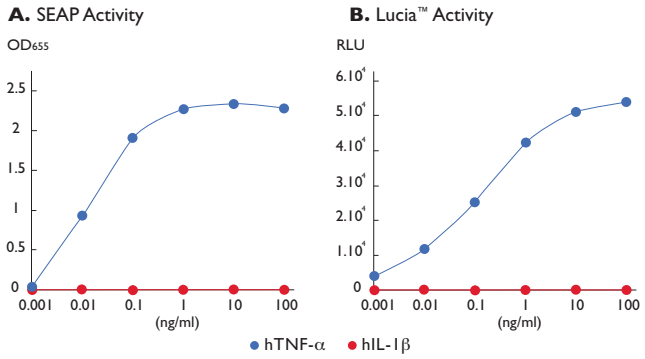
HEK-Dual™ TNF-α cells were specifically designed for the monitoring of bioactive TNF-α in biological samples by assessing NF-κB activation. HEK-Dual™ TNF-α cells were derived from the human embryonic kidney 293 cell line by stable co-transfection of two NF-κB-inducible reporter constructs. HEK-Dual™ TNF-α cells feature two different secreted reporter genes, the SEAP (secreted embryonic alkaline phosphatase) gene and the Lucia™ gene which encodes a novel secreted luciferase. Both reporter genes are under the control of the same NF-κB-inducible promoter consisting of an IFN-β minimal promoter fused to five copies of the NF-κB consensus transcriptional response element and three copies of the c-Rel binding site. Another feature of HEK-Dual™ TNF-α cells is their inability to respond to IL-1β due to a mutation in IL-1β signaling pathway. As a result, HEK-Dual™ TNF-α cells allow the specific study of TNF-α-induced NF-κB activation by monitoring the activity of either SEAP or Lucia™, each reporter system presenting specific advantages (see pages 53-57).
Both reporter proteins are readily measurable in the cell culture supernatant when using QUANTI-Blue™, a SEAP detection reagent, and QUANTI-Luc™, a Lucia™ detection reagent (see pages 55 and 56).

➢ **Detection range for hTNF-α using QUANTI-Luc™:** 0.001 - 10 ng/ml
➢ **Detection range for hTNF-α using QUANTI-Blue™:** 0.005 - 1 ng/ml

HEK-Dual™ TNF-α cells are resistant to Zeocin™.

Contents and Storage

HEK-Dual™ TNF-α cells are grown in standard DMEM medium, 2mM L-glutamine, 10% FBS supplemented with 100 µg/ml Normocin and 100 µg/ml Zeocin™. Cells are provided frozen in a cryotube containing 5-7 x 10^6 cells. They are supplied with 50 mg of Normocin™, 10 mg of Zeocin™, 1 pouch of QUANTI-Blue™ and 1 pouch of QUANTI-Luc™. Cells are shipped on dry ice. They are guaranteed mycoplasma-free.



Figures 2A & 2B: SEAP and Lucia™ Responses of HEK-Dual™ TNF-α cells to TNF-α and IL-1β. HEK-Dual™ TNF-α cells were incubated with increasing concentrations of recombinant human TNF-α or IL-1β. After 24h incubation, the levels of NF-κB-induced SEAP (A) and NF-κB-induced Lucia™ (B) were determined using QUANTI-Blue™ and QUANTI-Luc™, respectively. SEAP activity was assessed by measuring the OD at 655 while Lucia™ activity was determined by measuring Relative Light Units (RLU).

PRODUCT	QUANTITY	CAT. CODE
HEK-Dual™ TNFα Cells	5-7 x 10^6 cells	hkd-tnfa

HEK-Blue™ TNF-α/IL-1β

HEK-Blue™ TNF-α/IL-1β cells are designed for the detection of bioactive TNF-α and IL-1β (and IL-1α) by monitoring the activation of the NF-κB pathway. HEK-Blue™ TNF-α/IL-1β cells derive from HEK293 cells. They endogenously express the receptors for TNF-α and IL-1β and stably express a SEAP reporter gene under the control of the IFN-β minimal promoter fused to five NF-κB and five AP-1 binding sites.
HEK-Blue™ TNF-α/IL-1β cells secrete SEAP upon stimulation by human TNF-α and IL-1β (figure 2). SEAP production is also detected in the presence of murine TNF-α and IL-1β, and human or murine IL-1α. SEAP levels can be readily determined using a SEAP detection medium, such as QUANTI-Blue™ or HEK-Blue™ Detection medium.
Stimulation of HEK-Blue™ TNF-α/IL-1β cells with recombinant human TNF-α or IL-1β can be blocked by the neutralizing monoclonal anti-hTNF-α-IgA or anti-hIL-1β-IgA antibody, respectively (see page 99).

➢ **Detection range for hTNF-α :** 0.5 ng - 1 µg/ml
➢ **Detection range for hIL-1β :** 0.2 - 100 ng/ml

HEK-Blue™ TNF-α/IL-1β cells are resistant to Zeocin™.

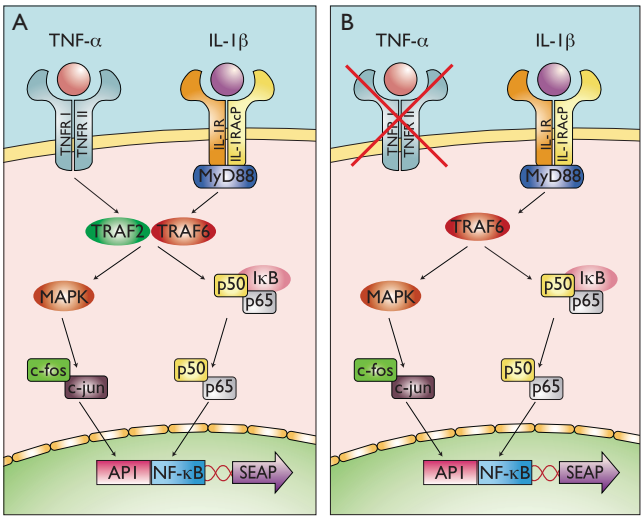


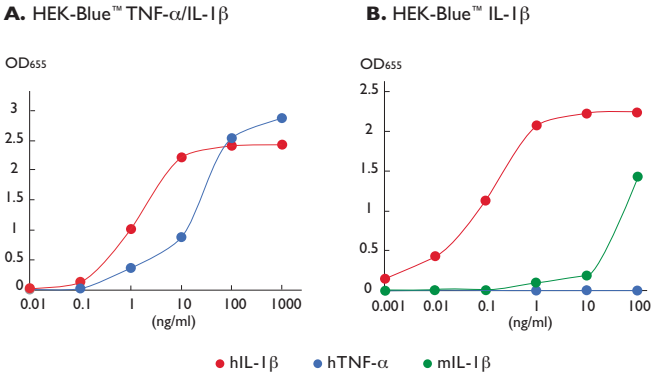
Figure 1: Simplified NF-κB signaling pathway induced by TNF-α and IL-1β in HEK-Blue™ TNF-α/IL-1β (A) and HEK-Blue™ IL-1β (B) cells.

HEK-Blue™ IL-1β

HEK-Blue™ IL-1β cells allow to detect bioactive IL-1β by monitoring the activation of the NF-κB and AP-1 pathways. They derive from HEK-Blue™ TNF-α/IL-1β cells in which the TNF-α response has been blocked. Therefore, HEK-Blue™ IL-1β cells respond specifically to IL-1β. They express a SEAP reporter gene under the control of the IFN-β minimal promoter fused to five NF-κB and five AP-1 binding sites.
Binding of IL-1β to its receptor on the surface of HEK-Blue™ IL-1β cells triggers the IL1-R signaling pathway leading to the activation of NF-κB/AP-1 and the subsequent production of SEAP. The levels of SEAP in the supernatant can be easily monitored using QUANTI-Blue™.
HEK-Blue™ IL-1β cells respond to IL-1β and also IL-1α (data not shown) but do not respond to TNF-α (figure 2).
Stimulation of HEK-Blue™ IL-1β cells with recombinant human IL-1β can be blocked by the neutralizing monoclonal anti-hIL-1β-IgA antibody (see page 99),

➢ **Detection range for human IL-1β :** 0.001 - 10 ng/ml
➢ **Detection range for murine IL-1β :** 10 ng - 1 µg/ml

HEK-Blue™ IL-1β cells are resistant to Zeocin™ and hygromycin B.



Figures 2A & 2B: Responses of HEK-Blue™ TNF-α/IL-1β cells and HEK-Blue™ IL-1β cells to TNF-α and IL-1β. HEK-Blue™ TNF-α/IL-1β (A) and HEK-Blue™ IL-1β (B) cells were incubated with increasing concentrations of recombinant human TNF-α or IL-1β. After 24h incubation the levels of NF-κB-induced SEAP were determined using QUANTI-Blue™.

Contents and Storage

HEK-Blue™ TNF-α/IL-1β and HEK-Blue™ IL-1β cells are grown in standard DMEM medium, 2mM L-glutamine, 10% FBS supplemented 100 µg/ml Normocin™ and the appropriate selective antibiotic(s). HEK-Blue™ TNF-α/IL-1β cells are cultivated with 100 µg/ml Zeocin™. HEK-Blue™ IL-1β cells are cultivated with 100 µg/ml Zeocin™ and 200 µg/ml HygroGold™ (ultrapure hygromycin B). Cells are provided frozen in a cryotube containing 5-7 x 10^6 cells and supplied with 50 mg of Normocin™, 10 mg of Zeocin™ (and 10 mg of HygroGold™) and 1 pouch of QUANTI-Blue™. Cells are shipped on dry ice. They are guaranteed mycoplasma-free.

PRODUCT	QUANTITY	CAT. CODE
HEK-Blue™ TNFα/IL-1β Cells	5-7 x 10^6 cells	hkb-tnfil1
HEK-Blue™ IL-1β Cells	5-7 x 10^6 cells	hkb-il1b

Related Products

Recombinant human TNF-α, page 96
Recombinant human IL-1β, page 96
Anti-hIL-1β-IgA2 , page 99
Anti-hTNF-α-IgG1 , page 99
Zeocin™, page 61
HygroGold™ , page 61
Normocin™, page 59
QUANTI-Blue™, page 56

HEK-Blue™ IL-18/IL-1β

HEK-Blue™ IL-18/IL-1β cells are designed to detect bioactive IL-18 and IL-1β (and IL-1α) by monitoring the activation of the NF-κB and AP-1 pathways. They were generated by stable transfection of HEK-Blue™ IL-1β cells with the gene encoding IL-18RAP. They express a SEAP reporter gene under the control of the IFN-β minimal promoter fused to five NF-κB and five AP-1 binding sites.
Stimulation of HEK-Blue™ IL-18/IL-1β cells with human IL-18 or IL-1β, but not with human TNF-α, activates the NF-κB and AP-1 pathways triggering the production of SEAP (figure 2). Levels of SEAP in the supernatant can be easily determined with QUANTI-Blue™.
Stimulation of HEK-Blue™ IL-18/IL-1β cells with recombinant human IL-18 or IL-1β can be blocked by the neutralizing antibodies anti-hIL-18-IgA2 and anti-hIL-1β-IgA2, respectively (see page 99).

➤ **Detection range for human IL-18 :** 0.5 - 100 ng/ml
➤ **Detection range for human IL-1β :** 0.5 - 100 ng/ml

HEK-Blue™ IL-18/IL-1β cells are resistant to blasticidin, Zeocin™ and hygromycin B.

HEK-Blue™ IL-33/IL-1β

HEK-Blue™ IL-33/IL-1β cells are designed to detect bioactive IL-33 and IL-1β (and IL-1α) by monitoring the activation of the NF-κB and AP-1 pathways. They were generated by stable transfection of HEK-Blue™ IL-1β cells with the IL1RL1 gene. They express a SEAP reporter gene under the control of the IFN-β minimal promoter fused to five NF-κB and five AP-1 binding sites.
Stimulation of HEK-Blue™ IL-33/IL-1β cells with human IL-33 or IL-1β, but not with IL-18 nor TNF-α, activates the NF-κB and AP-1 pathways triggering the production of SEAP (figure 2). Levels of SEAP in the supernatant can be easily determined with QUANTI-Blue™.
Stimulation of HEK-Blue™ IL-33/IL-1β cells with recombinant human IL-1β can be blocked by using the neutralizing antibody anti-hIL-1β-IgA.

➤ **Detection range for human IL-33 :** 0.5 - 100 ng/ml
➤ **Detection range for human IL-1β :** 0.5 - 100 ng/ml

HEK-Blue™ IL-33/IL-1β cells are resistant to blasticidin, Zeocin™ and hygromycin B.

Contents and Storage

HEK-Blue™ IL-18/IL-1β and HEK-Blue™ IL-33/IL-1β cells are grown in DMEM medium, 2mM L-glutamine, 10% FBS supplemented with 100 µg/ml Normocin™, 30 µg/ml blasticidin, 100 µg/ml Zeocin™ and 200 µg/ml HygroGold™ (ultrapure Hygromycin). Cells are provided frozen in a cryotube containing 5-7 x 10^6 cells and supplied with 50 mg of Normocin™, 1 mg blasticidin, 10 mg Zeocin™, 10 mg HygroGold™ and 1 pouch of QUANTI-Blue™. Cells are shipped on dry ice. They are guaranteed mycoplasma-free.

PRODUCT	QUANTITY	CAT. CODE
HEK-Blue™ IL-18/IL-1β Cells	5-7 x 10^6 cells	hkb-il18
HEK-Blue™ IL-33/IL-1β Cells	5-7 x 10^6 cells	hkb-il33

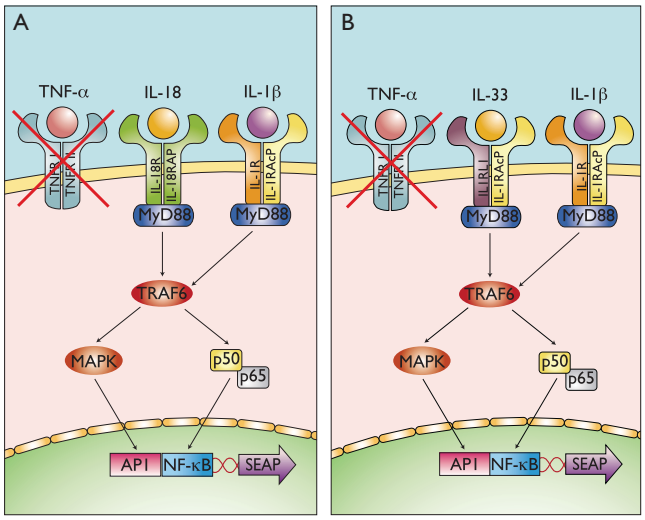
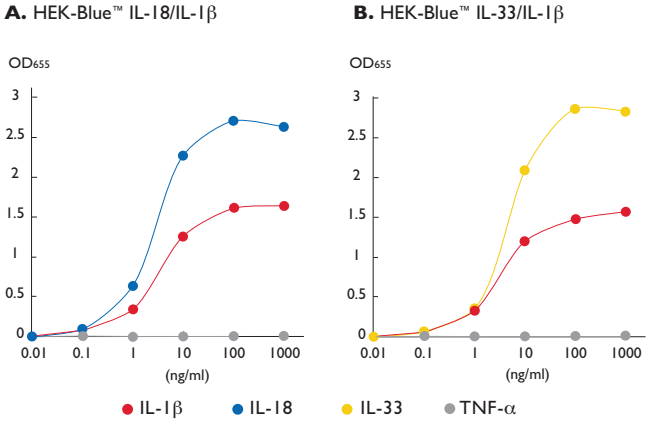


Figure 1: Simplified NF-κB signaling pathway induced by IL-1β, IL-18 or IL-33 in HEK-Blue™ IL-18/IL-1β (A) and HEK-Blue™ IL-33/IL-1β (B) cells. IL-1β binds to the type I IL-1 receptor which requires the IL-1 receptor accessory protein (IL-1RAcP) to transduce a signal. IL-18 binds to an heterodimeric receptor consisting of IL-18R and IL-18 receptor accessory protein (IL18RAP). IL-33 mediates its biological effects through ST2 (also known as IL1RL1), a receptor expressed on Th2 and mast cells. IL-33 and ST2 form a complex with IL-1R accessory protein (IL-1RAcP), a signaling receptor subunit that is also a member of the IL-1R complex. The signaling of IL-1β, IL-18 and IL-33 leads to the activation of NF-κB and MAP kinases.



Figures 2A & 2B: Responses of HEK-Blue™ IL-18/IL-1β cells and HEK-Blue™ IL33/IL1β cells. HEK-Blue™ IL-18/IL-1β (A) and HEK-Blue™ IL-33/IL-1β (B) cells were incubated with increasing concentrations of recombinant human IL-1β , IL-18, IL-33 or TNF-α. After 24h incubation the levels of NF-κB-induced SEAP were determined using QUANTI-Blue™.

Related Products

Recombinant human IL-1β, page 96
Recombinant human IL-18, page 96
Recombinant human IL-33, page 96
Anti-hIL-1β-IgA2 , page 99
Anti-hIL-18-IgA2 , page 99
Blasticidin, page 60
HygroGold™ , page 61
Zeocin™, page 61
Normocin™, page 59
QUANTI-Blue™ page 56

Th2 Lymphokine Reporter Cells

HEK-Blue™ IL-4/IL-13 cells - Human IL-4 and human/murine IL-13 SEAP-reporter cells

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

HEK-Blue™ IL-4/IL-13 cells

HEK-Blue™ IL-4/IL-13 cells allow the detection of bioactive IL-4 and IL-13 by monitoring the activation of the STAT-6 pathway. These cells were generated by stable transfection of HEK293 cells with the human STAT6 gene to obtain a fully active STAT6 pathway. The other genes of the pathway are naturally expressed in sufficient amounts. The cells were further transfected with a SEAP reporter gene under the control of the IFNβ minimal promoter fused to four STAT6 binding sites. HEK-Blue™ IL-4/IL-13 cells produce SEAP in response to IL-4 or IL-13 stimulation and to a lower extent IFN-α (Figure 2).
Stimulation of HEK-Blue™ IL-4/IL-13 cells with IL-4 or IL-13 can be blocked by the neutralizing monoclonal anti-hIL-4-IgA and anti-hIL-13-IgA antibodies, respectively (see page 99).

➢ **Detection range for hIL-4 :** 0.5 - 100 ng/ml
➢ **Detection range for hIL-13 :** 5 - 1000 ng/ml

HEK-Blue™ IL-4/IL-13 cells are resistant to blasticidin and Zeocin™.

HEK-Blue™ IL-6 cells

HEK-Blue™ IL-6 cells allow the detection of bioactive IL-6 by monitoring the activation of the STAT-3 pathway. These cells were generated by stable transfection of HEK293 cells with the human IL-6R gene. They were further transfected with a SEAP reporter gene under the control of the IFN-β minimal promoter fused to four STAT3 binding sites. Upon IL-6 stimulation, HEK-Blue™ IL-6 cells trigger the activation of STAT3 and the subsequent secretion of SEAP.
Stimulation of HEK-Blue™ IL-6 cells with recombinant human IL-6 can be blocked by anti-hIL-6-IgA, a neutralizing monoclonal antibody of the IgA isotype (see page 99).

➢ **Detection range for IL-6:** 0.5 - 50 ng/ml

HEK-Blue™/IL-6 cells are resistant to both hygromycin and Zeocin™.

Contents and Storage

HEK-Blue™ cells are grown in DMEM medium, 2 mM L-glutamine, 10% FBS supplemented with 100 µg/ml Normocin™ and the appropriate selective antibiotic(s). They are provided frozen in a cryotube containing 5-7 x 10^6 cells and supplied with 50 mg of Normocin™, 1 pouch of QUANTI-Blue™ and the corresponding selective antibiotic(s). Cells are shipped on dry ice. They are guaranteed mycoplasma-free.

HEK-Blue™ IL-4/IL-13 cells are cultivated with 10 µg/ml blasticidin and 100 µg/ml Zeocin. They are supplied with 1 mg of blasticidin and 10 mg of Zeocin.

HEK-Blue™ IL-6 cells are cultivated with 200 µg/ml HygroGold™ (ultrapure hygromycin) and 100 µg/ml Zeocin. They are supplied with 10 mg of HygroGold™ and 10 mg of Zeocin™.

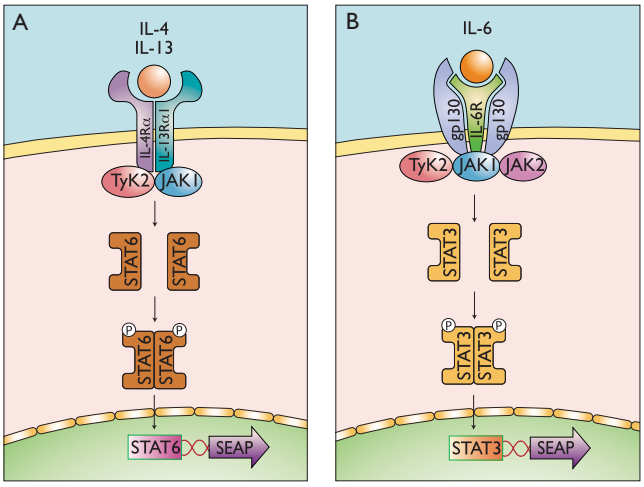
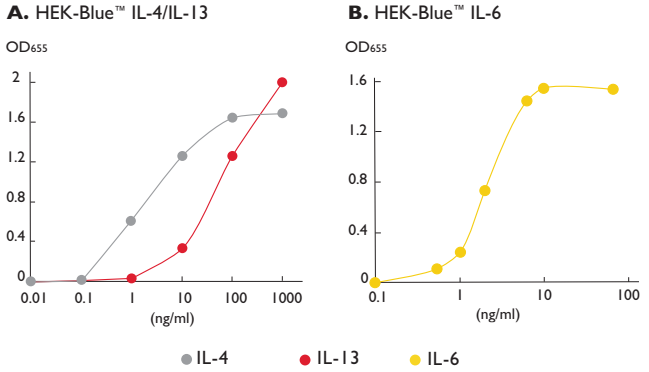


Figure 1: Simplified JAK/STAT signaling pathway induced by IL-4/IL-13 and IL-6. A- Binding of IL-4 or IL-13 to the heterodimeric receptor IL-4Ralpha/IL-13Ralpha1 leads to the recruitment of JAK1 and Tyk2 and the subsequent activation of STAT6. Activated STAT6 forms homodimers that translocate to the nucleus where they bind the promoter of responsive genes inducing their transcription. B- IL-6 exerts its action by first binding to the IL-6R. The complex of IL-6 and IL-6R associates with the signal-transducing membrane protein gp130, inducing its dimerization. This leads to the activation of JAK1, JAK2, and Tyk2 and the translocation to the nucleus of STAT3 where it binds enhancer elements of IL-6-inducible genes.



Figures 2A & 2B: Responses of HEK-Blue™ IL-4/IL-13 cells and HEK-Blue™ IL-6 cells. A- HEK-Blue IL-4/IL-13 cells were incubated with increasing concentrations of recombinant human IL-4 or IL-13. After 24h incubation the levels of STAT6-induced SEAP were determined using QUANTI-Blue™. B- HEK-Blue IL-6 cells were stimulated with increasing concentrations of recombinant human IL-6. After 24h incubation the levels of STAT3-induced SEAP were assessed using QUANTI-Blue™.

PRODUCT	QUANTITY	CAT. CODE
HEK-Blue™ IL-4/IL-13 cells	5-7 x 10^6 cells	hkb-stat6
HEK-Blue™ IL-6 cells	5-7 x 10^6 cells	hkb-il6

REPORTER CELLS RELATED PRODUCTS

Reporter Detection Reagents

InvivoGen offers two reporter systems that feature two secreted reporter genes, the Lucia™ gene encoding a novel secreted luciferase and the SEAP gene coding for the human secreted embryonic alkaline phosphatase. Each reporter system includes the gene available in a mammalian expression plasmid, the recombinant protein and a reporter detection reagent provided lyophilized.

- **Lucia™ Reporter Gene System**
- **SEAP Reporter Gene System**

Mycoplasma Detection & Elimination

Mycoplasma contamination remains a significant problem to the culture of mammalian cells as they can cause disastrous effects on eukaryotic cells, leading to unreliable experimental results, and can remain undetected in the cell cultures for long periods. InvivoGen has developed PlasmoTest™, the first mycoplasma detection kit that uses engineered cells and therefore can be easily established as a routine procedure in the lab. Furthermore, InvivoGen offers a choice of antimicrobial solutions designed to eliminate and prevent mycoplasma contaminations, as well as bacteria.

- **PlasmoTest™ -** Mycoplasma Detection
- **Plasmocin™ & Normocin™ -** Antimicrobial Reagents

Selective Antibiotics

InvivoGen provides the largest choice of antibiotics for selection of mammalian cell lines. Our selective antibiotics are provided as cell-culture tested, sterile solutions that are ready-to-use.

- **Blasticidin**
- **G418 Sulfate**
- **Hygromycin B**
- **Puromycin**
- **Zeocin™**

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's Reporter Cell Lines feature the **secreted embryonic alkaline phosphatase (SEAP) reporter gene**. SEAP is readily quantified by measuring the enzyme activity in the supernatant of cultured cells using the colorimetric assays, QUANTI-Blue™ and HEK-Blue™ Detection (see page 56).

InvivoGen introduces **Lucia™**, a new secreted synthetic luciferase. Lucia™ is a coelenterazine luciferase that can be directly measured in the cell culture supernatant using bioluminescent assays. InvivoGen has developed QUANTI-Luc™, an innovative and ready-to-use coelenterazine-based luminescence assay reagent (see page 55).

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 1000-fold higher bioluminescent signal compared 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 determined fast and easily 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 the following:

➢ **Lucia™ Gene**

➢ **Recombinant Lucia™ Protein**

➢ **Lucia™ Antibody**

➢ **QUANTI-Luc™,** a Lucia™ detection reagent

➢ **Lucia™ -expressing cell lines** (see pages 38, 40, 47 & 48)

➢ **Lucia™ Reporter Plasmids** (see page 87)

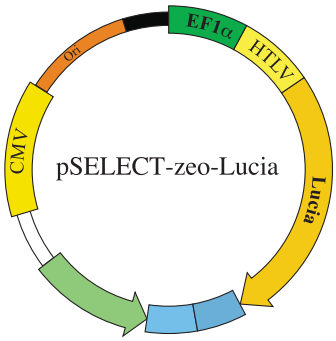
Lucia™ Reporter Gene System NEW

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 p22). 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

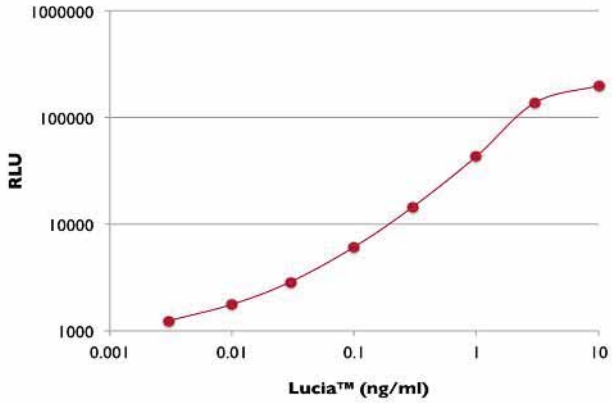
Recombinant Lucia™ Protein is a monomeric protein expressed in CHO cells. The mature protein is composed of 192 amino acids and has an estimated molecular weight of 21 kDa. Recombinant Lucia™ protein is provided in FBS-containing culture medium.

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™

InvivoGen's NEW and original lyophilized product, QUANTI-Luc™, is an assay reagent containing all the components required to quantitively measure the activity of Lucia™ and other secreted coelenterazine-utilizing luciferases. 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 (see page 47) and HEK-Dual™ TNF-α (see page 48),
- double promoter reporter cells, THP1-Dual™ (NF-κB, ISG) (see page 38) and Jurkat-Dual™ (NF-κB, ISG) (see page 40).

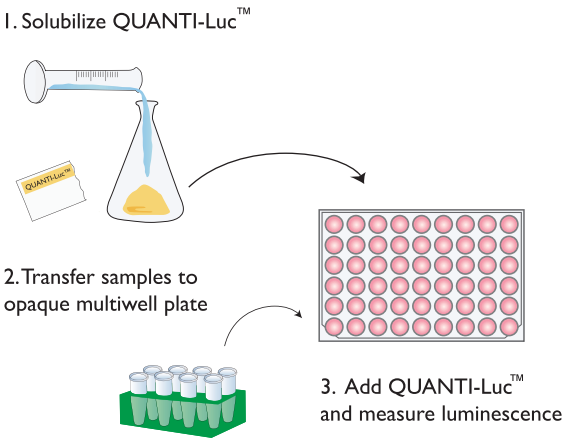
No cell lysis required
Lucia is secreted into the cell culture media. Small sample volumes of 20 µl are sufficient.

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. Shortens time-to-results by half compared to other coelenterazine-utilizing luciferases.

Related Products

Lucia™ Reporter Cell Lines, pages 38, 40, 47 & 48
Lucia™ Reporter Plasmids - pNiFty-Lucia, page 87
Lucia™ Expression Plasmids, Catalog 2 - Mammalian Cell Expression

QUANTI-Luc™ Procedure



1. Prepare QUANTI-Luc™ reagent by adding 25 ml water to the contents of one pouch.
2. Transfer samples, for instance 10 µl of media from cells containing the secreted luciferase to opaque 96-microwell plates suitable for your luminometer.
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**

SEAP Reporter Gene System

QUANTI-Blue™ - Detection and quantification of SEAP

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

QUANTI-Blue™ is a detection medium developed to determine the levels of SEAP in biological samples, including cell supernatants and mouse plasma. QUANTI-Blue™ offers many advantages over the conventional SEAP Reporter Assay Kit based on the pNPP substrate, such as 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 allows the detection of 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 color 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 medium	Cell culture medium
Applications	• SEAP detection in biological samples • SEAP kinetic studies	• 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. Add SEAP-expressing cells 3. Transfer to multi-well plate 4. Incubate 6 to 24 hours at 37°C, 5% CO2 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**

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.

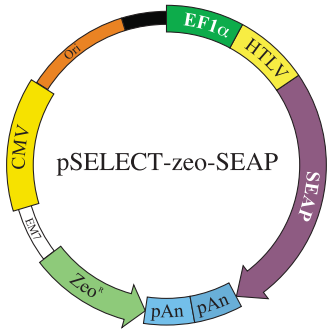
Luke JM. *et al.*, 2011. Coexpressed RIG-I Agonist Enhances Humoral Immune Response to Influenza Virus DNA Vaccine. J. Virol., 85: 1370 - 1383.

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.

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 22). 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

Recombinant SEAP Protein NEW

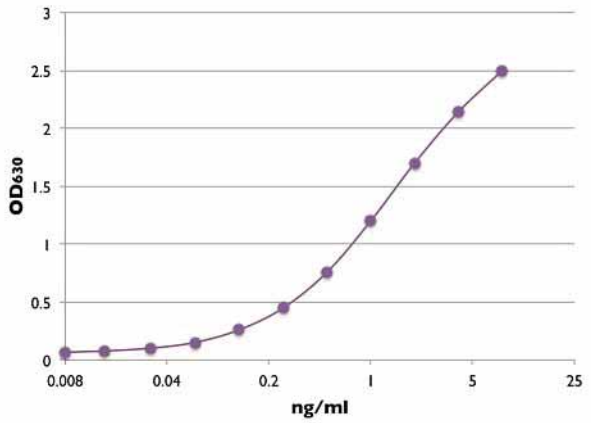
Recombinant SEAP (secreted embryonic alkaline phosphatase) protein is a truncated form of human placental alkaline phosphatase that comprises 520 amino acids. It is expressed in CHO cells and shows a 75 kDa band on SDS page. Recombinant SEAP protein is purified by affinity chromatography. It is formulated 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.

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



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

MYCOPLASMA DETECTION & ELIMINATION

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.

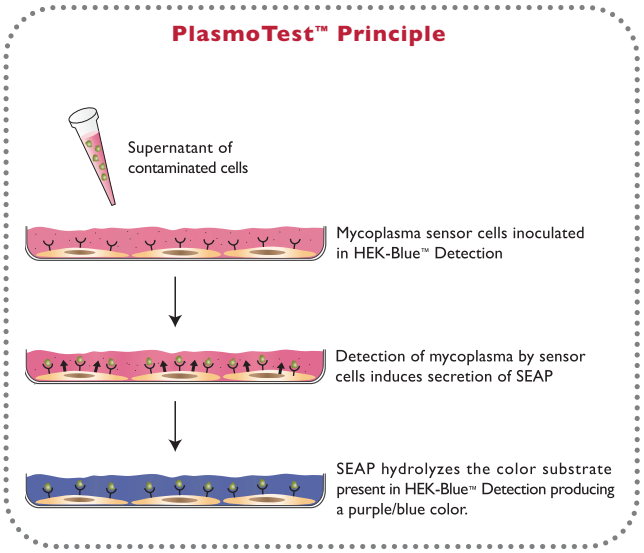
HEK-Blue™ Detection is a medium specifically designed for the detection of SEAP. It contains a color substrate that produces a purple/blue color following its hydrolysis by SEAP (see page 56).

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.



Contents

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

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

PRODUCT	QUANTITY	CAT. CODE
PlasmoTest™	1 kit	rep-pt2
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

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 is observed. No resistance in liquid cultures of mycoplasmas has ever been identified in repeated experiments attempting to measure the mutation rate. Therefore, development of resistant mycoplasma strains is virtually eliminated.

Plasmocin™ Treatment (ant-mpt)

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

Plasmocin™ Prophylactic (ant-mpp)

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

Contents 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 at least one year at -20°C.

PRODUCT	QUANTITY	CAT. CODE
Plasmocin™ Treatment	50 mg (2 x 1 ml)	ant-mpt
Plasmocin™ Prophylactic	25 mg (5 x 2 ml)	ant-mpp

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.

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

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

Related Products

Fungin™, Catalog 2 - Mammalian Cell Expression
Plasmocure™, Catalog 2 - Mammalian Cell Expression
Primocin™, Catalog 2 - Mammalian Cell Expression

Recent articles using Plasmocin™ or Normocin™

Plasmocin™

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.

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.

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.

Normocin™

Matsunaga N. *et al.*, 2011. TAK-242 (resatorvid), a small-molecule inhibitor of Toll-like receptor (TLR) 4 signaling, binds selectively to TLR4 and interferes with interactions between TLR4 and its adaptor molecules. Mol Pharmacol. 79(1):34-41.

Li YJ. *et al.*, 2012. Gold nanoparticles as a platform for creating a multivalent poly-SUMO chain inhibitor that also augments ionizing radiation. PNAS [Epub ahead of print].

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

SELECTIVE ANTIBIOTICS

High Quality - Our antibiotics meet rigorous standards and have passed stringent quality control, which includes verification of potency, purity and stability using microbiological and chromatographic methods. This leads to consistently high quality.

Ready-to-use Cell Culture Tested Solutions - No weighing needed - Our antibiotics are available in solution filtered to sterility for customer convenience and validated for cell culture usage.

Endotoxin Tested - InvivoGen's selective antibiotics contain no detectable levels of endotoxin (<0.125 EU/ml). Many commonly used cell lines, such as immune cells, express TLR4, the receptor for endotoxins (also known as lipopolysaccharide (LPS)). The guarantee that our antibiotics are endotoxin-free ensures that their addition to cell cultures will not bias the results of your experiments.

Blasticidin

Description

Blasticidin is a peptidyl nucleoside antibiotic isolated from *Streptomyces griseochromogenes*. Blasticidin 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 at least one year when stored at -20°C.

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

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.

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 for at least one year when stored at -20°C.

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 Antibiotics

Blasticidin

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.

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.

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

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.

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.

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 for at least one year when stored at -20°C.

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

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 for at least one year at -20°C.

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

Puromycin

Description

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

Contents 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 at least one year when stored at -20°C.

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 Antibiotics

Hygromycin B / HygroGold™

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. 10.1093/nar/gkr1282.

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

Ropers F. *et al.*, 2011, Identification of a novel candidate gene for non-syndromic autosomal recessive intellectual disability: the WASH complex member SWIP. Hum. Mol. Genet., 20: 2585 - 2590.

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

Puromycin

DeMars G. *et al.*, 2011. The Extreme C-Terminal Region of Gαs Differentially Couples to the Luteinizing Hormone and β2-Adrenergic Receptors. Mol. Endocrinol. 25: 1416 - 1430.

van Lith M. *et al.*, 2011. Real-time monitoring of redox changes in the mammalian endoplasmic reticulum. J. Cell Sci., 124: 2349 - 2356.

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: 307 - 318.

Zeocin™

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: 16663 - 16668.

Bielig H. *et al.*, 2011. NOD-Like Receptor Activation by Outer Membrane Vesicles from Vibrio cholerae Non-O1 Non-O139 Strains Is Modulated by the Quorum-Sensing Regulator HapR. Infect. Immun., 79: 1418 - 1427.

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

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PRR LIGANDS

TLRs, NODs, RLRs and Dectin-1 are pattern recognition receptors (PRRs) that recognize a wide variety of microbial molecules, called pathogen-associated molecular patterns (PAMPs), discriminating Gram-positive and Gram-negative bacteria from fungi and other pathogens. InvivoGen offers a comprehensive choice of high quality PAMPs know to activate these PRRs.

All PAMPs are tested for TLR stimulation using Blue™ reporter cells. The endotoxin levels are determined using a kinetic chromogenic LAL assay. EndoFit™ agonists contain less than 0.001 EU/μg.

TLR2 Agonists

TLR2 is involved in the recognition of a wide array of microbial molecules representing broad groups of species such as Gram- and Gram+ bacteria, as well as mycoplasma and yeast.

TLR3 Agonists

TLR3 recognizes double-stranded RNA (dsRNA), a molecular pattern associated with viral infection. Polyinosine-polycytidylic acid (poly(I:C)), a synthetic analog of dsRNA, is the ligand of choice for TLR3.

TLR4 Agonists

TLR4 is the receptor for Gram-negative lipopolysaccharide (LPS) and lipid A, its toxic moiety. InvivoGen offers LPS from various bacteria and monophosphoryl lipid A.

TLR5 Agonists

TLR5 recognizes flagellin, the major component of the bacterial flagellar filament, from both Gram+ and Gram- bacteria. InvivoGen provides flagellin purified from *B. subtilis* (Gram+) and *S. typhimurium* (Gram-) bacteria and a recombinant form.

TLR7/8 Agonists

TLR7 and TLR8 are involved in the response to viral infection. They recognize GU-rich short single-stranded RNA as well as small synthetic molecules such as imidazoquinolines and nucleoside analogues.

TLR9 Agonists

TLR9 recognizes specific unmethylated CpG-ODN sequences that distinguish microbial DNA from mammalian DNA. Three types of stimulatory ODNs have been described: type A, B and C. InvivoGen also provides control ODNs and inhibitory ODNs. **Larger quantities are available upon request.**

NOD1/2 Agonists

NOD1 and NOD2 are intracellular pathogen-recognition molecules that sense bacterial peptidoglycan (PGN). InvivoGen provides insoluble and soluble PGNs from Gram$^-$ and Gram$^+$ bacteria and bioactive fragments of PGN such as iE-DAP and MDP.

RIG-I/MDA-5 Agonists

RIG-I and MDA-5 are cytoplasmic RNA helicases that recognize intracellular double-stranded RNA (dsRNA), a molecular pattern associated with viral infection. InvivoGen provides poly(I:C)/LyoVec™, a ligand that mimics viral dsRNA, as well as 5'ppp-dsRNA.

Cytosolic DNA Sensor (CDS) Agonists

Double-stranded DNA (dsDNA) is a potent inducer of type I interferons. Several sensors of cytosolic dsDNA have been identified, including DAI, RIG-I and LRRFIP1. These sensors recognize AT-rich B-form dsDNA and GC-rich Z-form dsDNA.

Dectin-1 Agonists

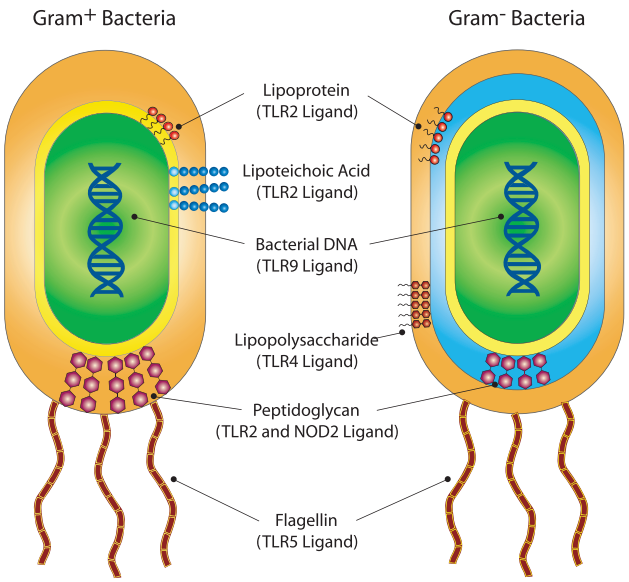
Dectin-1 is a specific receptor of β-glucans, which are glucose polymers found in the cell walls of fungi, including the yeasts *Saccharomyces cerevisiae* and *Candida albicans*.

Mincle Agonist

Mincle is a multi-task danger receptor that recognizes a wide variety of ligands such as damaged cells, fungus, yeast and mycobacteria.

Contents and Storage

Most products are provided as a powder form and are supplied with endotoxin-free water for their reconstitution.
Products are shipped at room temperature and should be stored at 4°C or -20°C as mentioned in the technical datasheet.



PRODUCT	ORIGIN/DESCRIPTION	ENDOTOXIN LEVELS	WORKING CONCENTRATION	QTY	CATALOG CODE	INFO
TLR2 Agonists						
FSL-1	Synthetic diacylated lipoprotein - TLR2/6	EndoFit™	1 - 100 ng/ml	100 µg	tlrl-fsl	p 70
HKAL	Heat Killed *Acholeplasma laidlawii*	EndoFit™	10^6 - 10^8 cells/ml	10^9 cells	tlrl-hkal	p 70
HKEB	Heat Killed *Escherichia coli* 0111:B4	>1 EU/10^9 cells	10^5 - 10^7 cells/ml	10^{10} cells	tlrl-hkeb	p 70
HKHP	Heat Killed *Helicobacter pylori*	EndoFit™	10^6 - 10^8 cells/ml	10^9 cells	tlrl-hkhp	p 70
HKLM	Heat Killed *Listeria monocytogenes*	EndoFit™	10^7 - 10^8 cells/ml	10^{10} cells	tlrl-hklm	p 70
HKLP	Heat Killed *Legionella pneumophila*	EndoFit™	10^7 - 10^8 cells/ml	10^9 cells	tlrl-hklp	p 70
HKLR	Heat Killed *Lactobacillus rhamnosus*	>1 EU/10^9 cells	10^8 - 10^9 cells/ml	10^{10} cells	tlrl-hklr	p 70
HKMF	Heat Killed *Mycoplasma fermentans*	EndoFit™	10^6 - 10^8 cells/ml	10^9 cells	tlrl-hkmf	p 70
HKPA	Heat Killed *Pseudomonas aeruginosa*	>1 EU/10^8 cells	10^5 - 10^7 cells/ml	10^{10} cells	tlrl-hkpa	p 70
HKPG	Heat Killed *Porphyromonas gingivalis*	EndoFit™	10^6 - 10^8 cells/ml	10^{10} cells	tlrl-hkpg	p 70
HKSA	Heat Killed *Staphylococcus aureus*	>1 EU/10^9 cells	10^6 - 10^8 cells/ml	10^{10} cells	tlrl-hksa	p 70
HKSP	Heat Killed *Streptococcus pneumoniae*	EndoFit™	10^7 - 10^9 cells/ml	10^{10} cells	tlrl-hksp	p 70
LAM-MS	Lipoarabinomannan from *M. smegmatis*	EndoFit™	100 ng - 10 µg/ml	500 µg	tlrl-lams	p 70
LM-MS	Lipomannan from *Mycobacterium smegmatis*	>5 EU/mg	1 - 10 ng/ml	250 µg	tlrl-lmms2	p 70
LPS-PG	LPS from *P. gingivalis*	>10^4 EU/mg	10 ng - 10 µg/ml	1 mg	tlrl-pglps	p 70
LTA-BS	Lipoteichoic acid from *Bacillus subtilis*	10 EU/mg	100 ng - 1 µg/ml	5 mg	tlrl-lta	p 70
LTA-SA	Lipoteichoic acid from *S. aureus*	10 EU/mg	100 ng - 1 µg/ml	5 mg	tlrl-slta	p 70
LTA-SA Purified	Purified lipoteichoic acid from *S. aureus*	EndoFit™	1 ng - 1 µg/ml	5 mg	tlrl-pslta	p 70
Pam2CSK4	Synthetic diacylated lipoprotein - TLR2(6)	EndoFit™	1 - 100 ng/ml	100 µg 1 mg	tlrl-pm2s tlrl-pm2s-1	p 71
Pam2CSK4 Biotin	Biotinylated Pam2CSK4	EndoFit™	1 - 100 ng/ml	50 µg	tlrl-bpam2	p 71
Pam2CSK4 Rhodamine	Rhodamine-labeled Pam2CSK4	EndoFit™	1 - 100 ng/ml	50 µg	tlrl-rpam2	p 71
Pam3CSK4	Synthetic triacylated lipoprotein - TLR1/2	EndoFit™	1 - 300 ng/ml	1 mg	tlrl-pms	p 71
Pam3CSK4 Biotin	Biotinylated Pam3CSK4	EndoFit™	1 - 100 ng/ml	50 µg	tlrl-bpms	p 71
Pam3CSK4 Rhodamine	Rhodamine-labeled Pam3CSK4	EndoFit™	1 - 300 ng/ml	50 µg	tlrl-rpms	p 71
Pam3CSK4 VacciGrade™	Sterile Pam3CSK4	EndoFit™	1 - 300 ng/ml	1 mg	vac-pms	p105
PGN-BS	Peptidoglycan from *B. subtilis*	EndoFit™	1 - 10 µg/ml	5 mg	tlrl-pgnbs	p 71
PGN-EB	Peptidoglycan from *E. coli* 0111:B4	10^2 - 10^3 EU/mg	1 - 10 µg/ml	1 mg	tlrl-pgnec	p 71
PGN-EK	Peptidoglycan from *E. coli* K12	10^2 - 10^3 EU/mg	1 - 10 µg/ml	1 mg	tlrl-pgnek	p 71
PGN-SA	Peptidoglycan from *S. aureus*	1 EU/mg	1 - 10 µg/ml	5 mg	tlrl-pgnsa	p 71
Zymosan	Cell wall preparation of *S. cerevisiae*	EndoFit™	10 µg/ml	100 mg	tlrl-zyn	p 71
TLR3 Agonists						
Poly(A:U)	Polyadenylic–polyuridylic acid	<0.005 EU/µg	300 ng - 100 µg/ml	10 mg	tlrl-pau	p 72
Poly(I:C) (HMW)	Polyinosine-polycytidylic acid High molecular weight (1.5-8 kb)	EndoFit™	10 ng - 10 µg/ml	10 mg 50 mg	tlrl-pic tlrl-pic-5	p 72
Poly(I:C) (LMW)	Polyinosine-polycytidylic acid Low molecular weight (0.2-1 kb)	EndoFit™	30 ng - 10 µg/ml	25 mg 250 mg	tlrl-picw tlrl-picw-250	p 72
Poly(I:C) (HMW) Fluorescein	Fluorescein-labeled poly(I:C) (HMW)	EndoFit™	10 ng - 10 µg/ml	10 µg	tlrl-picf	p 72
Poly(I:C) (HMW) Rhodamine	Rhodamine-labeled poly(I:C) (HMW)	EndoFit™	10 ng - 10 µg/ml	10 µg	tlrl-picr	p 72
Poly(I:C) (LMW) Rhodamine	Rhodamine-labeled poly(I:C) (LMW)	EndoFit™	10 ng - 10 µg/ml	10 µg	tlrl-piwr	p 72
Poly(I:C) (HMW) VacciGrade™	Sterile poly(I:C) (HMW)	EndoFit™	10 ng - 10 µg/ml	10 mg	vac-pic	p 72
TLR4 Agonists						
LPS-EB	Standard lipopolysaccharide from *E. coli* 0111:B4	1 x 10^6 EU/mg	10 ng - 10 µg/ml	5 mg	tlrl-eblps	p 72
LPS-EB Ultrapure	Ultrapure lipopolysaccharide from *E. coli* 0111:B4	1 x 10^6 EU/mg	10 ng - 10 µg/ml	5 mg	tlrl-3pelps	p 72
LPS-EB Biotin	Biotinylated ultrapure LPS from *E. coli* 0111:B4	1 x 10^6 EU/mg	10 ng - 10 µg/ml	500 µg	tlrl-bblps	p 72
LPS-EK	Standard lipopolysaccharide from *E. coli* K12	1 x 10^6 EU/mg	1 ng - 10 µg/ml	5 mg	tlrl-eklps	p 72

PRODUCT	ORIGIN/DESCRIPTION	ENDOTOXIN LEVELS*	WORKING CONCENTRATION	QTY	CATALOG CODE	INFO
TLR4 Agonists						
LPS-EK Ultrapure	Ultrapure lipopolysaccharide from *E. coli K12*	1 x 10^6 EU/mg	1 ng - 10 µg/ml	1 mg	tlrl-peklps	p 72
LPS-SM Ultrapure	Ultrapure lipopolysaccharide from *S. minnesota*	1 x 10^5 EU/mg	10 ng - 10 µg/ml	5 mg	tlrl-smlps	p 72
MPLA	Monophosphoryl lipid A from *S. minnesota*	1 x 10^6 EU/mg	100 ng - 1 µg/ml	1 mg	tlrl-mpl	p 72
MPLA VacciGrade™	Sterile detoxified MPLA	1 x 10^6 EU/mg	2 - 20 µg/mouse	1 mg	vac-mpl	p 72
MPLAs	Synthetic monophosphoryl lipid A	1 x 10^6 EU/mg	10 ng - 10 µg/ml	1 mg	tlrl-mpls	p 72
MPLAs VacciGrade™	Sterile synthetic MPLA	1 x 10^6 EU/mg	2 - 20 µg/mouse	1 mg	vac-mpls	p 72
TLR4 Antagonist						
LPS-RS	Lipopolysaccharide from *Rhodobacter sphaeroides*	1 x 10^6 EU/mg	10 ng - 10 µg/ml	5 mg	tlrl-rslps	p 73
LPS-RS Ultrapure NEW	Ultrapure lipopolysaccharide from *R. sphaeroides*	1 x 10^6 EU/mg	10 ng - 10 µg/ml	1 mg	tlrl-prslps	p 73
TLR5 Agonists						
FLA-BS	Standard flagellin from *B. subtilis*	<10 EU/mg	10 ng - 10 µg/ml	100 µg	tlrl-bsfla	p 73
FLA-ST	Standard flagellin from *S. typhimurium - 10% pure*	1 x 10^3-10^4 EU/mg	10 ng - 10 µg/ml	100 µg	tlrl-stfla	p 73
FLA-ST Ultrapure	Ultrapure flagellin from *S. typhimurium - >95% pure*	<50 EU/mg	10 - 100 ng/ml	10 µg 50 µg	tlrl-pstfla tlrl-pstfla-5	p 73
RecFLA-ST	Recombinant flagellin from *S. typhimurium*	EndoFit™	10 - 100 ng/ml	1 µg 10 µg	tlrl-flic tlrl-flic-10	p 73
Flagellin Flic VacciGrade™	Sterile recombinant flagellin from *S. typhimurium*	EndoFit™	1 - 10 µg/mouse	50 µg	vac-fla	p 105
TLR7 Agonists						
CL264	Adenine analog	EndoFit™	50 ng - 10 µg/ml	500 µg 5 mg	tlrl-c264s tlrl-c264-5	p 73
CL264 Biotin	Biotinylated CL264	EndoFit™	1 - 10 µg/ml	100 µg	tlrl-bc264	p 73
CL264 FITC	FITC-labeled CL264	EndoFit™	1 - 10 µg/ml	100 µg	tlrl-fc264	p 73
CL264 Rhodamine	Rhodamine-labeled CL264	EndoFit™	1 - 10 µg/ml	100 µg	tlrl-rc264	p 73
Gardiquimod™	Imidazoquinoline compound	EndoFit™	0.1 - 3 µg/ml	500 µg 5 mg	tlrl-gdqs tlrl-gdq-5	p 73
Gardiquimod™ VacciGrade™	Sterile Gardiquimod™	EndoFit™	10 - 100 µg/mouse	5 mg	vac-gdq	p 105
Imiquimod (R837)	Imidazoquinoline compound	EndoFit™	1 - 5 µg/ml	500 µg 5 mg	tlrl-imqs tlrl-imq	p 74
Imiquimod VacciGrade™	Sterile Imiquimod	EndoFit™	1 - 5 µg/ml	5 mg	vac-imq	p 105
Loxoribine	Guanosine analog	EndoFit™	10 - 100 µg/mouse	50 mg	tlrl-lox	p 74
TLR8 Agonists						
ORN02/LyoVec	ssRNA with 6 UUAU repeats / LyoVec™	EndoFit™	0.25 - 5 µg/ml	4 x 25 µg	tlrl-orn2	p 74
ORN06/LyoVec	ssRNA with 6 UUGU repeats / LyoVec™	EndoFit™	0.25 - 5 µg/ml	4 x 25 µg	tlrl-orn6	p 74
ssPolyU Naked	RNA homopolymer	EndoFit™	1 - 10 µg/ml	10 mg	tlrl-sspu	p 74
ssPolyU/LyoVec	RNA homopolymer / LyoVec™	EndoFit™	1 - 10 µg/ml	4 x 25 µg	tlrl-lpu	p 74
ssRNA40/LyoVec	HIV-1 LTR-derived ssRNA / LyoVec™	EndoFit™	0.25 - 5 µg/ml	4 x 25 µg	tlrl-lrna40	p 74
ssRNA41/LyoVec	ssRNA40 control / LyoVec™	EndoFit™	0.25 - 5 µg/ml	4 x 25 µg	tlrl-lrna41	p 74
ssRNA-DR/LyoVec	ssRNA with 2 GUCCUUCAA repeats / LyoVec™	EndoFit™	1 - 10 µg/ml	4 x 25 µg	tlrl-ssdr	p 74
TLR7/8 Agonists						
CL075	Thiazoquinoline compound	EndoFit™	100 ng - 5 µg/ml	500 µg 5 mg	tlrl-c75 tlrl-c75-5	p 73
CL097	Imidazoquinoline compound	EndoFit™	50 ng - 5 µg/ml	500 µg 5 mg	tlrl-c97 tlrl-c97-5	p 73
Poly(dT)	Thymidine homopolymer ODN (17 mer)	EndoFit™	10 µM	100 nmol	tlrl-pt17	p 74
R848 (resiquimod)	Imidazoquinoline compound	EndoFit™	10 ng - 10 µg/ml	500 µg 5 mg	tlrl-r848 tlrl-r848-5	p 74
R848 VacciGrade™	Sterile R848	EndoFit™	10 - 100 µg/mouse	5 mg	vac-r848	p 105

PRODUCT	ORIGIN/DESCRIPTION	ENDOTOXIN LEVELS	WORKING CONCENTRATION	QTY	CATALOG CODE	INFO
TLR9 Agonists						
***E. coli* DNA ef**	Endotoxin-free DNA from *E. coli K12*	EndoFit™	0.25 - 10 µg/ml	1 mg	tlrl-ednaef	p 75
***E. coli* ssDNA/LyoVec**	*E. coli* single stranded DNA/LyoVec™ complexes	EndoFit™	1 - 10 µg/ml	200 µg	tlrl-ssec	p 75
ODN 1585	Stimulatory CpG ODN Type A Mouse specific	EndoFit™	5 µM (10 µg/ml)	200 µg 1 mg 5 mg	tlrl-1585 tlrl-1585-1 tlrl-1585-5	p 75
ODN 1585 control	Negative control for ODN 1585	EndoFit™	5 µM (10 µg/ml)	200 µg 1 mg 5 mg	tlrl-1585c tlrl-1585c-1 tlrl-1585c-5	p 75
ODN 1585 FITC	FITC-labeled CpG ODN - mouse specific, type A	EndoFit™	10 ng - 10 µg/ml	50 µg	tlrl-1585f	p 75
ODN 1585 VacciGrade™	Sterile ODN 1585	EndoFit™	20 - 50 µg/mouse	1 mg	vac-1585-1	p 105
ODN 1668	Stimulatory CpG ODN Type B Mouse specific	EndoFit™	5 µM (10 µg/ml)	200 µg 1 mg 5 mg	tlrl-1668 tlrl-1668-1 tlrl-1668-5	p 75
ODN 1668 control	Negative control for ODN 1668	EndoFit™	5 µM (10 µg/ml)	200 µg 1 mg 5 mg	tlrl-1668c tlrl-1668c-1 tlrl-1668-5	p 75
ODN 1668 FITC	FITC-labeled CpG ODN - mouse specific, type B	EndoFit™	10 ng - 10 µg/ml	50 µg	tlrl-1668f	p 75
ODN 1826	Stimulatory CpG ODN Type B Mouse specific	EndoFit™	5 µM (10 µg/ml)	200 µg 1 mg 5 mg	tlrl-1826 tlrl-1826-1 tlrl-1826-5	p 75
ODN 1826 control	Negative control for ODN 1826	EndoFit™	5 µM (10 µg/ml)	200 µg 1 mg 5 mg	tlrl-1826c tlrl-1826c-1 tlrl-1826c-5	p 75
ODN 1826 Biotin	Biotinylated CpG ODN - mouse specific, type B	EndoFit™	10 ng - 10 µg/ml	50 µg	tlrl-1826b	p 75
ODN 1826 FITC	FITC-labeled CpG ODN - mouse specific, type B	EndoFit™	10 ng - 10 µg/ml	50 µg	tlrl-1826f	p 75
ODN 1826 VacciGrade™	Sterile ODN 1826	EndoFit™	20 - 50 µg/mouse	1 mg	vac-1826-1	p 105
ODN 2006	Stimulatory CpG ODN Type B Human specific	EndoFit™	5 µM (10 µg/ml)	200 µg 1 mg 5 mg	tlrl-2006 tlrl-2006-1 tlrl-2006-5	p 75
ODN 2006 control	Negative control for ODN 2006	EndoFit™	5 µM (10 µg/ml)	200 µg 1 mg 5 mg	tlrl-2006c tlrl-2006c-1 tlrl-2006c-5	p 75
ODN 2006 Biotin	Biotinylated CpG ODN - human specific, type B	EndoFit™	10 ng - 10 µg/ml	50 µg	tlrl-2006b	p 75
ODN 2006 FITC	FITC-labeled CpG ODN - human specific, type B	EndoFit™	10 ng - 10 µg/ml	50 µg	tlrl-2006f	p 75
ODN 2006-G5	Stimulatory CpG ODN Type B Human specific	EndoFit™	5 µM (10 µg/ml)	200 µg 1 mg 5 mg	tlrl-2006g5 tlrl-2006g5-1 tlrl-2006g5-5	p 75
ODN 2006 VacciGrade™	Sterile ODN 2006	EndoFit™	20 - 50 µg/mouse	1 mg	vac-2006-1	p 105
ODN 2007	Stimulatory CpG ODN Type B Bovine / porcine	EndoFit™	5 µM (10 µg/ml)	200 µg 1 mg 5 mg	tlrl-2007 tlrl-2007-1 tlrl-2007-5	p 75
ODN 2007 control	Negative control for ODN 2007	EndoFit™	5 µM (10 µg/ml)	200 µg 1 mg 5 mg	tlrl-2007c tlrl-2007c-1 tlrl-2007c-5	p 75
ODN 2216	Stimulatory CpG ODN Type A Human specific	EndoFit™	5 µM (10 µg/ml)	200 µg 1 mg 5 mg	tlrl-2216 tlrl-2216-1 tlrl-2216-5	p 75
ODN 2216 control	Negative control for ODN 2216	EndoFit™	5 µM (10 µg/ml)	200 µg 1 mg 5 mg	tlrl-2216c tlrl-2216c-1 tlrl-2216c-5	p 75
ODN 2216 Biotin	Biotinylated CpG ODN - human specific, type A	EndoFit™	10 ng - 10 µg/ml	50 µg	tlrl-2216b	p 75
ODN 2216 FITC	FITC-labeled CpG ODN - human specific, type A	EndoFit™	10 ng - 10 µg/ml	50 µg	tlrl-2216f	p 75

PRODUCT	ORIGIN/DESCRIPTION	ENDOTOXIN LEVELS	WORKING CONCENTRATION	QTY	CATALOG CODE	INFO
TLR9 Agonists						
ODN 2336	Stimulatory CpG ODN Type A Human specific	EndoFit™	5 µM (10 µg/ml)	200 µg 1 mg 5 mg	tlrl-2336 tlrl-2336-1 tlrl-2336-5	p 75
ODN 2336 control	Negative control for ODN 2336	EndoFit™	5 µM (10 µg/ml)	200 µg 1 mg 5 mg	tlrl-2336c tlrl-2336c-1 tlrl-2336c-5	p 75
ODN 2336 FITC	FITC-labeled CpG ODN - human specific, type A	EndoFit™	10 ng - 10 µg/ml	50 µg	tlrl-2336f	p 75
ODN 2395	Stimulatory CpG ODN Type C Human / mouse	EndoFit™	5 µM (10 µg/ml)	200 µg 1 mg 5 mg	tlrl-2395 tlrl-2395-1 tlrl-2395-5	p 75
ODN 2395 control	Negative control for ODN 2395	EndoFit™	5 µM (10 µg/ml)	200 µg 1 mg 5 mg	tlrl-2395c tlrl-2395c-1 tlrl-2395c-5	p 75
ODN 2395 FITC	FITC-labeled CpG ODN - human specific, type C	EndoFit™	10 ng - 10 µg/ml	50 µg	tlrl-2395f	p 75
ODN M362	Stimulatory CpG ODN Type C Human / mouse	EndoFit™	5 µM (10 µg/ml)	200 µg 1 mg 5 mg	tlrl-m362 tlrl-m362-1 tlrl-m362-5	p 75
ODN M362 control	Negative control for ODN M362	EndoFit™	5 µM (10 µg/ml)	200 µg 1 mg 5 mg	tlrl-m362c tlrl-m362c-1 tlrl-m362c-5	p 75
ODN M362 FITC	FITC-labeled CpG ODN - human specific, type C	EndoFit™	10 ng - 10 µg/ml	50 µg	tlrl-m362f	p 75
pCpG Giant	CpG-free *dcm*- giant plasmid	EndoFit™	5 - 10 µg/ml	1 mg	tlrl-cpgg	p 75
Salmon sperm DNA	TLR9 negative control	EndoFit™	5 - 100 µg/ml	50 mg	tlrl-sdef	p 75
TLR9 Antagonists						
ODN 2088	Inhibitory ODN, mouse preferred	EndoFit™	50 nM - 1 µM	200 µg 1 mg	tlrl-2088 tlrl-2088-1	p 76
ODN 2088 control	Negative control for ODN 2088	EndoFit™	50 nM - 1 µM	200 µg 1 mg	tlrl-2088c tlrl-2088c-1	p 76
ODN 4084-F	Class B inhibitory ODN	EndoFit™	50 nM - 1 µM	200 µg	tlrl-4084	p 76
ODN INH-1	Class R inhibitory ODN	EndoFit™	50 nM - 1 µM	200 µg	tlrl-inh1	p 76
ODN INH-47	Negative control for ODN INH-1	EndoFit™	50 nM - 1 µM	200 µg	tlrl-inh47	p 76
ODN TTAGGG	Inhibitory ODN, human preferred	EndoFit™	50 nM - 1 µM	200 µg 1 mg	tlrl-ttag tlrl-ttag-1	p 76
ODN TTAGGG control	Negative control for ODN TTAGGG	EndoFit™	50 nM - 1 µM	200 µg 1 mg	tlrl-ttagc tlrl-ttagc-1	p 76
G-ODN	Inhibitory guanosine-rich ODN	EndoFit™	50 nM - 1 µM	200 µg	tlrl-godn	p 76
NOD1 Agonists						
C12-iE-DAP	Acylated derivative of iE-DAP	EndoFit™	1 ng - 1 µg/ml	1 mg	tlrl-c12dap	p 76
iE-DAP	D-γ-Glu-mDAP	EndoFit™	1 - 100 µg/ml	5 mg	tlrl-dap	p 76
iE-Lys	iE-DAP negative control	EndoFit™	1 - 100 µg/ml	5 mg	tlrl-lys	p 76
Tri-DAP	L-Ala-γ-D-Glu-mDAP	EndoFit™	100 ng - 10 µg/ml	1 mg	tlrl-tdap	p 77
Tri-Lys	Tri-DAP negative control	EndoFit™	100 ng - 10 µg/ml	1 mg	tlrl-tlys	p 77
NOD2 Agonists						
L18-MDP	Muramyldipeptide with a C18 fatty acid chain	EndoFit™	1 - 100 ng/ml	1 mg	tlrl-lmdp	p 76
MDP	Muramyldipeptide (L-D isoform, active)	EndoFit™	10 ng - 10 µg/ml	5 mg	tlrl-mdp	p 76
MDP control	Muramyldipeptide (D-D isoform, inactive)	EndoFit™	10 ng - 10 µg/ml	5 mg	tlrl-mdpc	p 76
MDP Biotin	Biotinylated Muramyldipeptide	EndoFit™	100 ng - 10 µg/ml	500 µg	tlrl-bmdp	p 76
MDP FITC	FITC-labeled Muramyldipeptide	EndoFit™	10 ng - 10 µg/ml	500 µg	tlrl-fmdp	p 76
MDP Rhodamine	Rhodamine-labeled Muramyldipeptide	EndoFit™	100 ng - 10 µg/ml	500 µg	tlrl-rmdp	p 76

PRODUCT	ORIGIN/DESCRIPTION	ENDOTOXIN LEVELS	WORKING CONCENTRATION	QTY	CATALOG CODE	INFO
NOD2 Agonists						
M-Tri$_{LYS}$ NEW	Synthetic muramyl tripeptide	EndoFit™	100 ng - 10 µg/ml	1 mg	tlrl-mtl	p 77
M-Tri$_{LYS}$-D-ASN NEW	Synthetic muramyl tetrapeptide	EndoFit™	100 ng - 10 µg/ml	1 mg	tlrl-mtn	p 77
Murabutide	Synthetic derivative of muramyldipeptide	EndoFit™	10 ng - 1 µg/ml	5 mg	tlrl-mbt	p 76
Murabutide control	Murabutide analog (D isoform, inactive)	EndoFit™	10 ng - 1 µg/ml	5 mg	tlrl-mbtc	p 76
N-Glycolyl-MDP	N-glycolylated muramyldipeptide	EndoFit™	100 ng - 10 µg/ml	5 mg	tlrl-gmdp	p 76
N-Glycolyl-MDP VacciGrade™	Sterile N-glycolylated muramyldipeptide	EndoFit™	100 ng - 10 µg/ml	5 mg	tlrl-gmdp	p 105
NOD1/2 Agonists						
M-Tri$_{DAP}$	MurNAc-L-Ala-γ-D-Glu-mDAP	EndoFit™	1 - 100 µg/ml	1 mg	tlrl-mtd	p 77
PGN-ECndi ultrapure	Insoluble peptidoglycan from *E. coli K12*	EndoFit™	1 - 5 µg/ml	5 mg	tlrl-kipgn	p 77
PGN-ECndss ultrapure	Soluble sonicated peptidoglycan from *E. coli K12*	EndoFit™	1 - 5 µg/ml	1 mg	tlrl-ksspgn	p 77
PGN-SAndi ultrapure	Insoluble peptidoglycan from *S. aureus*	EndoFit™	1 - 5 µg/ml	5 mg	tlrl-sipgn	p 77
RIG-I/MDA-5 and CDS Agonists						
5'ppp-dsRNA	5'Triphosphate blunt-end double-stranded RNA	EndoFit™	300 ng - 1 µg/ml	25 µg 100 µg	tlrl-3prna tlrl-3prna-100	p 77
5'ppp-dsRNA Control	Blunt-end double-stranded RNA, control	EndoFit™	300 ng - 1 µg/ml	25 µg 100 µg	tlrl-3prnac tlrl-3prnac-100	p 77
Poly(dA:dT) Naked	Poly(dA-dT)•poly(dT-dA)	EndoFit™	1 - 5 µg/ml	200 µg 1 mg	tlrl-patn tlrl-patn-1	p 77
Poly(dA:dT)/LyoVec	Poly(dA-dT)•poly(dT-dA)/LyoVec™ complexes	EndoFit™	1 - 5 µg/ml	100 µg	tlrl-patc	p 77
Poly(dG:dC) Naked	Poly(dG-dC)•poly(dG-dC)	EndoFit™	1 - 5 µg/ml	200 µg	tlrl-pgcn	p 77
Poly(dG:dC)/LyoVec	Poly(dG-dC)•poly(dG-dC)/LyoVec™ complexes	EndoFit™	1 - 5 µg/ml	100 µg	tlrl-pgcc	p 77
Poly(I:C) (HMW)/LyoVec	Poly(I:C) (HMW)/LyoVec™ complexes	EndoFit™	100 ng - 1 µg/ml	100 µg 1 mg	tlrl-piclv tlrl-piclv-10	p 78
Poly(I:C) (LMW)/LyoVec	Poly(I:C) (LMW)/LyoVec™ complexes	EndoFit™	100 ng - 1 µg/ml	100 µg 1 mg	tlrl-picwlv tlrl-picwlv-10	p 78
Dectin-1 Agonists						
Curdlan	Beta-1,3-glucan from *Alcaligenes faecalis*	<0.05 EU/µg	100 µg/ml	100 mg	tlrl-curd	p 78
HKCA	Heat-killed *Candida albicans*	EndoFit™	10^8 cells/ml	10^9 cells	tlrl-hkca	p 78
HKSC	Heat-killed *Saccharomyces cerevisiae*	EndoFit™	10^8 cells/ml	10^9 cells	tlrl-hksc	p 78
WGP Dispersable NEW	Whole Glucan Particles, insoluble	N/A	1 - 200 µg/ml	50 mg	tlrl-wgp	p 78
WGP Soluble NEW	Whole Glucan Particles, soluble	N/A	1 µg - 1 mg/ml	50 mg	tlrl-wgps	p 78
Zymosan	Cell wall preparation from *S. cerevisiae*	N/A	1 - 100 µg/ml	100 mg	tlrl-zyn	p 78
Zymosan Depleted	Hot alkali treated zymosan	EndoFit™	100 µg/ml	10 mg	tlrl-dzn	p 78
Mincle Agonist						
TDB NEW	Synthetic analog of the cord factor	N/A	1 - 100 µg/ml	1 mg	tlrl-tdb	p 78
NLRP3 Inflammasome Inducers						
Alum Crystals	Aluminium potassium sulfate	N/A	10 - 200 µg/ml	1 g	tlrl-alk	p 79
ATP	Adenosine 5′-triphosphate disodium salt	N/A	5 mM	1 g	tlrl-atp	p 79
CPPD Crystals	Calcium pyrophosphate dihydrate	N/A	50 - 200 µg/ml	5 mg	tlrl-cppd	p 79
Hemozoin	Synthetic heme crystal	N/A	50 - 400 µg/ml	5 mg	tlrl-hz	p 79
MSU Crystals	Monosodium urate (uric acid)	N/A	50 - 200 µg/ml	5 mg	tlrl-msu	p 79
Nano-SiO2 NEW	Nanoparticles of silica dioxide	N/A	10 - 200 µg/ml	10 mg	tlrl-sio	p 79
Nigericin	Nigericin, sodium salt	N/A	1 µM	10 mg	tlrl-nig	p 79
AIM2 Inflammasome Inducer						
Poly(dA:dT)/LyoVec	Poly(dA-dT)•poly(dT-dA)/LyoVec™ complex	EndoFit™	1 - 5 µg/ml	100 µg	tlrl-patc	p 79

TLR & NOD Agonist Kits

The TLR and NOD agonist kits provide convenient and economical tools to study the stimulation of the TLRs and NOD1/NOD2, respectively. Each kit contains one or several known agonists for a given TLR or NOD and allows to perform 100 tests (100 µl in a 96-well plate).

PRODUCT	CONTENTS		CAT. CODE
TLR1-9 Agonist Kit Human (10 ligands)	1- **Pam3CSK4** (10 µg) - TLR1/2 Agonist 2- **FSL-1** (10 µg) - TLR6/2 Agonist 3- **HKLM** (10^9 cells) - TLR2 Agonist 4- **Poly(I:C) (HMW)** (500 µg) - TLR3 Agonist 5- **Poly(I:C) (LMW)** (500 µg) - TLR3 Agonist	6- **LPS-EK standard** (100 µg) - TLR4 Agonist 7- **FLA-ST standard** (10 µg) - TLR5 Agonist 8- **Imiquimod** (25 µg) - TLR7 Agonist 9- **ssRNA40/LyoVec** (25 µg) - hTLR8 Agonist 10- **ODN 2006** (100 µg) - TLR9 Agonist	tlrl-kit1hw
TLR1-9 Agonist Kit Mouse (9 ligands)	1- **Pam3CSK4** (10 µg) - TLR1/2 Agonist 2- **FSL-1** (10 µg) - TLR6/2 Agonist 3- **HKLM** (10^9 cells) - TLR2 Agonist 4- **Poly(I:C) (HMW)** (500 µg) - TLR3 Agonist 5- **Poly(I:C) (LMW)** (500 µg) - TLR3 Agonist	6- **LPS-EK standard** (100 µg) - TLR4 Agonist 7- **FLA-ST standard** (10 µg) - TLR5 Agonist 8- **ssRNA40/LyoVec** (25 µg) - mTLR7 Agonist 9- **ODN 1826** (100 µg) - TLR9 Agonist	tlrl-kit1mw
TLR2 Agonist Kit Human/Mouse (7 ligands) **NEW CONTENT**	1- **Pam2CSK4** (10 µg) - TLR6/2 Agonist 2- **Pam3CSK4** (10 µg) - TLR1/2 Agonist 3- **FSL-1** (10 µg) - TLR6/2 Agonist 4- **HKLM** (10^9 cells) - TLR2 Agonist	5- **LPS-PG** (100 µg) - TLR2 Agonist 6- **LTA-SA standard** (100 µg) - TLR2 Agonist 7- **PGN-SA** (100 µg) - TLR2 Agonist	tlrl-kit2hm
TLR3/7/8/9 Agonist Kit Human (14 ligands) **NEW CONTENT**	1- **Poly(I:C) (HMW)** (500 µg) - TLR3 Agonist 2- **Poly(I:C) (LMW)** (500 µg) - TLR3 Agonist 3- **Poly(A:U)** (500 µg) - TLR3 Agonist 4- **Imiquimod** (25 µg) - TLR7 Agonist 5- **R848** (25 µg) - TLR7/8 Agonist 6- **CL075** (25 µg) - TLR7/8 Agonist 7- **ssRNA40/LyoVec** (25 µg) - hTLR8 Agonist	8- **ssRNA41/LyoVec** (25 µg) - Control 9- **ODN 2006** (100 µg) - TLR9 Agonist, type B 10- **ODN 2006 control** (100 µg) - Control 11- **ODN 2216** (100 µg) - TLR9 Agonist, type A 12- **ODN 2216 control** (100 µg) - Control 13- **ODN 2395** (100 µg) - TLR9 Agonist, type C 14- **ODN 2395 control** (100 µg) - Control	tlrl-kit3hw3
NOD1/2 Agonist Kit Human/Mouse (10 ligands) **NEW CONTENT**	1- **C12-iE-DAP** (25 µg) - NOD1 Agonist 2- **iE-DAP** (100 µg) - NOD1 Agonist 3- **L18-MDP** (25 µg) - NOD2 Agonist 4- **MDP** (100 µg) - NOD2 Agonist 5- **M-Tri$_{DAP}$** (25 µg) - NOD1/2 Agonist	6- **M-Tri$_{LYS}$** (25 µg) - NOD2 Agonist 7- **Murabutide** (100 µg) - NOD2 Agonist 8- **PGN-ECndi ultrapure** (100 µg) - NOD1/2 Agonist 9- **PGN-SAndi ultrapure** (100 µg) - NOD1/2 Agonist 10- **Tri-DAP** (25 µg) - NOD1 Agonist	tlrl-nodkit2

Contents and Storage

All agonists are provided in a powdered form. Products are shipped at room temperature and should be stored at 4°C or -20°C according to the product label.

Recent articles using TLR & NOD Agonist Kits

Lindgren A. *et al.*, 2011. Interferon-gamma secretion is induced in IL-12 stimulated human NK cells by recognition of Helicobacter pylori or TLR2 ligands. Innate Immunity, 17: 191 - 203.

Digby JE. *et al.*, 2012. Anti-Inflammatory Effects of Nicotinic Acid in Human Monocytes Are Mediated by GPR109A Dependent Mechanisms. Arterioscler Thromb Vasc Biol, 32: 669 - 676.

Geraghty P. *et al.*, 2011. TLR4 Protein Contributes to Cigarette Smoke-induced Matrix Metalloproteinase-1 (MMP-1) Expression in Chronic Obstructive Pulmonary Disease. J. Biol. Chem., 286: 30211 - 30218.

TLR Ligands

TLR2 Agonists

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

HKAL (*Acholeplasma laidlawii*)
Acholeplasma laidlawii, a member of the mycoplasma family, is a cell wall-less bacteria. Heat-killed mycoplasma such as HKAL induce higher stimulation of macrophage than lipoproteins from Gram- bacteria, even at low concentrations[4]. This response is mediated by TLR2 and MyD88.

HKEB (*Escherichia coli*)
HKEB is a heat-killed preparation of the Gram-negative bacterium, *E.coli O111:B4*. Cell wall components from this bacterium, such as peptidoglycan (PGN) and lipopolysaccharide (LPS), are recognized by TLR2 and TLR4[5]. It has been demonstrated that HKEB can stimulate TLR2 and induce the production of NF-κB and pro-inflammatory cytokines, such as IL-8[6]. HKEB is a potent stimulator of TLR2, and has a weak stimulatory effect on TLR4.

HKHP (*Helicobacter pylori*)
Helicobacter pylori, a Gram-negative bacterium, is an important human pathogen that causes gastritis and is strongly associated with peptic ulcer, gastric adenocarcinoma, and mucosa-associated lymphoid tissue lymphoma. Heat-killed *Helicobacter pylori* (HKHP) induces the production of IL-8 through the activation of the ERK and p38 MAPK pathway. TLR2 was shown to be the sensor involved in HKHP-mediated secretion of IL-8 in monocytes[7].

HKLM (*Listeria monocytogenes*)
HKLM is a freeze-dried heat-killed preparation of *Listeria monocytogenes* (LM), a facultative intracellular Gram-positive bacterium. Infection with LM induces the secretion of inflammatory cytokines, such as TNF-α, IL-12, and several chemokines, allowing the recruitment and activation of immune cells. This response is mediated mainly by the interaction between MyD88 and TLR2[8,9].

HKLP (*Legionella pneumophila*)
Legionella pneumophila, a Gram-negative bacterium, is the causative agent of Legionnaires' disease which is characterized by severe pneumonia. Although TLR4 is involved in host defense against gram negative bacteria infection, it is not activated or is activated only to a limited extent by *L. pneumophila*[10]. *L. pneumophila* requires TLR2 rather than TLR4 to induce the production of cytokines[11].

HKLR (*Lactobacillus rhamnosus*)
Lactobacillus rhamnosus is a nonpathogenic Gram-positive inhabitant of the human microflora. It is used as a natural preservative in yogurt and other dairy products to extend their shelf life. *L. rhamnosus* is known to have health beneficial effects, such as the nonspecific enhancement of the immune system. Indeed, heat-killed *L. rhamnosus* (HKLR) has been shown to be a potent inducer of TNF-α from mouse mononuclear cells. This immune response is dependent on TLR2 and CD14[12].

HKMF (*Mycoplasma fermentans*)
Mycoplasma fermentans, a member of the mycoplasma family, is a cell wall-less bacterium. It contains lipopeptides, in particular 2-kDa macrophage-activating lipopetide (MALP-2), a potent stimulator of macrophages through TLR2 and TLR6[3]. Stimulation with heat-killed *M. fermentans* (HKMF) induces rapid activation of NF-κB and the production of pro-inflammatory cytokines.

HKPA (*Pseudomonas aeruginosa*)
Pseudomonas aeruginosa is a virulent Gram-negative pathogen that infects patients through the respiratory tract, in particular patients with cystic fibrosis. Heat-killed *Pseudomonas aeruginosa* (HKPA) initiates host inflammatory responses through TLR2 and TLR5 but not TLR4[13,14]. The TLR5-mediated response was shown to be induced by flagellin while LPS appears to play an important role in the TLR2-mediated response.

HKPG (*Porphyromonas gingivalis*)
HKPG is a freeze-dried heat-killed preparation of the periodontopathic Gram-negative bacteria *Porphyromonas gingivalis*. In CHO cells expressing TLR2 and CD14, exposure to HKPG induces the activation of NF-κB through TLR2. Expression of TLR4 fails to enhance the response to HKPG suggesting that either the whole bacterial components of *P. gingivalis* are not recognized by TLR4 or some components of these bacteria inhibit TLR4-mediated activation[15].

HKSA (*Staphylococcus aureus*)
HKSA is a lyophilized heat-killed preparation of *Staphyloccocus aureus*, a Gram-positive extra-cellular growing bacterium. HKSA is recognized mainly by TLR2[16]. HKSA induces tolerance to a secondary HKSA stimulation but causes priming to LPS, suggesting a differential regulation of cytokines and chemokines in gram-positive- and gram-negative-induced inflammatory events[17].

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

LM-MS & LAM-MS (*Mycobacterium smegmatis*)
Lipoarabinomannans (LAM) and lipomannans (LM) are lipoglycans found in mycobacterial cell walls. LM and LAM derived from the non-pathogenic *Mycobacterium smegmatis* are proinflammatory molecules[20]. Both LM-MS and LAM-MS activate macrophages in a TLR2-dependent manner[21].

LPS-PG (*Porphyromonas gingivalis*)
Recognition of lipopolysaccharide from *Porphyromonas gingivalis* (LPS-PG), a Gram-negative bacterium, is mediated by TLR2 and CD14, and unlike enteric LPS, is able to induce a septic shock in C3H/HeJ mice which are deficient for TLR4 and hyporesponsive to *E. coli* LPS. This property is attributed mainly to the unique lipid A motif of PG-LPS[22].

LTA-BS & LTA-SA (*B. subtilis and S. aureus*)
Lipoteichoic acid (LTA) is a major immunostimulatory component of Gram-positive bacteria. Like LPS, LTA is an amphiphile formed by a hydrophilic polyphosphate polymer linked to a neutral glycolipid. LTA stimulates immune cells through TLR2 to produce TNF-α and other inflammatory cytokines[23]. Recognition of LTA also involves LPS-binding protein (LBP) and CD14[24]. InvivoGen provides LTA from *B. subtilis* (LTA-BS) and *S. aureus* (LTA-SA) as well as a purified form of LTA-SA, which is EndoFit™ (<0.001 EU/μg).

Pam2CSK4 - TLR2 Agonist

Pam2CSK4 is a synthetic diacylated lipopeptide (LP). According to the current model, diacylated LPs induce signaling through TLR2/6. However, it was also reported that Pam2CSK4 induces signaling in a TLR6-independent manner[25]. This finding suggests that both the lipid and peptide part of lipoproteins take part in the specificity of recognition by TLR2 heterodimers.
Pam2CSK4 Biotin and **Pam2CSK4 Rhodamine** are available.

Pam3CSK4 - TLR1/2 Agonist

Pam3CSK4 is a synthetic triacylated lipopeptide (LP) that mimics the acylated amino terminus of bacterial LPs. Pam3CSK4 is a potent activator of the proinflammatory transcription factor NF-κB[26]. Activation is mediated by the interaction between TLR2 and TLR1 which recognize LPs with three fatty acids, a structural characteristic of bacterial LPs[27].
Pam3CSK4 Biotin and **Pam3CSK4 Rhodamine** are available.

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

Peptidoglycan (PGN) is a major surface component of Gram-positive bacteria. It is embedded in a relatively thick cell wall and is usually covalently attached to other polymers, such as lipoproteins and LTAs. PGN is known to be a potent activator of NF-κB and TNF-α through TLR2[5]. However, other pattern recognition proteins have been reported to mediate the immunostimulatory activity of PGN[28-30]. This discrepancy is attributed to the method of purification.
PGN-BS, PGN-EB, PGN-EK and PGN-SA are purified by detergent lysis, enzymatic treatment, LiCl/EDTA and acetone cleaning. These preparations of PGN induce the production of NF-κB through TLR2.

Zymosan - TLR2 & Dectin-1 Agonist

Zymosan, an insoluble preparation of cell wall from *Saccharomyces cerevisiae*, activates macrophages via TLR2. TLR2 cooperates with TLR6 and CD14 in response to zymosan[31]. Zymosan is also recognized by Dectin-1, a phagocytic receptor expressed on macrophages and dendritic cells, which collaborates with TLR2 and TLR6 enhancing the immune responses triggered by the recognition of Zymosan by each receptor[32].

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Recent articles using InvivoGen's TLR2 Agonists

FSL-1

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Allensworth JJ. *et al.*, 2011. Investigation of the differential potentials of TLR agonists to elicit uveitis in mice. J. Leukoc. Biol. 90: 1159 - 1166.

HKLM - O'Hara SP. *et al.*, 2011. Cholangiocyte N-Ras protein mediates lipopolysaccharide-induced interleukin 6 secretion and proliferation. J. Biol. Chem. 286: 30352 - 30360.

LTA-SA - Avbelj M. *et al.*, 2011. The role of intermediary domain of MyD88 in cell activation and therapeutic inhibition of TLRs. J. Immunol. 187: 2394 - 2404.

Pam3CSK4

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Pam2CSK4 - Duggan JM. *et al.*, 2011. Synergistic interactions of TLR2/6 and TLR9 induce a high level of resistance to lung infection in mice. J. Immunol. 186: 5916 - 5926.

PGN-EB & LPS-PG

Nahid MA. *et al.*, 2011. Mechanistic Role of MicroRNA-146a in endotoxin-induced differential cross-regulation of TLR signaling. J. Immunol. 186: 1723 - 1734.

Doisne JM. *et al.*, 2011. Cutting Edge: Crucial role of IL-1 and IL-23 in the innate IL-17 response of peripheral lymph node NK1.1– invariant NKT cells to bacteria. J. Immunol. 186: 662 - 666.

TLR3 Agonists

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

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

Poly(I:C) (HMW) has an average size of 1.5-8 kb.
Poly(I:C) (LMW) has an average size of 0.2-1 kb.

Poly(A:U)

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

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Recent articles using InvivoGen's poly(I:C)

Bogunovic D. *et al.*, 2011. TLR4 Engagement during TLR3-Induced Pro-inflammatory Signaling in Dendritic Cells Promotes IL-10–Mediated Suppression of Antitumor Immunity. Cancer Res., 71: 5467 - 5476.

Loschko J. *et al.*, 2011. Antigen Delivery to Plasmacytoid Dendritic Cells via BST2 Induces Protective T Cell-Mediated Immunity. J. Immunol., 186: 6718 - 6725.

McGowan KA. *et al.*, 2011. Reduced ribosomal protein gene dosage and p53 activation in low-risk myelodysplastic syndrome. Blood, 118: 3622 - 3633.

Ng ACY. *et al.*, 2011. Human leucine-rich repeat proteins: a genome-wide bioinformatic categorization and functional analysis in innate immunity. PNAS, 108: 4631 - 4638.

Kim J. et al., 2012. Wnt5a Is Secreted by Follicular Dendritic Cells To Protect Germinal Center B Cells via Wnt/Ca2+/NFAT/NF-{kappa}B–B Cell Lymphoma 6 Signaling. J. Immunol., 188: 182 - 189.

TLR4 Agonists

Bacterial lipopolysaccharide (LPS) is the major structural component of the outer wall of all Gram-negative bacteria and a potent activator of the immune system. LPS is recognized by Toll-like receptor 4 (TLR4) which interacts with three different extracellular proteins: LPS binding protein (LBP), CD14 and, myeloid differentiation protein 2 (MD-2), to induce a signaling cascade leading to the activation of NF-κB and the production of proinflammatory cytokines. LPS consists of a polysaccharide region that is anchored in the outer bacterial membrane by a specific carbohydrate lipid moiety termed lipid A. Lipid A, also known as endotoxin, is responsible for the immunostimulatory activity of LPS. The most active form of lipid A contains six fatty acyl groups and is found in pathogenic bacteria such as Escherichia coli and Salmonella species[1]. Underacylated lipid A structures, containing four or five fatty acids, induce markedly less host defense responses and can inhibit in a dose-dependent manner the strong endotoxic response triggered by hexa-acylated LPS[2].

LPS-EB & LPS-EK Standard (*E. coli 0111:B4 and E. coli K12*)

LPS-EB and LPS-EK are standard preparations of lipopolysaccharide. They are extracted by a phenol-water mixture. LPS-EB and LPS-EK contain other bacterial components, such as lipopeptides, and therefore stimulate both TLR4 and TLR2.

LPS-EB, LPS-EK & LPS-SM Ultrapure (*E. coli 0111:B4, E. coli K12 and S. minnesota*)

Ultrapure LPS-EB (*E. coli* 0111:B4), LPS-EK (*E. coli* K12) and LPS-SM (*S. minnesota* Re type) are extracted by successive enzymatic hydrolysis steps and purified by the phenol-TEA-DOC extraction protocol described by Hirschfeld M. *et al.*[3]

MPLA & MPLAs (synthetic)

MPLA (monophosphoryl lipid A) is a derivative of lipid A from *Salmonella minnesota* R595 lipopolysaccharide (LPS or endotoxin). While LPS is a complex heterogeneous molecule, its lipid A portion is relatively similar across a wide variety of pathogenic strains of bacteria[4]. MPLA, used extensively as a vaccine adjuvant, has been shown to activate TLR4.

MPLAs is a synthetic monophosphoryl lipid A from *E. coli* with 6 fatty acyl groups. It is structurally very similar to natural MPLA except that natural MPLA contains a mixture of 5, 6, and 7 acyl Lipid A. This synthetic *E. coli* MPLA activates TLR4 but does not activate TLR2 even at high concentrations reflecting its high purity.

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Recent articles using InvivoGen's TLR4 Agonists

LPS-EB Ultrapure

Liu YC. *et al.*, 2012. TLR2 Signaling Depletes IRAK1 and Inhibits Induction of Type I IFN by TLR7/9. J. Immunol. 188: 1019 - 1026.

Beverly S. *et al.*, 2011. Presentation of type B peptide–MHC complexes from hen egg white lysozyme by TLR ligands and type I IFNs independent of H2-DM regulation. J. Immunol. 187: 2193 - 2201.

LPS-EK Ultrapure

Takahashi K. *et al.*, 2011. Epigenetic control of the host gene by commensal bacteria in large intestinal epithelial cells. J. Biol. Chem. 286: 35755 - 35762.

Costa A. *et al.*, 2012. Activation of the NLRP3 inflammasome by group B streptococci. J. Immunol. 188: 1953 - 1960.

MPLA - Duggan JM. *et al.*, 2011. Synergistic interactions of TLR2/6 and TLR9 induce a high level of resistance to lung infection in mice. J. Immunol. 186: 5916 - 5926.

MPLA - Allensworth JJ. *et al.*, 2011. Investigation of the differential potentials of TLR agonists to elicit uveitis in mice. J. Leukoc. Biol. 90: 1159 - 1166.

TLR4 Antagonist

LPS-RS (*Rhodobacter sphaeroides*) - TLR4 Antagonist
LPS from the photosynthetic bacterium *Rhodobacter sphaeroides* (LPS-RS) is a potent antagonist of LPS from pathogenic bacteria[1]. Complete competitive inhibition of LPS activity is possible at a 100 fold excess of the antagonist. LPS-RS does not induce TLR4 signaling but is detected by the LAL assay, the standard endotoxin detection assay.

1. Coats SR. *et al.*, 2005. MD-2 mediates the ability of tetra-acylated and penta-acylated lipopolysaccharides to antagonize Escherichia coli lipopolysaccharide at the TLR4 signaling complex. J Immunol.;175(7):4490-8.

TLR5 Agonists

Flagellin is the major component of the bacterial flagellar filament, which confers motility on a wide range of bacterial species. Flagellin is recognized by TLR5[1] and induces the activation of NF-κB and the production of cytokines and nitric oxide depending on the nature of the TLR5 signaling complex[2].

FLA-BS & FLA-ST (*B. subtilis and S. typhimurium)*
FLA-BS and FLA-ST are flagellins isolated from the Gram-positive bacteria *B. subtilis* and from the Gram-negative bacteria *S. typhimurium*, respectively. They are purified by acid hydrolysis, heating and ultrafiltration according to Ibrahim GF. *et al.*[3]. The purity of FLA-ST is estimated at 10%.

FLA-ST Ultrapure (*S. typhimurium)*
FLA-ST ultrapure was purified by monoclonal anti-FliC affinity chromatography. The purity of FLA-ST ultrapure is >95%.

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

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Recent articles using InvivoGen's Flagellins

FLA-BS

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Bosmann M. *et al.*, 2011. MyD88-dependent production of IL-17F is modulated by the anaphylatoxin C5a via the Akt signaling pathway. FASEB J. 25: 4222 - 4232.

FLA-ST

Cai Z. *et al.*, 2011. Activation of Toll-like Receptor 5 on Breast Cancer Cells by Flagellin Suppresses Cell Proliferation and Tumor Growth. Cancer Res., 71: 2466 - 2475.

Avbelj M. *et al.*, 2011. The role of intermediary domain of MyD88 in cell activation and therapeutic inhibition of TLRs. J. Immunol. 187: 2394 - 2404.

Jarchum I. *et al.*, 2011. Toll-Like receptor 5 stimulation protects mice from acute Clostridium difficile colitis. Infect. Immun. 79(4):1498.

recFLA-ST

Nahid MA. *et al.*, 2011. Mechanistic Role of MicroRNA-146a in endotoxin-induced differential cross-regulation of TLR signaling. J. Immunol. 186: 1723 - 1734.

Kalb ML. et al., 2012. TRAIL+ Human plasmacytoid dendritic cells kill tumor cells in vitro: mechanisms of Imiquimod- and IFN-α–mediated antitumor reactivity. J. Immunol. 188: 1583 - 1591.

TLR7/8 Agonists

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

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

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

Gardiquimod - TLR7 Agonist
Gardiquimod is a new imidazoquinoline compound developed and manufactured by InvivoGen. Similarly to Imiquimod, Gardiquimod induces the activation of NF-κB in HEK293 cells expressing human or mouse TLR7. However Gardiquimod is 10 times more active as a concentration of 0.1 μg/ml is sufficient to detect NF-κB activation whereas Imiquimod requires a concentration of 1 μg/ml. Gardiquimod shares the same actions as R848[7].

TLR7/8 Agonists

Imiquimod - TLR7 Agonist

Imiquimod (R837), an imidazoquinoline amine analogue to guanosine, is an immune response modifier with potent indirect antiviral activity. This low molecular weight synthetic molecule induces the production of cytokines such as IFN-α through the activation of TLR7[8]. This activation is MyD88-dependent and leads to the induction of the transcription factor NF-κB[9].

Loxoribine - TLR7 Agonist

Loxoribine is a guanosine analog derivatized at position N7 and C8. This L-nucleoside is a strong stimulator of the immune system[10]. It signals through TLR7 leading to the activation of NF-κB[8, 11]. Similarly to the imidazoquinoline compound imiquimod, loxoribine recognition is restricted to TLR7.

Poly(dT) - TLR7/8 Modulator

Poly(dT), a thymidine homopolymer phosphorothioate ODN, is a modulator of human TLR7 and TLR8. In combination with an imidazoquinoline, such as R848 and CL075, it increases TLR8-mediated signaling but abolishes TLR7-mediated signaling[12, 13]. Alone poly(dT) has no significant effect on either of these TLRs. Furthermore, co-incubation of poly(dT) and an imidazoquinoline was shown to induce NF-κB activation in HEK293 cells transfected with murine TLR8- and primary TLR8-expressing mouse cells, demonstrating that murine TLR8 is functional[14].

R848 - TLR7/8 Agonist

R848 is an imidazoquinoline compound with potent anti-viral activity. This low molecular weight synthetic molecule activates immune cells via the TLR7/TLR8 MyD88-dependent signaling pathway[9, 12]. Recently, R848 was shown to trigger NF-κB activation in cells expressing murine TLR8 when combined with poly(dT)[14]. Unlike other commercially available R848 preparations, InvivoGen's R848 is water soluble (~5 mg/ml).

Single-stranded RNAs: ssPolyU, ssRNA40, ssRNA-DR & ORN02/06 - TLR8 Agonists

Single-stranded RNA (ssRNA) has been identified as the natural ligand of TLR7 and TLR8[15, 16]. ssRNA derived from HIV-1 or the influenza virus were shown to induce the production of proinflammatory cytokines in pDC. This induction was reproduced using polyU or GU-rich (ssRNA40) ODNs complexed with cationic lipids to protect them from degradation. Upon stimulation with ssRNA, murine TLR7 and human TLR8 induced the activation of NF-κB, whereas human TLR7 and murine TLR8 failed, implying a species specificity difference in ssRNA recognition.

ssPolyU & ssRNA40: ssPolyU is a single-stranded poly-uridine (polyU) oligonucleotide while ssRNA40 is a 20-mer phosphorothioate protected single-stranded RNA oligonucleotide containing a GU-rich sequence. Both single-stranded RNAs are complexed with the cationic lipid LyoVec™, to protect them from degradation and facilitate their uptake. They are provided as lyophilized powder.

ssRNA41 is a 20-mer phosphorothioate protected single-stranded RNA oligonucleotide. It derives from ssRNA40 by replacement of all U nucleotides with adenosine[2]. ssRNA41 is complexed with the cationic lipid LyoVec™, to protect it from degradation and facilitate its uptake, and lyophilized to generate ssRNA41/LyoVec. Unlike ssRNA40, ssRNA41 is unable to induce the production of type I IFNs, and therefore can be used as a negative control for ssRNA40[16, 17].

ssRNA-DR is a short single-stranded RNA (<30 bp) that contains two copies of the 9 mer sequence GUCCUUCAA. This sequence is a putative immunostimulatory motif recognized by human TLR8 and mouse TLR7 that induces type I interferons[18]. ssRNA-DR is provided pre-complexed with the cationic lipid LyoVec™ to facilitate its uptake.

ORN06 contains 6 repeats of the UUGU sequence motif, identified as the minimal motif responsible for ssRNA40 immunoactivity[19].

ORN02 derives from ORN06 by substitution of G to A.

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CL097 - Phoolcharoen W. *et al.*, 2011. A nonreplicating subunit vaccine protects mice against lethal Ebola virus challenge. PNAS, 108: 20695 - 20700.

CL264 - Costa A. *et al.*, 2012. Activation of the NLRP3 inflammasome by group B streptococci. J. Immunol. 188: 1953 - 1960.

Gardiquimod™ - Beverly S. *et al.*, 2011. Presentation of type B peptide–MHC complexes from hen egg white lysozyme by TLR ligands and type I IFNs independent of H2-DM regulation. J. Immunol. 187: 2193 - 2201.

Imiquimod - Rahman S. *et al.*, 2011. Murine FLT3 ligand-derived dendritic cell-mediated early immune responses are critical to controlling cell-free human T cell leukemia virus Type 1 infection. J. Immunol. 186: 390 - 402.

Imiquimod & Loxoribine - Allacher P. *et al.*, 2011. Stimulation and inhibition of FVIII-specific memory B-cell responses by CpG-B (ODN 1826), a ligand for Toll-like receptor 9. Blood 117: 259 - 267.

Imiquimod, CL075 & ssPolyU/LyoVec- Kalb ML. *et al.*, 2012. TRAIL+ Human plasmacytoid dendritic cells kill tumor cells in vitro: mechanisms of Imiquimod- and IFN-α–mediated antitumor reactivity. J. Immunol. 188: 1583 - 1591.

Imiquimod & ssRNA 40 - O'Hara SP. *et al.*, 2011. Cholangiocyte N-Ras protein mediates lipopolysaccharide-induced interleukin 6 secretion and proliferation. J. Biol. Chem. 286: 30352 - 30360.

R848 - Miles K. *et al.*, 2012. A tolerogenic role for Toll-like receptor 9 is revealed by B-cell interaction with DNA complexes expressed on apoptotic cells. PNAS 109: 887 - 892.

ssRNA40 - Liu YC. *et al.*, 2012. TLR2 Signaling Depletes IRAK1 and Inhibits Induction of Type I IFN by TLR7/9. J. Immunol. 188: 1019 - 1026.

E. coli DNA ef (endotoxin-free)

Bacterial DNA contains 20-fold more unmethylated CpG motifs than mammalian DNA and thus activates TLR9[1]. *E. coli* DNA ef is an ultrapure, endotoxin-free (ef) preparation of bacterial double-stranded DNA devoid of TLR2 and TLR4 activities.

E. coli ssDNA

E. coli ssDNA is an ultrapure, endotoxin-free preparation of bacterial single-stranded DNA (ssDNA). It is a better TLR9 ligand than *E. coli* DNA ef (dsDNA endotoxin-free) as TLR9 binds directly and sequence-specifically to single-stranded unmethylated CpG-DNA[2]. *E. coli* ssDNA is complexed with the cationic lipid LyoVec™ to allow a better internalization of the immunostimulatory DNA to the acidic compartment where TLR9 is expressed.

Stimulatory CpG ODNs & Control CpG ODNs

Toll-Like Receptor 9 (TLR9) detects unmethylated CpG dinucleotides in bacterial or viral DNA inducing strong immunostimulatory effects. TLR9 activation can be mimicked by synthetic phosphorothioate-stabilized oligodeoxynucleotides (ODN) containing immune stimulatory "CpG motifs". Three types of stimulatory CpG ODNs have been identified, types A (or D), B (or K) and C, which differ in their immune-stimulatory activities:

- Type A CpG ODNs are characterized by a phosphodiester central CpG-containing palindromic motif and a phosphorothioate 3' poly-G string. They induce high IFN-α production from plasmacytoid dendritic cells (pDC) but are weak stimulators of TLR9-dependent NF-κB signaling.

- Type B CpG ODNs contain a full phosphorothioate backbone with one or more CpG dinucleotides. They strongly activate B cells but weakly stimulate IFN-α secretion.

- Type C CpG ODNs combine features of both types A and B. They contain a complete phosphorothioate backbone and a CpG-containing palindromic motif. Type C CpG ODNs induce strong IFN-α production from pDC and B cell stimulation.

These stimulatory CpG ODNs differentially induce the stimulation of human and murine immune cells *in vitro*. This species-specificity is also observed with nonresponsive cells such as HEK293 cells transfected with human or mouse TLR9. InvivoGen offers a comprehensive collection of stimulatory CpG ODNs and control CpG ODNs that provide useful tools for studying TLR9-mediated activation. InvivoGen's CpG ODNs are endotoxin-free and tested for activity in various cell lines expressing human or mouse TLR9.

Control CpG ODNs that do not stimulate TLR9 have been designed for each stimulatory CpG ODN. They feature the same sequence as their stimulatory counterparts but contain GpC dinucleotides in place of CpG dinucleotides.

ODN 1585[3]	(Type A, mouse specific)	5'-ggGGTCAACGTTGAgggggg-3'
ODN 1585 control		5'-ggGGTCAAGCTTGAgggggg-3'
ODN 1668[4]	(Type B, mouse specific)	5'-tccatgacgttcctgatgct-3'
ODN 1668 control		5'-tccatgagcttcctgatgct-3'
ODN 1826[5]	(Type B, mouse specific)	5'-tccatgacgttcctgacgtt-3'
ODN 1826 control		5'-tccatgagcttcctgagctt-3'
ODN 2006[6, 10]	(Type B, human specific)	5'-tcgtcgttttgtcgttttgtcgtt-3'
ODN 2006 control		5'-tgctgctttttgtgcttttgtgctt-3'
ODN 2007[7, 8]	(Type B, bovine/porcine)	5'-tcgtcgttgtcgttttgtcgtt-3'
ODN 2007 control		5'-tgctgcttgtgcttttgtgctt-3'
ODN 2216[9]	(Type A, human specific)	5'-ggGGGACGA:TCGTCgggggg-3'
ODN 2216 control		5'-ggGGGAGCA:TGCTGgggggc-3'
ODN 2336[10]	(Type A, human specific)	5'-gggGACGAC:GTCGTGgggggg-3'
ODN 2336 control		5'-gggGAGCAG:CTGCTGgggggg-3'
ODN 2395[6, 10]	(Type C, human/mouse)	5'-tcgtcgttttcggcgc:gcgccg-3'
ODN 2395 control		5'-tgctgcttttgggggggcccccc-3'
ODN M362[13]	(Type C, human/mouse)	5'-tcgtcgtcgttc:gaacgacgttgat-3'
ODN M362 control		5'-tgctgctgcttg:caagcagcttgat-3'

Bases in capital letters are phosphodiester, bases in lower case are phosphorothioate. Palindrome is underlined.

pCpG Giant - TLR9 Control

pCpG giant is a high molecular weight plasmid entirely devoid of CpG dinucleotides. This DNA also features no detectable amounts of endotoxin, as determined using a kinetic chromogenic LAL assay and the HEK -Blue™-4 cell line-based assay (LPS Detection Kit, see page 90) and no detectable TLR2 activity. In addition, this plasmid DNA displays no Dcm methylation and a reduced level of Dam methylation. pCpG Giant can be used as a control in studies on CpG methylations.

Salmon Sperm DNA - TLR9 Control

Salmon sperm DNA does not activate any TLR and can be used as a negative control for TLR induction experiments in particular in TLR9 studies. Salmon sperm DNA has been tested for endotoxin and has been found to be EndoFit™ (<0.001 EU/μg). Non-induction of TLR-expressing cells has been confirmed at concentrations up to 1 mg/ml.

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ODN 1668 - Liang Q. *et al.*, 2011. Characterization of Sparstolonin B, a Chinese herb-derived compound, as a selective Toll-like Receptor antagonist with potent anti-inflammatory properties. J. Biol. Chem. 286: 26470 - 26479.

ODN 1826 - Miles K. *et al.*, 2012. A tolerogenic role for Toll-like receptor 9 is revealed by B-cell interaction with DNA complexes expressed on apoptotic cells. PNAS 109: 887 - 892.

ODN 2006 - O'Hara SP. *et al.*, 2011. Cholangiocyte N-Ras protein mediates lipopolysaccharide-induced interleukin 6 secretion and proliferation. J. Biol. Chem. 286: 30352 - 30360.

ODN 2216 - Kalb ML. *et al.*, 2012. TRAIL+ Human plasmacytoid dendritic cells kill tumor cells in vitro: mechanisms of Imiquimod- and IFN-α–mediated antitumor reactivity. J. Immunol. 188: 1583 - 1591.

ODN 2395 - Duggan JM. *et al.*, 2011. Synergistic interactions of TLR2/6 and TLR9 induce a high level of resistance to lung infection in mice. J. Immunol. 186: 5916 - 5926.

***E. coli* DNA ef -** Rahman MM. & McFadden G. 2011. Myxoma virus lacking the pyrin-like protein M013 is sensed in human myeloid cells by both NLRP3 and multiple Toll-like receptors, which independently activate the inflammasome and NF-kB innate response pathways. J. Virol. 85: 12505 - 12517.

TLR9 Antagonists

Inhibitory ODNs

ODN 2088, ODN TTAGGG and G-ODN

Recent studies suggest the existence of DNA sequences that can neutralize the stimulatory effect of CpG ODNs. The most potent inhibitory sequences are (TTAGGG)4 found in mammalian telomeres[1] and ODN 2088 which derives from a murine stimulatory CpG ODN by replacement of 3 bases[2]. Recently, another inhibitory guanosine-rich ODN, named G-ODN, was described[3]. G-ODN was suppressive in murine DC and macrophages as well as in human plasmacytoid DC. Inhibitory ODNs seem to act by disrupting the colocalization of CpG ODNs with TLR9 in endosomal vesicles without affecting cellular binding and uptake. Inhibitory ODNs are often utilized to demonstrate a TLR9 dependence in murine systems.

ODN 2088[1] (Mouse preferred)	5'-tcctggcggggaagt-3'
ODN 2088 control	5'-tcctgagcttgaagt-3'
ODN TTAGGG[2] (Human preferred)	5'-tttagggttagggttagggttaggg-3'
ODN TTAGGG control	5'-gctagatgttagcgt-3'
G-ODN	5'-ctcctattgggggtttcctat-3'

Bases are phosphorothioate.

ODN 4084-F and ODN INH-1

ODN 4084-F and ODN INH-1 belong to a new class of inhibitory ODNs[4]. They contain an inhibitory DNA motif consisting of two nucleotide triplets, a proximal CCT and a more distal GGG, spaced from each other by four nucleotides. ODN 4084-F is the shortest active inhibitory ODN. ODN INH-1 derives from ODN 4084-F by addition of a complementary strand of nucleotides forming a complete palindrome. ODN INH-47 is a palindromic variant of ODN INH-1 in which the CCT and GGG have been replaced by random nucleotide triplets. ODN 4084-F is linear and a class B ('broadly-active) inhibitory ODN, while ODN INH-1 is palindromic and a class R ('restricted') inhibitory ODN. ODN 4084-F and ODN INH-1 are potent inhibitors of TLR9-induced B cells and macrophages, whereas ODN INH-47 has no effect[5].

ODN 4084-F	5'-cctggatgggaa-3'
ODN INH-1	5'-cctggatgggaa:ttcccatccagg-3'
ODN INH-47 (control)	5'-tatggattttaa:ttaaaatccata-3'

Bases are phosphorothioate.

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NOD Ligands

NOD1/2 Agonists

iE-DAP & iE-Lys & C12-iE-DAP - NOD1 Agonists

iE-DAP (D-γ-Glu-mDAP) is a dipeptide present in the PGN of a subset of bacteria that include Gram-negative bacilli and particular Gram-positive bacteria such as *Bacillus subtilis* and *Listeria monocytogenes*[1]. iE-DAP is the minimal motif recognized by NOD1.

iE-Lys is a peptide found in the PGN of Gram-positive bacteria. It is not recognized by NOD1 and thus can be used as negative control for iE-DAP.

C12-iE-DAP is an acylated derivative of iE-DAP. It was generated by addition of a lauroyl (C12) group to the glutamic residue of iE-DAP. C12-iE-DAP specifically stimulates NOD1 at concentrations 100- to 1000-fold lower than the original iE-DAP.

MDP, MDP control & L18-MDP - NOD2 Agonists

MDP (MurNAc-L-Ala-D-isoGln, also known as muramyl dipeptide), is the minimal bioactive peptidoglycan motif common to all bacteria and the essential structure required for adjuvant activity in vaccines. MDP has been shown to be recognized by NOD2, but not TLR2, nor TLR2/1 or TLR2/6 associations[2, 3]. This recognition is highly stereospecific of the L-D isomer, excluding any reaction to the D-D or L-L analogs[3, 4]. NOD2 mutants associated with susceptibility to Crohn's disease have been found to be deficient in their recognition of MDP[2, 3]. The potent adjuvant activity of MDP may also be linked to an activation of the CIAS1/NALP3/Cryopyrin inflammasome[5].

MDP control (MurNAc-D-Ala-D-isoGln), the D-D isomer of muramyl dipeptide, is unable to induce NOD2 signaling.

L18-MDP, a synthetic derivative of MDP, has been shown to display a higher adjuvant activity than MDP[6].

N-glycolyl-MDP - NOD2 Agonist

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[7].

Murabutide & Murabutide control - NOD2 Ligands

Murabutide (MurNAc-L-Ala-D-GlnOBu) is a synthetic immunomodulator derived from muramyl dipeptide (MDP). In contrast to MDP, murabutide is devoid of pyrogenic activity[8] and lacks somnogenic activity[9]. Murabutide is recognized by the intracellular receptor NOD2 inducing the activation of NF-κB.

Murabutide control contains D-alanine instead of L-alanine and is inactive on NOD2.

M-Tri$_{DAP}$ - NOD1/ NOD2 Agonist
M-TriDAP (MurNAc-L-Ala-D-γ-Glu-mDAP), also called DAP-containing muramyl tripeptide is a peptidoglycan (PGN) degradation product found mostly in Gram-negative bacteria. M-TriDAP is recognized by NOD1 and to a lesser extent NOD2. M-TriDAP induces the activation of NF-κB at similar levels to Tri-DAP[10].

M-Tri$_{LYS}$ & M-Tri$_{LYS}$-D-ASN - NOD2 Agonists **NEW**
M-TriLYS (MurNAc-Ala-D-isoGln-Lys) and M-TriLYS-D-ASN (MurNAc-Ala-D-isoGln-L-Lys(D-Asn)) are muropeptides released by *Lactobacillus salivarus* and *L. acidophilus*, respectively, after digestion of their PGN. Both chemically synthesized muropeptides are sensed by NOD2 and induce the activation of NF-κB[11]. However, M-TriLYS has been shown to produce the anti-inflammatory cytokine IL-10 and protect mice from colitis, unlike M-TriLYS-D-ASN[11]. The opposite effect of both muropeptides may be linked to a differential transport.

PGN-Ecndi & PGN-SAndi insoluble, ultrapure - NOD1/2 Agonists
PGN-ECndi from *E. coli K12* and PGN-SAndi from *S. aureus* are insoluble preparations of PGNs purified by detergent lysis and hydrolysis under basic conditions to eliminate lipophilic constituents. These PGN preparations have lost their ability to activate TLR2-transfected HEK293 cells but still activate NOD2-transfected cells. PGN-ECndi activates also NOD1-transfected cells.

PGN-Ecndss soluble, sonicated, ultrapure - NOD1/2 Agonists
PGN-ECndss from *E. coli K12* is obtained after sonication of insoluble PGNs. At the working concentrations, it activates HEK293 cells transfected with either NOD1 or NOD2 but not cells transfected with TLR2 or TLR4.

Tri-DAP & Tri-Lys - NOD1 Agonists
Tri-DAP (L-Ala-γ-D-Glu-mDAP) comprises the iE-DAP dipeptide and an L-Ala residue. Similarly to iE-DAP, this tripeptide is specifically recognized by NOD1 but exhibits a ~3-fold higher ability to activate NF-κB than iE-DAP[10].
Tri-Lys is a peptide found in the PGN of Gram-positive bacteria. It is not recognized by NOD1 and thus can be used as negative control for Tri-DAP.

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RLR Ligands

RIG-I/MDA5 and CDS Agonists

5'ppp-dsRNA - RIG-I Agonist
5'ppp-dsRNA is a short (19 mer) blunt-end double-stranded RNA with a 5' triphosphate. Transfected 5'ppp-dsRNA is a ligand for RIG-I[1]. This dsRNA sensor is specifically activated by the uncapped 5' triphosphate moiety on viral RNA. This triphosphate occurs during viral replication and is absent from most cytosolic self-RNA. A synthetic approach to the exact structure requirement to RIG-I recognition demonstrated that a short blunt double-stranded conformation containing a triphosphate at the 5' end is required[2]. 5'ppp-dsRNA Control is a 19 mer blunt-end dsRNA without a 5' triphosphate.

5'ppp-dsRNA	5'- pppGCAUGCGACCUCUGUUUGA -3' 3'- CGUACGCUGGAGACAAACU -5'
5'ppp-dsRNA Control	5'- GCAUGCGACCUCUGUUUGA -3' 3'- CGUACGCUGGAGACAAACU -5'

Poly(dA:dT) - CDS Agonist
Poly(dA:dT) is a repetitive synthetic double-stranded DNA sequence of poly(dA-dT)•poly(dT-dA) and a synthetic analog of B-DNA. Poly(dA:dT) is recognized by several sensors, including DAI, LRRFIP1 and AIM2[3-5]. It has also been shown to be transcribed by RNA polymerase III into dsRNA with a 5'-triphosphate moiety which is a ligand for RIG-I[6]. Poly(dA:dT) is available naked or complexed with the cationic lipid LyoVec™ to facilitate their uptake.

Poly(dG:dC) - CDS Agonist
Poly(dG:dC) is a repetitive synthetic double-stranded DNA sequence of poly(dG-dC)•poly(dC-dG). Poly(dG:dC) is a synthetic analog of the Z-DNA form. It has been reported to be recognized by LRRFIP1[4]. Poly(dG:dC) is available naked or complexed with the cationic lipid LyoVec™ to facilitate their uptake.

Poly(I:C)/LyoVec Complexes

Unlike naked poly(I:C) which is recognized by TLR3, transfected poly(I:C) is sensed by RIG-I/MDA-5 in a cell-type-specific manner[7, 8]. Poly(I:C) (HMW)/LyoVec and poly(I:C) (LMW)/LyoVec are preformed complexes between poly(I:C) (HMW) or poly(I:C) (LMW) and the transfection reagent LyoVec™. These complexes induce the activation of the RIG-I/MDA-5 signaling pathway at concentrations ranging from 100 ng to 1 µg/ml in C57/WT murine embryonic fibroblasts (MEFs), InvivoGen's RLR reporter cell line.

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CLR Ligands

Dectin-1 & Mincle Agonists

Curdlan - TLR2/4 & Dectin-1 Agonist

Curdlan is a high molecular weight linear polymer consisting of ß-(1 -> 3)-linked glucose residues. Curdlan is produced as a water-insoluble polysaccharide by the soil bacterium, *Alcaligenes faecalis*. Curdlan is recognized by the membrane bound Dectin-1 receptor leading to the CARD9-dependent activation of NF-κB and MAP kinases[1]. Furthermore, Dectin-1 signaling activates the NFAT transcription factor. Recent data suggest that Curdlan is also recognized by the cytosolic NLRP3 inflammasome complex which cooperates with Dectin-1 resulting in robust activation of IL-1β–mediated inflammatory response[2].

HKCA (*Candida albicans*) - Dectin-1 Agonist

HKCA is a heat-killed preparation of *Candida albicans*. *C. albicans* is an opportunistic yeast that causes serious infections in immunocompromised patients. Beta-glucans represent 40% of the cell wall of *C. albicans*. Heat killing of this yeast result in the exposure of the beta-glucans on the surface of the cell wall and their subsequent recognition by the beta-glucan receptor, Dectin-1[3]. HKCA derives from the strain ATCC 10231.

HKSC (*Saccharomyces cerevisiae*) - Dectin-1 Agonist

HKSC is a heat-killed preparation of the yeast *Saccharomyces cerevisiae*. The cell wall of *S. cerevisiae* consists mainly of equal amounts of α-mannans and β-glucans[4]. Early studies have suggested that the phagocytosis of unopsonized HKSC is mediated by both mannose and β-glucans receptors. However, recent data show that the β-glucan receptor, Dectin-1, is the predominant receptor involved in this process[5].

TDB - Mincle Agonist NEW

Trehalose-6,6-dibehenate (TDB) is a synthetic analog of trehalose-6,6-dimycolate (TDM, also known as cord factor), which is the most studied immunostimulatory component of Mycobacterium tuberculosis[6]. TDB binds the C-Type lectin, Mincle (macrophage-inducible C-type lectin)[6,7]. Upon TDB recognition Mincle interacts with the Fc receptor common γ-chain (FcRγ), which triggers intracellular signaling through Syk leading to CARD9-dependent NF-κB activation.

WGP Dispersable & WGP Soluble - Dectin-1 Agonist NEW

WGP Dispersable (Wellmune WGP® Dispersable, Biothera), whole glucan particles, is a particulate *Saccharomyces cerevisiae* β-glucan preparation. It consists of hollow yeast cell wall "ghosts" composed primarily of long polymers of β-1,3 glucose obtained after a series of alkaline and acid extractions from *S. cerevisiae* cell wall[8]. In contrast to Zymosan, WGP lacks TLR-stimulating activity[9]. Similarly to Zymosan, WGP induces Dectin-1-dependent responses, including phagocytosis and induction of TNF-α, IL-6 and ROS by macrophages and dendritic cells.

WGP Soluble (Wellmune WGP® Soluble, Biothera) is a pure soluble WGP preparation. Similarly to particulate WGP, WGP Soluble binds efficiently Dectin-1, however it is uncapable of activating the receptor[9]. Furthermore, WGP Control is able to significantly block the binding of WGP to macrophages and its immunostimulatory effect[8].

Zymosan - TLR2 & Dectin-1 Agonist

Zymosan is an insoluble preparation of *Saccharomyces cerevisiae* cell wall. Zymosan primarily contains β-glucans as well as other components such as mannans, mannoproteins and chitin. Zymosan activates macrophages via TLR2 which cooperates with TLR6 and CD14 to mediate the activation of NF-κB[10]. Zymosan is also recognized by Dectin-1, a phagocytic receptor expressed on macrophages and dendritic cells, which collaborates with TLR2 and TLR6 enhancing the immune responses triggered by the recognition of Zymosan by each receptor[11].

Zymosan Depleted

Zymosan depleted is a *S. cerevisiae* cell wall preparation treated with hot alkali to remove all its TLR-stimulating properties[12]. Zymosan depleted activates Dectin-1 but not TLR2.

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Inflammasome Inducers

NLRP3 & AIM2 Inflammasome Inducers

Alum Crystals - NLRP3 Inflammasome Inducer

Aluminum hydroxide and potassium salts (alum) are commonly used vaccine adjuvants. Adjuvants are vaccine additives that stimulate the immune system without having any specific antigenic effect. Alum has been demonstrated to activate caspase-1 and trigger IL-1β and IL-18 secretion[1]. All alum preparations contain crystals. The alum-induced release of IL-1β in macrophages is dependent on NLRP3 and ASC, indicating that alum triggers inflammation through activation of the NLRP3 inflammasome[2]. Alum has been shown to trigger NLRP3 activation through lysosomal destabilization[2].

ATP - NLRP3 Inflammasome Inducer

Adenosine triphosphate (ATP), a potassium efflux agent, can trigger the activation of NLRP3 inflammasome in response to PAMPs, such as LPS and peptidoglycan. It stimulates the caspase-1-dependent cleavage and secretion of IL-1β from LPS-stimulated cells[3]. ATP triggers the opening of the non-selective cation channel of the purinergic P2X7 receptor, followed by the gradual opening of a larger pore. The larger pore is attributed to pannexin-1, which is recruited upon P2X7 receptor activation[4]. Activation of the P2X7 receptor results in potassium efflux which is necessary for activation of the post-translational maturation of IL-1β[5].

Hemozoin - NLRP3 Inflammasome Inducer

Hemozoin is a dark-brown heme crystal produced by the intraerythrocytic parasite *Plasmodium*, the causative agent of malaria. Hemozoin is taken up by macrophages initiating signals that lead to the production of IL-1β. Hemozoin-induced IL-1β production is dependent on the activation of the NLRP3 inflammasome[6, 7]. Synthetic hemozoin has been shown to possess adjuvant properties that differ depending on the method of synthesis[8]. InvivoGen provides a chemically synthesized hemozoin using an acidic method.

MSU & CPPD Crystals - NLRP3 Inflammasome Inducers

Crystals of monosodium urate (MSU) and calcium pyrophosphate dihydrate (CPPD) are the aetiological agents of the inflammatory joint diseases gout and pseudo-gout, respectively. Both pathogenic crystals have been recently shown to be potent activators of caspase-1 through the NLRP3 inflammasome[9]. Involvement of the inflammasome is suggested by the finding that macrophages from mice deficient in various components of the inflammasome are defective in crystal-induced IL-1β induction. NLRP3 activation requires the phagocytosis of crystals that leads to lysosomal damage which appears to be the signal recognized by the inflammasome resulting in its activation[2].

Nano-SiO2 - NLRP3 Inflammasome Inducer **NEW**

SiO2 nanoparticles (Nano-SIO2) are single particles of silica dioxide, an inorganic metal oxide, with a diameter less than 100 nm. Nano-SiO2 was recently shown to trigger caspase-1 cleavage and IL-1β secretion in human macrophages and keratinocytes[10]. The pro-inflammatory activity of nano-SiO2 is mediated by the NLRP3 inflammasome. THP1 cells stably infected with shRNAs against caspase-1 or NLRP3 did not secrete IL-1β in response to nano-SiO2.

Nigericin - NLRP3 Inflammasome Inducer

Nigericin is a microbial toxin derived from *Streptomyces hygroscopicus*. Nigericin acts as a potassium ionophore. The release of IL-1β in response to nigericin has been demonstrated to be NLRP3-dependent[3]. Similar to ATP, nigericin induces a net decrease in intracellular levels of potassium which is crucial for the activation of caspase-1[5]. Nigericin requires signaling through pannexin-1 to induce caspase-1 maturation and IL-1β processing and release[11].

Poly(dA:dT) - AIM2 Inflammasome Inducer

Poly(dA:dT) is a repetitive synthetic double-stranded DNA sequence of poly(dA-dT)•poly(dT-dA). Poly(dA:dT) is complexed with the cationic lipid LyoVec™ to facilitate its uptake. Transfection of macrophages with poly(dA:dT) leads to the production of IL-1β[12]. This response to transfected poly(dA:dT) is ASC-dependent, but NLRP3 independent. AIM2 was recently shown to sense poly(dA:dT), form an inflammasome with ASC and trigger caspase-1 activation[13-15]. Poly(dA:dT) binds to AIM2 and induces its oligomerization, which is the first demonstration of an inflammasome bound to its ligand[13].

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MSU & Alum

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ATP & Nigericin

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4 PRR & PAMPs DETECTION

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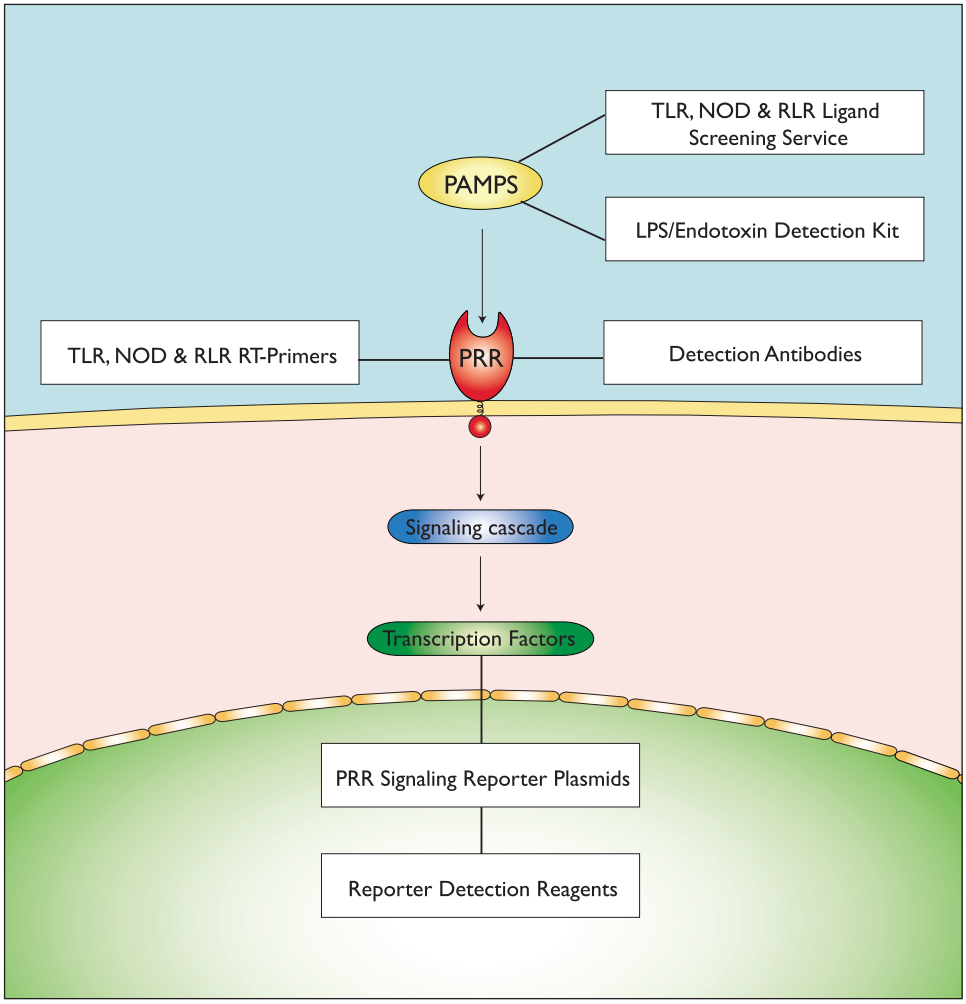
PRR & PAMPs DETECTION

InvivoGen provides a comprehensive range of tools for the rapid, convenient and reliable detection of pattern recognition receptors (PRRs) and pathogen-associated molecular patterns (PAMPs). These tools include the following service and products:

- **Immunomodulatory Compound Screening -** TLR, NOD and RLR Ligand Screening Service
- **HEK-Blue™ LPS Detection Kit -** LPS/Endotoxin Detection
- **PRR RT-Primers -** RT-PCR Primers for the Detection of TLR, NOD and RLR
- **pNiFty -** PRR Signaling Reporter Plasmids Featuring Lucia™ or SEAP
- **Reporter Detection Reagents -** Lucia™ & SEAP Reporter Gene Systems (see pages 53-57)
- **Antibodies -** Antibodies for PRR Detection (see page 98)



Immunomodulatory Compound Screening

TLR, NOD and RLR Ligand Screening Service

There is a growing interest in the targeting of Toll-like receptors (TLRs) and other pattern recognition receptors (PRRs) for drug discovery research. As a recognized industry leader in innate immunity, InvivoGen provides a high quality immunomodulatory compound screening service to assist our clients' drug discovery and development needs.

- **Short turnaround time -** Screening turnaround: ONLY 3 weeks
- **Screening flexibility -** Screening parameters can be selected and/or modified based on customer requirements.
- **Cost effective -** A set-up charge applies for the first compound. Subsequent compounds are heavily discounted.
- **Reliable -** Our screening service has been utilized consistently by leading Biotech and Pharmaceutical companies and academic institutes for many years.

Description

Over the past several years, InvivoGen has developed a large collection of cellular assays to detect compounds that activate or block the immune system through activation of PRRs with an emphasis on TLRs, NOD1/2 and RIG-I/MDA-5. These sensitive cellular assays feature an NF-κB-inducible SEAP (secreted embryonic alkaline phosphatase) or an IRF (interferon regulatory factor)-inducible sLUC (secreted luciferase) reporter gene as the read-out. Upon stimulation, activation of the NF-kB or IRF pathways is monitored using proprietary detection assays designed to provide rapid and reliable results.

➢ TLR Ligand Screening

The TLR ligand screening service utilizes the HEK-Blue TLR cells (see page XX). These HEK293-derived cells are NF-κB-SEAP reporter cells that stably express a human or mouse TLR gene. The TLR genes that are stably expressed are TLR2, 3, 4, 5, 7, 8 or 9.

Compound profiling: The compound is tested on all human and/or mouse TLRs to determine which TLR recognizes the compound. TLR ligands are typically recognized by a single TLR or potentially two (TLR7 and TLR8). Recognition by TLR4 usually reflects the presence of endotoxins in the sample.

Antagonist assays can also be performed.

➢ NOD1/2 Ligand Screening

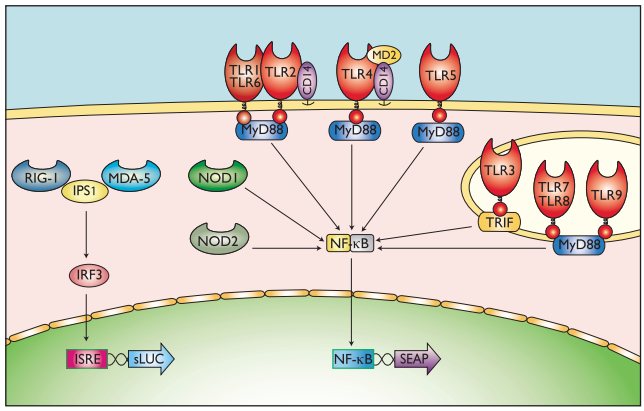
The NOD1/2 ligand screening service utilizes the HEK-Blue NOD cells (see page XX). These HEK-Blue cells stably express an NF-κB-inducible SEAP reporter gene and the human or mouse NOD1 or NOD2 gene.

Compound profiling: The compound is tested on human and/or mouse NOD1 and NOD2.

➢ RIG-I/MDA-5 Ligand Screening NEW

InvivoGen introduces the RIG-I/MDA-5 ligand screening service. This service utilizes recently developed HEK293 cell lines that exploit the IRF pathway and a secreted luciferase reporter assay. They overexpress the human RIG-I or MDA-5 gene and are highly sensitive to 5'ppp-dsRNA and transfected poly(I:C), respectively, unlike their parental cell line that expresses low levels of both RIG-I and MDA-5.

Compound profiling: The compound is tested on human RIG-I and/or MDA-5.



Simplified representation of the TLR, NOD and RLR pathways

Screening Services

Two choices of services are offered, Compound Profiling and Compound Dose Response, that can be performed sequentially or individually.

- **Compound Profiling (level 1):** Single dose testing on a set of PRRs. Screening is performed at a single concentration, typically a 1/10 dilution of the original compound solution provided, or as specified by customer.
- **Compound Dose Response (level 2):** Dose response on one or several PRRs.

Three concentrations of the compound(s), typically 1/10, 1/100 and 1/1000 dilutions of the original compound solution, are tested on the PRR(s) recognizing the compound(s) as determined in level 1 or specified by the customer.

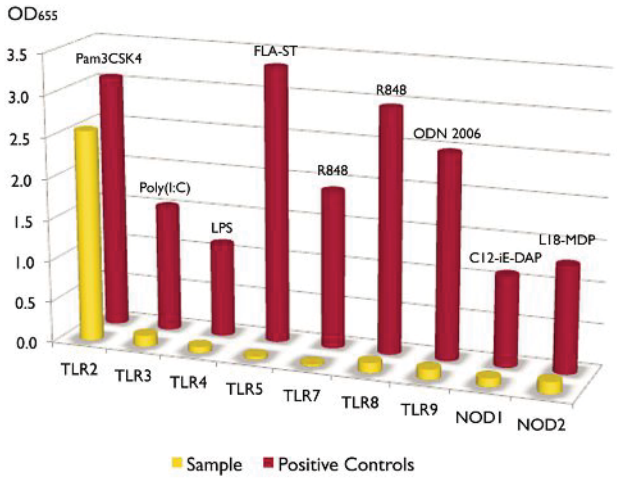
A detailed report is prepared and provided to the customer electronically and in hard copy. All procedures are performed accordingly to strict guidelines. Confidentiality is guaranteed.

PRODUCT	CAT. CODE
Compound Profiling	tlrl-test1
Compound Dose Response	tlrl-test2

Question 1: Is my compound a TLR or NOD agonist?

To answer this question, all 7 HEK-Blue™ TLR or NOD cells will be stimulated with a single dose of your compound as well as a set of known TLR agonists that serve as positive controls. After overnight incubation, the production of SEAP is assessed using QUANTI-Blue™, a SEAP detection medium. The OD is measured and analyzed.

Example 1: As seen in graph 1, only HEK-Blue™ hTLR2 cells produce SEAP in response to the compound tested, suggesting that this compound activates TLR2 and no other TLR. Thus, the compound tested is a TLR2 agonist.

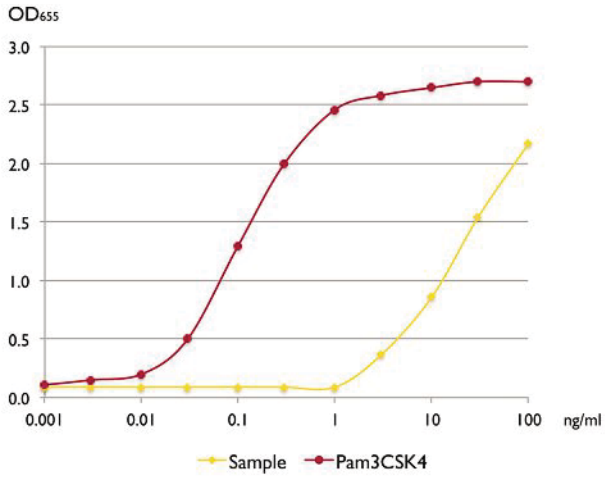


Graph 1. HEK-Blue™ TLR (human TLR2, 3, 4, 5, 7, 8, 9) and HEK-Blue™ NOD (human NOD1 or NOD2) cells were stimulated with 1/10 dilution of the sample solution provided and a fixed concentration of the positive controls: 100 ng/ml Pam3CSK4 (TLR2), 1 μg/ml poly(I:C) (TLR3), 100 ng/ml LPS-EK (TLR4), 100 ng/ml FLA-ST UP (TLR5), 10 μg/ml R848 (TLR7/8), 10 μg/ml ODN 2006 (TLR9), 10 μg/ml C12-iE-DAP (NOD1) and 10 μg/ml L18-MDP (NOD2). After 24h incubation, TLR-induced NF-κB activation was assessed by measuring the levels of SEAP in the supernatants of HEK-Blue™ cells using QUANTI-Blue™.

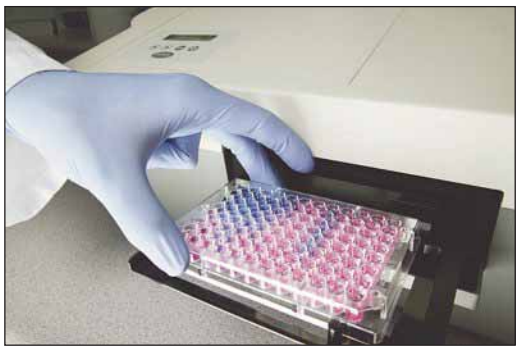
Question 2: Is my compound a potent TLR2 agonist?

To determine the potency of the compound tested as a TLR2 agonist, a dose response of the compound and a TLR2 positive control is performed on the HEK-Blue™ hTLR2 cells assayed using QUANTI-Blue™. The TLR2 positive control used is Pam3CSK4, a synthetic lipopeptide, that activates TLR2 at sub-nanogram per ml concentrations.

Example 2: As seen in graph 2, the compound tested is a good TLR2 agonist although not as potent as the positive control. The linear range of TLR2 activation is 5-100 ng/ml for the sample and 0.05-0.5 ng/ml for Pam3CSK4.



Graph 2. HEK-Blue™ hTLR2 cells were stimulated with increasing concentrations of the sample provided or the TLR2 positive control, Pam3CSK4. After 24h incubation, TLR2-induced NF-κB activation was assessed by measuring the levels of SEAP in the supernatants of HEK-Blue™ hTLR2 cells using the QUANTI-Blue™ assay.



Contact us for more information at info@invivogen.com

Recent articles using InvivoGen's Screening Service

Schreibelt G. *et al.*, 2010, Commonly used prophylactic vaccines as an alternative for synthetically produced TLR ligands to mature monocyte-derived dendritic cells. Blood, 116: 564 - 574.

Keller JF. *et al.*, 2011, Expression of NOD2 is increased in inflamed human dental pulps and lipoteichoic acid-stimulated odontoblast-like cells. Innate Immunity, 17: 29 - 34.

Tamayo J. *et al.*, 2011. Poly(Anhydride) Nanoparticles Act as Active Th1 Adjuvants through Toll-Like Receptor Exploitation. Clin. Vaccine Immunol., 17: 1356 - 1362.

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.

HEK-Blue™ LPS Detection Kit - LPS/Endotoxin Detection

Lipopolysaccharide (LPS), also known as endotoxin, the major cell wall component of Gram-negative bacteria, induces the activation of NF-κB and the production of proinflammatory cytokines. *In vivo*, this response can cause fever, septic shock and eventually death of the animal. *In vitro*, it can introduce a bias in experiments involving cells sensitive to low levels of LPS such as monocytes. In addition, repeated passages of cell lines in a medium containing LPS might render these cells unresponsive to further stimulation by LPS. This desensitization, termed LPS tolerance, does not only affect inflammatory responses but also other essential functions including antigen presentation by monocytes. Thus, monitoring the presence of LPS in biological reagents is crucial. InvivoGen provides the HEK-Blue™ LPS Detection Kit, a simple, rapid and reliable system to detect the presence of endotoxins in your samples.

➤ Detection range: 1.5 EU/ml in the sample

Principle

The HEK-Blue™ LPS Detection Kit is based on the ability of TLR4 to recognize structurally different LPS from gram-negative bacteria and in particular lipid A, their toxic moiety. Proprietary cells engineered to become extremely sensitive to LPS, called HEK-Blue™-4 cells, are the main feature of this endotoxin detection kit. The presence of very low concentrations of LPS, starting as low as 0.03 ng/ml, are detected by the HEK-Blue™-4 cells leading to the activation of NF-κB. Using HEK-Blue™ Detection, a specific detection medium, NF-κB activation can be observed with the naked eye or quantified by reading the OD at 650 nm.

Key Features

HEK-Blue™-4 cells, the endotoxin sensor cells, are engineered HEK293 cells. These cells stably express TLR4, MD2, CD14 and multiple genes from the TLR4 pathway. They 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™-4 cells. Furthermore, Normocin™ is included in the kit to protect HEK-Blue™-4 cells from any potential microbial contamination, whether caused by mycoplasmas, bacteria or fungi (see page 59).

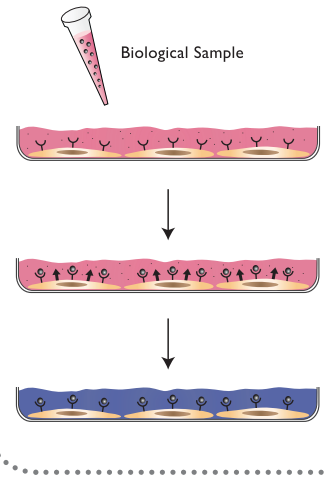
HEK-Blue™ Detection is a medium specifically designed for the detection of SEAP. It contains a color substrate that produces a purple/blue color following its hydrolysis by SEAP (see page 56).

Contents

The HEK-Blue™ LPS Detection Kit is composed of the following components:
- 1 vial of HEK-Blue™-4 cells (3-5x 10^6 cells)
- 4 tubes of 250X HEK-Blue™ Selection (2 ml)
- 4 tubes of 500X Normocin™ (1 ml)
- 2 pouches of HEK-Blue™ Detection (50 ml each)
- 1 tube of *E. coli* K12 LPS (LPS-EK, 100 µg) as a positive control
- 1 tube of endotoxin-free water (1.5 ml) as a negative control

Buy the HEK-Blue™ LPS Detection Kit once then reorder only the reagents to perform further assays.

HEK-Blue™ LPS Detection Kit Procedure



PRODUCT	QTY	CAT. CODE
HEK-Blue™ LPS Detection Kit	1 kit	rep-lps
HEK-Blue™ Selection	5 x 2 ml	hb-sel
Normocin™	10 x 1 ml	ant-nr-1
HEK-Blue™ Detection	5 pouches	hb-det2
LPS-EK (control)	5 mg	tlrl-eklps

Recent articles using HEK-Blue™ LPS Detection Kit

Roh HC. *et al.*, 2011. Bacteroides fragilis Enterotoxin Upregulates Intercellular Adhesion Molecule-1 in Endothelial Cells via an Aldose Reductase-, MAPK-, and NF-{kappa}B–Dependent Pathway, Leading to Monocyte Adhesion to Endothelial Cells. J. Immunol., 187: 1931 - 1941.

Swedan S. *et al.*, 2011. Multiple Functional Domains and Complexes of the Two Nonstructural Proteins of Human Respiratory Syncytial Virus Contribute to Interferon Suppression and Cellular Location. J. Virol., 85: 10090 - 10100.

Nembrini C. *et al.*, 2011. Nanoparticle conjugation of antigen enhances cytotoxic T-cell responses in pulmonary vaccination. PNAS, 108: E989 - E997.

PRR RT-Primers - RT-PCR Primers for TLR, NOD & RLR Detection

InvivoGen offers a collection of RT-PCR primers to determine the mRNA expression pattern of human and murine TLRs, NODs and RLRs. Each RT-PCR primer pair is provided with a positive control.

Description

RT-Primers are provided as pairs for each individual TLR, NOD or RLR. TLR RT-Primers are also available as a set containing a primer pair for all ten human TLRs and nine murine TLRs.

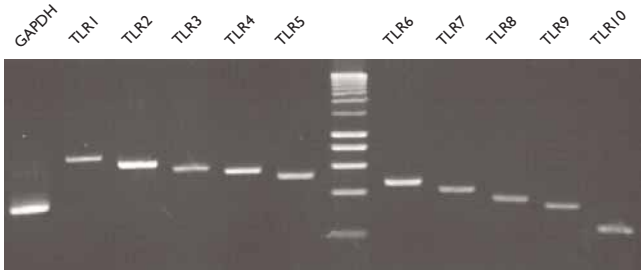
Each RT-PCR Primer pair is carefully designed and tested.

The size of the amplified fragments varies from 200 to 700 bp.

Contents and Storage

Each RT-Primer Pair contains the following:
- 2.5 nmoles of each primer, allowing 50 reactions at 1 µM final primer concentration. 5' sense primer and 3' antisense primer are shipped together in a single vial.
- 500 ng positive control double stranded DNA

The TLR RT-Primer Set contains the following:
- 2.5 nmoles of each primer (20 primers total for the human set and 18 primers total for the mouse set). Each primer pair is provided in a single vial.
- 5 µg positive control double stranded DNA



Expression of TLR mRNAs in human monocytic cell line THP-1 stimulated with PMA using the human TLR RT-PCR Primer Set.

PRODUCT	QUANTITY	CAT. CODE (HUMAN)	CAT. CODE (MOUSE)
TLR RT-Primers			
TLR1 RT-Primer Pair	2 x 2.5 nmol	rtp-htlr1	rtp-mtlr1
TLR2 RT-Primer Pair	2 x 2.5 nmol	rtp-htlr2	rtp-mtlr2
TLR3 RT-Primer Pair	2 x 2.5 nmol	rtp-htlr3	rtp-mtlr3
TLR4 RT-Primer Pair	2 x 2.5 nmol	rtp-htlr4	rtp-mtlr4
TLR5 RT-Primer Pair	2 x 2.5 nmol	rtp-htlr5	rtp-mtlr5
TLR6 RT-Primer Pair	2 x 2.5 nmol	rtp-htlr6	rtp-mtlr6
TLR7 RT-Primer Pair	2 x 2.5 nmol	rtp-htlr7	rtp-mtlr7
TLR8 RT-Primer Pair	2 x 2.5 nmol	rtp-htlr8	rtp-mtlr8
TLR9 RT-Primer Pair	2 x 2.5 nmol	rtp-htlr9	rtp-mtlr9
TLR10 RT-Primer Pair	2 x 2.5 nmol	rtp-htlr10	-
TLR RT-Primer Set	20 or 18 x 2.5 nmol	rts-htlrs	rts-mtlrs
NLR RT-Primers			
NOD1 RT-Primer Pair	2 x 2.5 nmol	rtp-hnod1	rtp-mnod1
NOD2 RT-Primer Pair	2 x 2.5 nmol	rtp-hnod2	rtp-mnod2
RLR RT-Primers			
RIG-I RT-Primer Pair	2 x 2.5 nmol	rtp-hrigi	rtp-mrigi
MDA-5 RT-Primer Pair	2 x 2.5 nmol	rtp-hmda5	rtp-mmda5

pNiFty - PRR Signaling Reporter Plasmids

PRR activation triggers a complex signaling cascade that leads to the activation of different transcription factors, each playing an important role in the subsequent immune response. To monitor the induction of PRR signaling in response to ligand stimulation in a simple and efficient manner, InvivoGen has designed pNiFty, a family of reporter plasmids expressing a reporter gene under the control of a minimal promoter inducible by these different transcription factors, either individually or in combination. Most pNiFty plasmids are selectable with Zeocin™ in both *E. coli* and mammalian cells, and can be used to generate stable clones.

Description

pNiFty plasmids are composed of three key elements: a minimal promoter, repeated transcription factor binding sites (TFBS) and a reporter gene. The proximal promoters are shorter than 500 bp and contain transcription factor binding sites. Upon stimulation in 293 cells, their expression level remains undetectable. With the addition of repeated TFBS, the proximal promoters become inducible by the appropriate stimulus and drive the expression of the reporter gene.

Minimal promoters

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

- IFN-β promoter: the mouse IFN-β minimal promoter comprises several positive regulatory domains that bind different cooperating transcription factors such as NF-κB, IRF3 and IRF7[3].

- ISG-56K promoter: the minimal promoter of the human interferon-stimulated gene ISG-56K contains two interferon-stimulated regulatory element (ISRE) sites and is fully inducible by type I IFNs and interferon regulatory factors (IRFs)[4, 5].

Transcription factor binding sites (TFBS)

- AP-1 binding site: Activator protein 1 (AP-1) is a transcription factor activated by most PRRs. AP-1 is a heterodimeric complex composed of members of Fos, Jun and, ATF protein families. AP-1 binds to the TPA responsive element (TRE: ;TGAG/CTCA)[6]. AP-1 activation in TLR signaling is mostly mediated by MAP kinases such as c-Jun N-terminal kinase (JNK), p38 and extracellular signal regulated kinase (ERK).

- NF-κB binding site: Nuclear factor (NF)-κB is a "rapid-acting" primary transcription factor activated by a wide variety of PRRs. NF-κB is a protein complex that belongs to the Rel-homology domain-containing protein family. The prototypical NF-κB is composed of the p65(RelA) and p50 subunits[7]. NF-κB binds specific decameric DNA sequences (GGGRNNYYCC, R-purine Y=pyrimidine) and activates genes involved in the regulation of the innate and adaptative immune response.

- ISRE binding site: PRRs involved in the antiviral response induce the activation of interferon regulatory factors (IRFs) and the production of type I interferons (IFNs). IFNs trigger the formation of the ISGF3 complex which contains signal transducer and activator of transcription (STAT) 1, STAT2 and IRF9. ISGF3 and IRFs bind to specific nucleotide sequences called interferon-stimulated response elements (ISREs; AGTTTCNNTTTCC) in the promoter of IFN-stimulated genes (ISGs) leading to their activation[8].

- NFAT binding site: Nuclear factor of activated T-cell (NFAT) is a family of transcription factors expressed in T cells, but also in other classes of immune and non-immune cells[9]. NFAT is activated by stimulation of receptors coupled to calcium mobilization, such as the PRRs Dectin-1 and Mincle[10,11]. Calcium mobilization induces the calmodulin-dependent phosphatase calcineurin leading to NFAT activation. NFAT binds to a 9 bp element, with the consensus sequence (A/T)GGAAA(A/N)(A/T/C)N.

Reporter Genes

- SEAP reporter gene: Secreted alkaline phosphatase (SEAP) is a reporter widely used to study promoter activity or gene expression. SEAP expression can be rapidly and readily measured in supernatants of transfected cells. SEAP levels can be evaluated qualitatively with the naked eye and quantitatively using SEAP detection reagents, such as HEK-Blue™ Detection or QUANTI-Blue™ (see "Related Products").

- Luc reporter gene: The firefly luciferase gene is a highly sensitive reporter gene and thus is ideal for detecting low-level gene expression. Luc activity can be quantified in cell extracts by using kits commercially available from other companies.

- Lucia™ reporter gene: Lucia™ is a novel secreted luciferase with strong bioluminescent activity. The Lucia reporter gene is ideal for promoter activity and gene expression studies. Lucia is encoded by a synthetic gene derived by genetic engineering of copepod luciferase genes. Lucia™ luciferase activity can be detected and quantified directly in the culture medium of transfected cells using InvivoGen's Quanti-Luc™ detection reagent (see "Related Products").

1. Schindler U., Baichwal VR., 1994. Three NF-kappa B binding sites in the human E-selectin gene required for maximal tumor necrosis factor alpha-induced expression. Mol Cell Biol. 14(9):5820-31. **2. Jensen LE. & Whitehead AS., 2003.** ELAM-1/E-selectin promoter contains an inducible AP-1/CREB site and is not NF-kB-specific. Biotechniques 35:54-58. **3. Vodjdani G. *et al.*, 1988.** Structure and characterization of a murine chromosomal fragment containing the interferon beta gene. J Mol Biol. 204(2):221-31. **4. Wathelet MG. *et al.*, 1987.** New inducers revealed by the promoter sequence analysis of two interferon-activated human genes. Eur J Biochem. 1987 Dec 1;169(2):313-21. **5. Grandvaux N, *et al.*, 2002.** Transcriptional profiling of interferon regulatory factor 3 target genes: direct involvement in the regulation of interferon-stimulated genes. J Virol. 2002 Jun;76(11):5532-9. **6. Hess J, *et al.*, 2004.** AP-1 subunits: quarrel and harmony among siblings. J Cell Sci. 117(Pt 25):5965-73. **7. Kawai T. & Akira S., 2007.** Signaling to NF-kappaB by Toll-like receptors. Trends Mol Med. 13(11):460-9. **8. Wesoly J. *et al.*, 2007.** STAT activation and differential complex formation dictate selectivity of interferon responses. Acta Biochim Pol. 54(1):27-38. **9. Rao A. *et al.*, 1997.** Transcription factors of the NFAT family: regulation and function. Annu Rev Immunol. 15:707-47. **10. Goodridge HS. *et al.*, 2007.** Dectin-1 stimulation by Candida albicans yeast or zymosan triggers NFAT activation in macrophages and dendritic cells. J Immunol. 178(5):3107-15. **11. Yamasaki S. *et al.*, 2009.** C-type lectin Mincle is an activating receptor for pathogenic fungus, Malassezia. PNAS. 106(6):1897-902.

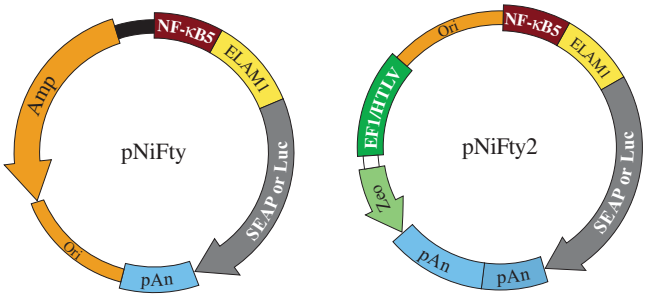
PLASMID NAME	TRANSCRIPTION FACTOR BINDING SITES	MINIMAL PROMOTER	SELECTION	REPORTER	CATALOG CODE
pNiFty-SEAP	NF-κB (x5)	ELAM	Ampicillin	SEAP	pnifty-seap
pNiFty-Luc	NF-κB (x5)	ELAM	Ampicillin	Luciferase	pnifty-luc
pNiFty2-SEAP	NF-κB (x5)	ELAM	Zeocin™	SEAP	pnifty2-seap
pNiFty2-Luc	NF-κB (x5)	ELAM	Zeocin™	Luciferase	pnifty2-luc
pNiFty2-56K	None	ISG56	Zeocin™	SEAP	pnf2-56ksp
pNiFty3-SEAP	None	IFN-β	Zeocin™	SEAP	pnf3-sp1
pNiFty3-Lucia **NEW**	None	IFN-β	Zeocin™	Lucia™	pnf3-lc1
pNiFty3-N-SEAP	NF-κB (x5)	IFN-β	Zeocin™	SEAP	pnf3-sp2
pNiFty3-N-Lucia **NEW**	NF-κB (x5)	IFN-β	Zeocin™	Lucia™	pnf3-lc2
pNiFty3-A-SEAP	AP-1 (x5)	IFN-β	Zeocin™	SEAP	pnf3-sp3
pNiFty3-A-Lucia **NEW**	AP-1 (x5)	IFN-β	Zeocin™	Lucia™	pnf3-lc3
pNiFty3-I-SEAP	ISRE (x5)	IFN-β	Zeocin™	SEAP	pnf3-sp4
pNiFty3-I-Lucia **NEW**	ISRE (x5)	IFN-β	Zeocin™	Lucia™	pnf3-lc4
pNiFty3-T-SEAP	NFAT (x5)	IFN-β	Zeocin™	SEAP	pnf3-sp5
pNiFty3-T-Lucia **NEW**	NFAT (x5)	IFN-β	Zeocin™	Lucia™	pnf3-lc5
pNiFty3-AN-SEAP	AP-1 (x5) NF-κB (x5)	IFN-β	Zeocin™	SEAP	pnf3-sp6
pNiFty3-AN-Lucia **NEW**	AP-1 (x5) NF-κB (x5)	IFN-β	Zeocin™	Lucia™	pnf3-lc6
pNiFty3-IAN-SEAP	ISRE (x5) AP-1 (x5) NF-κB (x5)	IFN-β	Zeocin™	SEAP	pnf3-sp7
pNiFty3-IAN-Lucia **NEW**	ISRE (x5) AP-1 (x5) NF-κB (x5)	IFN-β	Zeocin™	Lucia™	pnf3-lc7
pNiFty3-TAN-SEAP	NFAT (x5) AP-1 (x5) NF-κB (x5)	IFN-β	Zeocin™	SEAP	pnf3-sp8
pNiFty3-TAN-Lucia **NEW**	NFAT (x5) AP-1 (x5) NF-κB (x5)	IFN-β	Zeocin™	Lucia™	pnf3-lc8

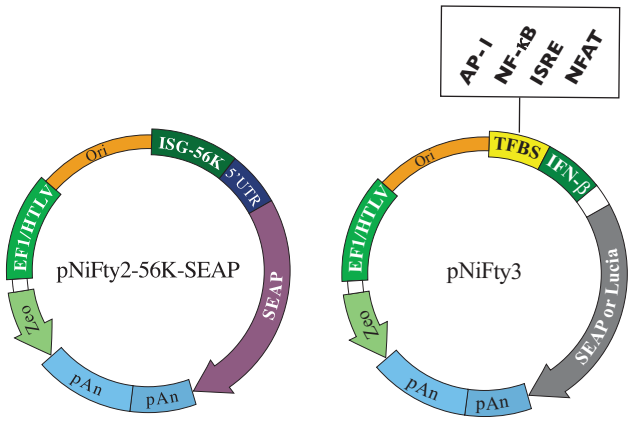
Contents and Storage

pNiFty plasmids are provided as lyophilized transformed *E. coli* strains on paper disk with 4 pouches of Fast-Media® (2 TB and 2 Agar), containing the appropriate antibiotic: ampicillin for pNiFty plasmids, or Zeocin™ for pNiFty2 and pNiFty3 plasmids. Products are shipped at room temperature and should be stored at -20°C.

Related Products

QUANTI-Blue™, page 56
HEK-Blue™ Detection, page 56
Zeocin™, page 61
QUANTI-Luc™, page 55
Fast-Media®, page 22





Recent articles using pNiFty plasmids

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5 IMMUNO-MODULATORS

Small Molecule & Peptide Immunomodulators 90

Short Hairpin RNAs 94

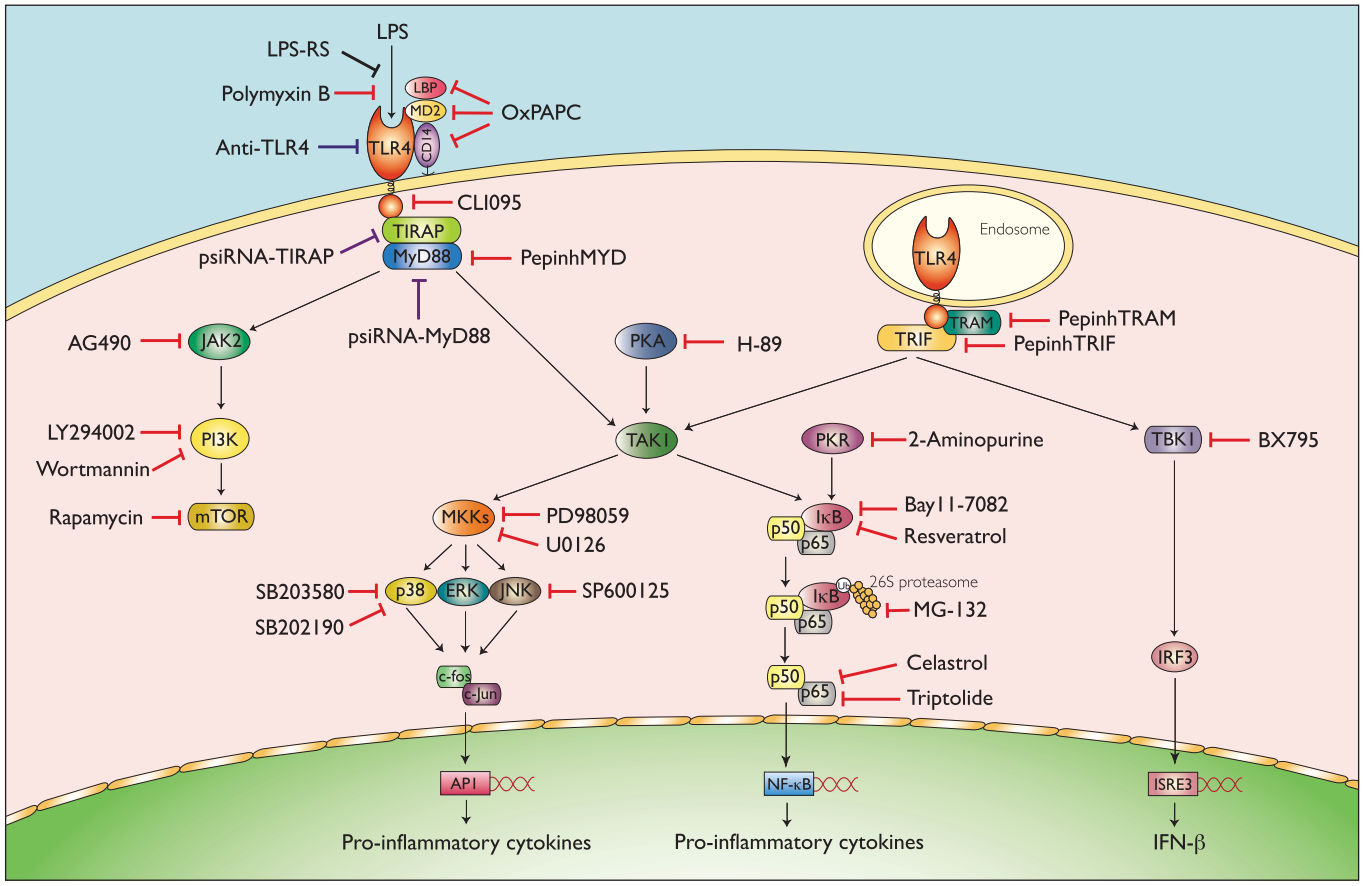
Cytokines 96

IMMUNOMODULATORS

InvivoGen offers a wide selection of immunomodulators of the signaling pathways initiated by pattern recognition receptors (PRRs). These molecules intervene in the different steps of PRR activation and signaling cascade, including ligand binding, interaction with adapters and activation of MAP kinases and NF-κB. Immunomodulators include a large variety of compounds that act as inducers or inhibitors (see example below):

- **Small molecule immunomodulators**
- **Peptide immunomodulators**
- **Short hairpin RNAs** (shRNAs) targeting PRR and related genes(see page 94)
- **Recombinant cytokines** (see page 96)
- **Neutralizing antibodies** (see page 99)
- **TLR antagonists** (see pages 65-67)

Inhibitors of TLR4 signaling pathways



Stimulation of TLR4 leads to the activation of different signaling pathways depending on the ligand and cell line used. These signaling pathways can be blocked at many levels using a variety of compounds:

- Small molecule, peptide or lipid inhibitors
- Short hairpin RNAs (an extensive collection is listed page 94)
- Neutralizing antibodies (a complete list is available page 99)
- TLR antagonists (more antagonists are described pages 65-67)

Small Molecule & Peptide Immunomodulators

PRODUCT	DESCRIPTION	WORKING CONCENTRATION	QTY	CATALOG CODE	INFO
2-Aminopurine	PKR inhibitor	1 - 10 mM	250 mg	tlrl-apr	p 91
3-Methyladenine	PI3K inhibitor / Autophagy inhibitor	5 mM	50 mg	tlrl-3ma	p 91
AG490	JAK2 inhibitor	1 -100 µM	10 mg	tlrl-ag4	p 91
Anti-hTLR4-IgG	Neutralizing monoclonal antibody against human TLR4	-	100 µg	mabg-htlr4	p 99
Bafilomycin A1	V-ATPase inhibitor / Autophagy inhibitor	0.1 - 1 µM	10 µg	tlrl-baf	p 91
Bay11-7082	IκB-α inhibitor	1 - 10 µM	10 mg	tlrl-b82	p 91
BX795	TBK1/IKKε inhibitor	10 nM - 1 µM	5 mg	tlrl-bx7	p 91
Celastrol	NF-κB inhibitor	2.5 - 10 µM	1 mg	ant-cls	p 91
Chloroquine	Endosomal acidification inhibitor	10 µM	250 mg	tlrl-chq	p 91
CLI-095	TLR4 signaling inhibitor	50 nM - 1 µM	1 mg	tlrl-cli95	p 91
Cyclosporin A	Calcineurin inhibitor	50 nM - 1.5 µM	100 mg	tlrl-cyca	p 91
Dexamethasone NEW	NF-κB and MAPK inhibitor	100 nM	100 mg	tlrl-dex	p 91
Gefitinib	RIP2 tyrosine kinase inhibitor	10 µM	10 mg	tlrl-gef	p 91
Glybenclamide	Proton pump inhibitor	25 µg/ml	1 g	tlrl-gly	p 91
H-89	PKA inhibitor	5 - 50 µM	5 mg	tlrl-h89	p 91
Leptomycin B	Nuclear export inhibitor	50 - 200 nM	5 µg	tlrl-lep	p 91
LL-37 NEW	Antimicrobial peptide	1 - 50 µg/ml	1 mg	tlrl-l37	p 91
LPS-RS Ultrapure NEW	Ultrapure liposaccharide from *R. sphaeroides* / TLR4 antagonist	10 ng - 10 µg/ml	5 mg	tlrl-rslps	p 65
LY294002	PI3K inhibitor / Autophagy inhibitor	50 - 100 µM	5 mg	tlrl-ly29	p 91
MAb-mTLR2	Neutralizing monoclonal antibody against human/mouse TLR2	-	100 µg	mab-mtlr2	p 99
MG-132 NEW	26S proteasome inhibitor	10 µM	5 mg	tlrl-mg132	p 91
ODN 2088	Inhibitory ODN / TLR9 antagonist	50 nM - 1 µM	1 mg	tlrl-2088-1	p 67
OxPAPC	TLR2 and TLR4 inhibitor	30 µg/ml	1 mg	tlrl-oxp1	p 92
PD98059	MAP kinase kinase inhibitor	5 - 100 µM	10 mg	tlrl-pd98	p 92
Pepinh-Control	Control for Pepinh inhibitory peptides	5 - 100 µM	2 mg	tlrl-pictrl	p 92
Pepinh-MYD	MyD88 inhibitory peptide	5 - 100 µM	2 mg	tlrl-pimyd	p 92
Pepinh-TRAM NEW	TRAM inhibitory peptide	40 µM	2 mg	tlrl-pitram	p 92
Pepinh-TRIF	TRIF inhibitory peptide	50 - 100 µM	2 mg	tlrl-pitrif	p 92
Piceatannol NEW	Syk inhibitor	1 - 25 µM	5 mg	tlrl-pct	p 92
PMA	PKC inducer	0.1 - 1 ng/ml	5 mg	tlrl-pma	p 92
Polymyxin B	LPS-induced TLR4 activation inhibitor	10 µg/ml	100 mg	tlrl-pmb	p 95
psiRNA-MyD88	Short hairpin RNA targeting Myd88	-	20 µg	psirna42-myd88	p 95
psiRNA-TIRAP	Short hairpin RNA targeting TIRAP	-	20 µg	psirna42-tirap	p 95
Rapamycin	mTOR inhibitor / Autophagy inducer	10-100 nM	5 mg	tlrl-rap	p 92
Resveratrol NEW	NF-κB inhibitor	10 - 100 µM	100 mg	tlrl-resv	p 92
SB202190 NEW	p38 MAP kinase inhibitor	1 - 20 µM	5 mg	tlrl-sb90	p 92
SB203580	p38 MAP kinase inhibitor	1 - 20 µM	5 mg	tlrl-sb20	p 92
SP600125	JNK inhibitor	10 - 500 µM	10 mg	tlrl-sp60	p 92
Tamoxifen	Estrogen receptor antagonist / Autophagy inducer	10 - 100 µM	200 mg	tlrl-txf	p 92
Triptolide	NF-κB inhibitor	10 - 100 nM	1 mg	tlrl-tpl	p 92
U0126	MEK1-MEK2 inhibitor	10 - 50 µM	5 mg	tlrl-u0126	p 92
Wortmannin	PI3K inhibitor / Autophagy inhibitor	0.1 - 2.5 µM	5 mg	tlrl-wtm	p 92
Z-VAD-FMK	Caspase inhibitor	10 µg/ml (20 µM)	1 mg	tlrl-vad	p 92

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) and contributes to the induction of type I interferons, such as IFN-β, which can further increase its expression[1]. PKR plays also a role in TLR-induced antiviral activities as an intermediary in TLR3, TLR4 and TLR9 signaling[2].

3-Methyladenine - PI3K Inhibitor / Autophagy Inhibitor

3-Methyladenine (3-MA) is commonly used as a specific inhibitor of autophagic sequestration[3]. It blocks autophagy by inhibition of phosphatidylinositol 3-kinase (PI3K) activity, an enzyme required for autophagy[4]. 3-MA supresses autophagy under starvation conditions but is also able to promote autophagy under nutritient-rich conditions through its its differential temporal effects on class I and class III PI3K[5].

AG490 - JAK2 Inhibitor

AG490 is a specific and potent inhibitor of the Janus kinase 2 protein (JAK2)[6]. 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[7] and TLR5-mediated responses to flagellin[8].

Bafilomycin A1 - V-ATPase Inhibitor / Autophagy 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 fusion of autophagosomes with both endosomes and lysosomes[9,10].

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[11]. TNF-α-dependent effects of NF-κB are important for TLR expression and cytokine production[12]. 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[13].

BX795 - TBK/IKKε Inhibitor

BX795, an aminopyrimidine compound, was developed as an inhibitor of 3-phosphoinositide-dependent kinase 1 (PDK1)[14]. 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[15]. BX795 inhibits the catalytic activity of TBK1/IKKε by blocking their phosphorylation.

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[16].

Chloroquine - Inhibitor of Endosomal Acidification

Chloroquine is a lysosomotropic agent that prevents endosomal acidification[17]. It accumulates inside the acidic parts of the cell, including endosomes and lysosomes. This accumulation leads to 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[18].

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[19]. 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[20].

Cyclosporin A - Calcineurin Inhibitor

Cyclosporin A, a calcineurin inhibitor, exerts its immunosuppressive effects through the down-regulation of NFAT (nuclear factor of activated T cells), thus preventing the transcription of T cell effector cytokines. NFAT has been implicated in the downstream signaling of Dectin-1[21]. Conversely, it has been demonstrated that the inhibition of calcineurin in macrophages can trigger TLR signaling and enhance NF-κB activation[22].

Dexamethasone - NF-κB & MAPK Inhibitor **NEW**

Dexamethasone is a synthetic glucocorticoid compound with potent anti-inflammatory activities. It represses 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[23].

Gefitinib - RIP2 Tyrosine Kinase Inhibitor

Gefitinib (also known as IRESSA) is a selective inhibitor of epidermal growth factor (EGFR), 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 phosphylation which is critical for activation of NOD2 downsream signaling pathways[24].

Glybenclamide (glyburide) - Proton Pump Inhibitor

Glybenclamide, also known as glyburide, blocks the maturation of caspase-1 and pro-IL-1β by inhibiting the K^+ efflux[25]. Glybenclamide was shown to potently block the activation of the NLRP3 inflammasome induced by PAMPs, DAMPs and crystalline substances[26,27]. Recent data suggest that glybenclamide works downstream of the $P2X_7$ receptor but upstream of NLRP3[26].

H-89 - PKA Inhibitor

H-89 is a selective, potent, cell-permeable inhibitor of cAMP-dependent protein kinase (PKA)[28]. 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[29].

Leptomycin B - Nuclear Export Inhibitor

Leptomycin B, an inhibitor of nuclear export, can be used to study nucleo-cytoplasmic translocation. It has been demonstrated that Leptomycin B can provoke the nuclear accumulation of proteins that shuttle between the cytosol and nucleus such as MAPK, MAPKAP kinase 2, IRAK-1 and NLRC5[30]. The cellular target of Leptomycin B has been identified as CRM1 (exportin 1), an evolutionarily conserved receptor for the nuclear export signal of proteins.

LL-37 - Antimicrobial Peptide **NEW**

LL-37, also known as hCAP18, is the C-terminal part of the only human cathelicidin identified to date called human cationic antimicrobial protein (hCAP). LL-37 exhibits a variety of immunomodulatory functions such as bactericidal action, chemotaxis, activation of chemokine secretion and antisepsis effect[31]. The synthetic LL-37 peptide has been shown to suppress the inflammatory response induced by LPS and other TLR ligands[32].

LY294002 - PI3K Inhibitor / Autophagy Inhibitor

LY294002 is a potent, cell-permeable inhibitor of phosphatidylinositol 3-kinase (PI3K) that acts on the ATP binding site of the enzyme[33]. The PI3K pathway is extensively studied for its property in inhibiting apoptosis[34]. PI3K is also known to regulate TLR-mediated inflammatory responses[35]. LY294002 is often used to study the role of PI3K in autophagy[4]

MG-132 - 26S Proteasome Inhibitor **NEW**

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[36].

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[37].

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). 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. MAPK ERK1/2 is involved in TLR-induced production of cytokines[38].

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)[39] preceeded by a protein transduction sequence (RQIKIWFQNRMKWKK) derived from antennapedia which enables the peptide to translocate through the cell membrane[40]. Pepinh-MYD is provided with a control peptide.

Pepinh-TRAM - TRAM inhibitory peptide **NEW**

Pepinh-TRAM is a 30 aa peptide comprised of 14 aa that corresponds to the BB loop of TRAM (IVFAEMPCGRLHLQ) and the cell-penetrating cationic peptide from antennapedia (RQIKIWFQNRMKWKK)[41]. TRAM is an adapter that interacts with TLR4 and TRIF in the MyD88-independent pathway. Pepinh-TRAM is equally effective in inhibiting both arms of the TLR4 signaling pathway[41].

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)[41] linked to the cell-penetrating segment of the antennapedia homoedomain (RQIKIWFQNRMKWKK)[40]. Pepinh-TRIF is provided with a control peptide.

Piceatannol - Syk Inhibitor **NEW**

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[42].

PMA - PKC Activator

Phorbol 12-myristate 13-acetate (PMA) is a specific activator of Protein Kinase C (PKC) and hence of NF-κB. PMA is the most commonly used phorbol ester. It is active at nM concentrations. PMA causes an extremely wide range of effects in cells and tissues, and is a very potent mouse skin tumor promoter[43].

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. The neutralizing effect of PMB on LPS is dose-related and specific for LPS[44]. PMB is widely used to eliminate the effects of endotoxin contamination, both *in vitro* and *in vivo*.

Rapamycin - mTOR Inhibitor / Autophagy Inducer

Rapamycin is a known antifungal and immunosuppressant agent. Its target is the Ser/Thr protein kinase named "mammalian target of rapamycin" (mTOR) that regulates cell growth and metabolism in response to environmental cues. Through inhibition of mTOR, rapamycin induces autophagy[45]. It has also been reported to interfere with TLR signaling[46].

Resveratrol - NF-κB Inhibitor **NEW**

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[46], block TRIF-dependent signaling pathway of TLR3 and TLR4[47] and modulate bacterial phagocytosis of macrophages[48]. Resveratrol may exert these diverse effects by suppressing NF-κB activation.

SB202190 - p38/RK MAP Kinase Inhibitor **NEW**

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)[50]. Further, SB202190 was shown to induce autophagic vacuoles through cross-inhibition of the PI3K/mTOR pathway[51].

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[52]. SB203580 also inhibits the phosphorylation and activation of protein kinase B (PKB, also known as Akt)[53]. Both kinases are involved in a wide array of signaling pathways, including the TLR signaling pathway.

SP600125 - JNK Inhibitor

SP600125 is a potent, cell-permeable, selective and reversible inhibitor of c-Jun N-terminal kinase (JNK)[54]. 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.

Tamoxifen - Estrogen Receptor Antagonist / Autophagy Inducer

Tamoxifen is an antagonist of the estrogen receptor known to induce autophagy and cell death[55]. Tamoxifen stimulates autophagy by increasing the intracellular level of ceramide and abolishing the inhibitory effect of the class-I PI3K pathway on autophagy[56].

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. Triptolide interferes with a number of transcription factors, such as NF-κB[57], NFAT[57] and HSF-1[58]. Recently, triptolide was shown to inhibit global gene trancription by inducing degradation of RNA polymerase 2[59].

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[60]. MAPK p42/p44 are involved in the signaling cascade triggered by LPS and other ligands through stimulation of the TLRs.

Wortmannin - PI3K Inhibitor / Autophagy Inhibitor

Wortmannin is a cell-permeable, fungal metabolite that acts as a potent, selective and irrevesible inhibitor of phosphatidylinositol 3-kinase (PI3K)[61]. Wortmannin has been used to determine the involvement of PI3K in many cellular processes, such as apoptosis[62], autophagy[4] and TLR signaling[63].

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[64]. 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[27].

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Recent articles using InvivoGen's immunomodulators

BAY11-7082, LY294002 & SB203580 - Ding J. & Chang TL., 2012. TLR2 activation enhances HIV nuclear import and infection through T cell activation-independent and -dependent pathways. J Immunol. 88(3):992-1001.

BAY11-7082 - Subbaramaiah K. *et al.*, 2011. Obesity is associated with inflammation and elevated aromatase expression in the mouse mammary gland. Cancer Prevention Research 4: 329 - 346.

LY294002, PD98059 & SB203580 - Nasso M. *et al.*, 2009. Genetically detoxified Pertussis toxin Induces Th1/Th17 immune response through MAPKs and IL-10-dependent mechanisms. J. Immunol. 183: 1892 - 1899.

Triptolide - Robins S. *et al.*, 2011. Steroid-insensitive ERK1/2 activity drives CXCL8 synthesis and neutrophilia by airway smooth muscle. Am J Respir Cell Mol Biol.45(5):984-90.

AG490 - Rinaldi CR. *et al.*, 2010. Preferential nuclear accumulation of JAK2V617F in CD34+ but not in granulocytic, megakaryocytic, or erythroid cells of patients with Philadelphia-negative myeloproliferative neoplasia. Blood 116: 6023 - 6026.

OxPAPC - Chahine MN. *et al.*, 2011. Oxidized LDL promotes the mitogenic actions of Chlamydia pneumoniae in vascular smooth muscle cells. Cardiovasc Res. 92(3):476-83.

Short Hairpin RNAs - shRNAs targeting PRR & Related Genes

RNA interference using small interfering RNA (siRNA) or short-hairpin RNA (shRNA) has become a common technique for gene silencing studies. InvivoGen provides an extensive list of plasmid-based shRNAs that target genes involved in innate immunity. These plasmid-based shRNAs, called ready-made psiRNA, are useful to study the role of these target genes in innate immunity.

Description

Ready-made psiRNA is a family of plasmids expressing a growing list of shRNAs whose 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%.

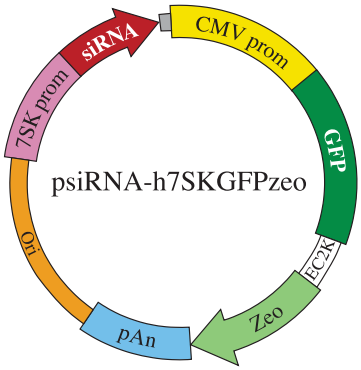
Ready-made psiRNAs are psiRNA-h7SKGFPzeo-derived plasmids that express high amounts of shRNAs through the human 7SK RNA Pol III promoter. They feature a GFP::Zeo fusion gene which confers both reporter and antibiotic resistance activities allowing simple monitoring of transfection efficiency and selection with Zeocin™ in both *E. coli* and mammalian cells.

The silencing efficiency of each Ready-made psiRNA plasmid has been tested using the psiTEST system (see Catalog 2 - Mammalian Cell Expression). The genes or fragments of the genes targeted by the Ready-made psiRNA have been fused to the 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.
Each **Ready-made psiRNA kit** contains the following components:
- 20 µg of the ready-made psiRNA plasmid of your choice
- 20 µg of a control psiRNA plasmid targeting Luciferase GL3
- 1 vial of LyoComp GT116 (*E. coli* competent cells)
- 4 pouches of Fast-Media® Zeo

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



Related Products

Zeocin™, page 61

Fast-Media® Zeo, page 22

GENE NAME	CAT. CODE* (human)	CAT. CODE* (mouse)
Toll-Like Receptors (TLRs)		
TLR1	psirna42-htlr1	psirna42-mtlr1
TLR2	psirna42-htlr2	psirna42-mtlr2
TLR3	psirna42-htlr3	psirna42-mtlr3
TLR4	psirna42-htlr4	psirna42-mtlr4
TLR5	psirna42-htlr5	psirna42-mtlr5
TLR6	psirna42-htlr6	psirna42-mtlr6
TLR7	psirna42-htlr7	psirna42-mtlr7
TLR8	psirna42-htlr8	psirna42-mtlr8
TLR9	psirna42-htlr9	psirna42-mtlr9
TLR10	psirna42-htlr10	-
NOD-Like Receptors (NLRs)		
IPAF / CARD12	psirna42-hipaf	-
NAIP5 / BIRC1E	psirna42-mnaip5	-
NALP1 / CARD7	psirna42-hnalp1	-
NALP2 / PAN1	psirna42-hnalp2	-
NALP3 / NLRP3	psirna42-hnalp3	psirna42-mnalp3
NALP11 / NLRP11	psirna42-hnalp11	-
NALP12 / Monarch1	psirna42-hnalp12	-
NOD1 / CARD4	psirna42-hnod1	psirna42-mnod1
NOD2 / CARD15	psirna42-hnod2	psirna42-mnod2
NOD9 / NLRX1	psirna42-hnod9	psirna42-mnod9
RIG-I-Like Receptors (RLRs) & DNA Sensors		
AIM2 / IFI210	psirna42-haim2	psirna42-maim2
DAI / ZBP1	psirna42-hdai	-
IFI16	psirna42-hifi16	-
LGP2 / DHX58	psirna42-hlgp2	psirna42-mlgp2
MDA5 / IFIH1	psirna42-hmda5	psirna42-mmda5
RIG-I / DDX58	psirna42-hrigi	psirna42-mrigi
C-Type Lectin Receptors (CLRs)		
DC-SIGN / CD209	psirna42-hdcsign	psirna42-mdcsign
Dectin-1	psirna42-hdectin1	psirna42-mdectin1
MBL2	psirna42-hmbl2	-
Mincle	psirna42-hmincle	psirna42-mmincle
SIGNR1	-	psirna42-msignr1
SIGNR2	-	psirna42-msignr2
SIGNR3	-	psirna42-msignr3
Co-Receptors		
CD14	psirna42-hcd14	psirna42-mcd14
CD36	psirna42-hcd36	psirna42-mcd36
MD2	psirna42-hmd2	-

GENE NAME	CAT. CODE * (human)	CAT. CODE* (mouse)
Adaptors		
ASC / CARD5	psirna42-hasc	psirna42-masc
CARD8 / Cardinal	psirna42-hcard8	-
IPS-1 / MAVS	psirna42-hips1	psirna42-mips1
MyD88	psirna42-hmyd88	psirna42-mmyd88
RAC1	psirna42-hrac1	psirna42-mrac1
SARM1	psirna42-hsarm1	-
TICAM1 / TRIF	psirna42-hticam1	psirna42-mticam1
TICAM2 / TRAM	psirna42-hticam2	psirna42-mticam2
TIRAP / Mal	psirna42-htirap	psirna42-mtirap
Signaling Effectors		
BCL10 / CLAP	psirna42-hbcl10	psirna42-mbcl10
CARD9	psirna42-hcard9	psirna42-mcard9
CD44	psirna42-hcd44	psirna42-mcd44
DDX3 / DDX3X	psirna42-hddx3x	-
FADD / MORT1	psirna42-hfadd	psirna42-mfadd
IKKε / IKBKE	psirna42-hikke	psirna42-mikke
IRAK-1	psirna42-hirak1	psirna42-mirak1
IRAK-4	psirna42-hirak4	psirna42-mirak4
LRRFIP2	psirna42-hlrrfip2	-
NAP1 / AZI2	psirna42-hnap1	psirna42-mnap1
Pannexin 1 / PANX1	psirna42-hpanx1	psirna42-mpanx1
Pellino1 / PELI1	psirna42-hpellino1	psirna42-mpellino1
Pellino2 / PELI2	psirna42-hpellino2	psirna42-mpellino2
Pellino3 / PELI3	psirna42-hpellino3	-
PKD1 / PKRD1	psirna42-hpkd1	-
PKR / EIF2AK2	psirna42-hpkr	-
PRKRA / PACT	psirna42-hpact	psirna42-mpact

GENE NAME	CAT. CODE* (human)	CAT. CODE* (mouse)
Signaling Effectors		
RIP1 / RIPK1	psirna42-hrip1	psirna42-mrip1
RIP2 / RIPK2	psirna42-mrip2	psirna42-mrip2
STING	psirna42-hsting	psirna42-msting
SUGT1	psirna42-hsugt1	-
TAK1 / MAP3K7	psirna42-htak1	psirna42-mtak1
TANK	psirna42-htank	psirna42-mtank
TBK1	psirna42-htbk1	psirna42-mtbk1
TRADD	psirna42-htradd	psirna42-mtradd
TRAF3 / CAP-1	psirna42-htraf3	psirna42-mtraf3
TRAF6 / RNF85	psirna42-htraf6	psirna42-mtraf6
Signaling Inhibitors		
A20 / TNFAIP3	psirna42-ha20	psirna42-ma20
ATF3	psirna42-hatf3	-
Bcl-3	psirna42-hbcl3	psirna42-mbcl3
DAK	psirna42-hdak	psirna42-mdak
DUBA	-	psirna42-mduba
FLII / Fliih	psirna42-hflii	-
IRAK-M	psirna42-hirakm	-
MULAN / Dublin	psirna42-hmul1	psirna42-mmul1
PIN1 / DOB	psirna42-hpin1	psirna42-mpin1
RNF125 / TRAC-1	psirna42-hrnf125	psirna42-mrnf125
RP105 / CD180	psirna42-hrp105	-
SIGIRR / TIR8		psirna42-msigirr
SIKE	psirna42-hsike	psirna42-msike
ST2 / IL1RL1 / T1	psirna42-hst2	-
Tollip / IL-1RAcPIP	psirna42-htollip	-
TRAFD1 / FLN29	psirna42-htrafd1	-

* For the kit catalog code, replace psirna42 by ksirna42

More Ready-Made psiRNA available on our website

Articles using Ready-made psiRNA plasmids

psiRNA-hTLR1 & psiRNA-hTLR6 - Turner JD. *et al.*, 2009. Wolbachia lipoprotein stimulates innate and adaptive immunity through Toll-like receptors 2 and 6 to induce disease manifestations of filariasis. J Biol Chem. 284(33):22364-78.

psiRNA-hTLR2 - Yang CS. *et al.*, 2009. NADPH oxidase 2 interaction with TLR2 Is required for efficient innate immune responses to mycobacteria via cathelicidin expression. J. Immunol. 182(6):3696-705.

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-mTLR2 - Chen K. *et al.*, 2006. Activation of Toll-like receptor 2 on microglia promotes cell uptake of Alzheimer disease-associated amyloid beta peptide. J. Biol. Chem. 281(6):3651-9.

psiRNA-mTLR3 & psiRNA-mTLR7 - Al-Salleeh F. & Petro TM., 2008. Promoter analysis reveals critical roles for SMAD-3 and ATF-2 in expression of IL-23 p19 in macrophages. J. Immunol. 181: 4523 - 4533.

psiRNA-hTLR3 & psiRNA-hMDA5 - Tormo D. *et al.*, 2009. Targeted activation of innate immunity for therapeutic induction of autophagy and apoptosis in melanoma cells. Cancer Cell. 16:103-14.

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.

psiRNA-hMyD88 - Bhattacharyya S. *et al.*, 2008. Toll-like receptor 4 mediates induction of the Bcl10-NFkB-interleukin-8 inflammatory pathway by carrageenan in human intestinal epithelial cells. J. Biol. Chem. 283(16):10550-8.

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-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.

Recombinant Human Cytokines

InvivoGen provides a selection of cytokines produced by recombinant DNA technology. They are fully proficient in inducing their cognate signaling pathway in susceptible cell lines, such as the HEK-Blue™ Cytokine Cells.

Recombinant human CD40L

CD40 ligand, CD40L (also known as CD154, TRAP or gp39), is a type II transmembrane glycoprotein belonging to the TNF family. CD40L is predominantly expressed on activated $CD4^+$ T lymphocytes. CD40L binds to CD40 present on B cells activating resting B cells[1]. Recombinant human CD40L is a 149 amino acid, non-glycosylated protein with a molecular mass of 16 kDa that is produced in *Escherichia coli*.

Recombinant human IFNγ

Interferon-γ (IFNγ, also known as Type II interferon) is a cytokine produced primarily by T-lymphocytes and natural killer (NK) cells. IFNγ has antiviral, immunoregulatory and anti-tumor properties. IFNγ induces the production of cytokines, modulates macrophage effector functions, and influences isotype switching[2]. Recombinant human IFNγ is a 144 amino acid non-glycosylated, polypeptide chain with a molecular mass of 17 kDa that is produced in *Escherichia coli.*

Recombinant human IL-1β

Interleukin-1beta (IL-1β) is produced mainly by monocytes and activated macrophages, as a proprotein which is proteolytically processed to its active form by caspase-1. IL-1β is a potent pro-inflammatory cytokine[3] and is also involved in cell proliferation, differentiation, and apoptosis. Recombinant human IL-1β is a 153 amino acid non-glycosylated polypeptide chain with a molecular mass of 17 kDa that is produced in *Escherichia coli.*

Recombinant human IL-4

Interleukin-4 (IL-4) is a key regulator of the adaptive and innate immune system. IL-4 plays an important role in cellular differentiation, including the polarization of Th2 cells from naïve CD4+ T cells and the generation immature dendritic cells from monocytes[4]. Recombinant human IL-4 is a 130 amino acid non-glycosylated, polypeptide chain with a molecular weight of 30 kDa that is produced in *Pichia*.

Recombinant human IL-6

Interleukin-6 (IL-6), which was originally identified as a B-cell differentiation factor, is a multifunctional cytokine that regulates the immune response, haemopoiesis, the acute phase response and inflammation[5]. Recombinant human IL-6 is a 185 amino acid non-glycosylated, polypeptide chain with a molecular weight of 21 kDa that is produced in *Escherichia coli*.

Recombinant human IL-13

Interleukin-13 (IL-13) is a Th2-type cytokine secreted from $CD4^+$ T cells, mast cells, basophils and eosinophils[4]. IL-13 is a key mediator in the pathogenesis of allergic inflammation. The functions of IL-13 overlap considerably with those of the closely related cytokine IL-4. Recombinant human IL-13 is a single, non-glycosylated, 112 amino acid protein with a molecular mass of 12 kDa that is produced in *Escherichia coli.*

Recombinant human IL-18 NEW

Interleukin-18 (IL-18) is a key pro-inflammatory cytokine and an important mediator of Th1 immune response[3]. IL-18 is produced by macrophages and other cells, as a proprotein which is proteolytically processed to its active form by caspase-1. Recombinant human IL-18 is a 157 amino acid protein with a molecular mass of 18 kDa that is produced in *Escherichia coli.*

Recombinant human IL-33 NEW

Interleukin 33 (IL-33) is a pro-inflammatory protein that induces helper T cells, mast cells, eosinophils and basophils to produce type 2 cytokines[3]. Similar to IL-1β and IL-18, IL-33 is produced as a proprotein which is proteolytically processed to its active form. Recombinant human IL-33 is a 159 amino acid protein with a molecular mass of 18 kDa that is produced in *Escherichia coli.*

Recombinant human TNF-α

Tumor necrosis factor-alpha (TNF-α) is a cytokine that plays a role in a variety of biological processes including cell proliferation, differentiation and apoptosis. It functions by activating transcription factors such as NF-κB and AP-1[6]. TNF-α consists of three identical polypeptide chains of 157 amino acids combined to form a homotrimer with subunit mass of 17 kDa. InvivoGen provides a recombinant human TNF-α produced in CHO cells and purified by affinity chromatography.

1. Bolduc A. *et al.*, 2010. Constitutive CD40L expression on B cells prematurely terminates germinal center response and leads to augmented plasma cell production in T cell areas. J Immunol. 185:220-30. **2. McCall M. & Sauerwein R., 2010.** Interferon-central mediator of protective immune responses against the pre-erythrocytic and blood stage of malaria. J. Leukoc. Biol. 88:1131-1143. **3. Arend W. *et al.*, 2008.** IL-1, IL-18, and IL-33 families of cytokines. Immunol Rev. 223:20-38. **4. Oliphant C. *et al.*, 2011.** Insights into the initiation of type 2 immune responses. Immunology. 134(4):378-85. **5. Scheller J. *et al.*, 2011.** The pro- and anti-inflammatory properties of the cytokine interleukin-6. Biochim Biophys Acta. 1813:878-88. **6. Balkwill F., 1989.** Tumour necrosis factor. Br Med Bull. 1989 Apr;45(2):389-400.

PRODUCT	SOURCE	WORKING CONCENTRATION	QUANTITY	CATALOG CODE
Recombinant human CD40L	*E. coli*	5 ng - 1 µg/ml	10 µg	rhcd-40l
Recombinant human IFNγ	*E. coli*	10 - 10^4 IU/ml	20 µg (4 x 10^5 IU)	rhifn-g
Recombinant human IL-1β	*E. coli*	0.2 - 100 ng/ml	10 µg	rhil-1b
Recombinant human IL-4	*Pichia*	0.5 - 100 ng/ml	10 µg	rhil-4
Recombinant human IL-6	*E. coli*	0.5 - 50 ng/ml	10 µg	rhil-6
Recombinant human IL-13	*E. coli*	5 - 1000 ng/ml	10 µg	rhil-13
Recombinant human IL-18	*E. coli*	5 - 1000 ng/ml	10 µg	rhil-18
Recombinant human IL-33	*E. coli*	5 - 1000 ng/ml	10 µg	rhil-33
Recombinant human TNF-α	CHO	0.5 - 1000 ng/ml	20 µg	rhtnf-a

Contents and Storage

Recombinant cytokines are provided as sterile lyophilized powder, endotoxin-free. Products are shipped at room temperature. Store at -20°C.

Related Products

Cytokine Antibodies, page 99
Cytokine Reporter Cells, page 44

6 ANTIBODIES

Antibodies for Detection 98

Antibodies for Neutralization 99

ANTIBODIES

InvivoGen provides a collection of monoclonal and polyclonal antibodies targeting human and murine TLRs. Most of them have been developed in house. They are listed below and classified according to their application(s):

➢ **Antibodies for neutralization.** Some of them can be used also for flow cytometry

➢ **Antibodies for detection.** The applications include Western blotting, immunoprecipitation, immunohistochemistry and 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.

Antibodies for Detection

TARGET	ANTIBODY	DESCRIPTION	SPECIFICITY	APPLICATIONS	QTY	CAT. CODE
CD14	Anti-hCD14-IgA	Monoclonal human IgA2	Human CD14	FC, Neutralization	100 μg	maba-hcd14
CD20	Anti-hCD20-hIgG1	Monoclonal human IgG1	Human CD20	FC, Neutralization	100 μg	hcd20-mab1
Dectin-1	MAb-mDectin-1	Monoclonal mouse IgG2b (clone 2A11)	Mouse Dectin-1	FC, Neutralization	100 μg	mab-mdect
FliC	Anti-Flagellin FliC	Monoclonal mouse IgG1	*S. typhimurium* flagellin	WB	100 μg	mabg-flic
HA Tag	Anti-HA Tag	Monoclonal mouse IgG1	Hemagglutinin epitope	WB, IP	250 μl	ab-hatag
TLR1	MAb-hTLR1	Monoclonal mouse IgG1 (GD2.F4)	Human TLR1	FC	100 μg	mab-htlr1
TLR1	MAb-hTLR1-FITC	Monoclonal mouse IgG1 (GD2.F4), FITC	Human TLR1	FC	100 μg	mab-htlr1f
TLR2	Anti-hTLR2-IgA	Monoclonal human IgA2	Human TLR2	FC, Neutralization	100 μg	maba2-htlr2
TLR2	MAb-hTLR2	Monoclonal mouse IgG2a (TL2.1)	Human TLR2	FC, IHC, WB	100 μg	mab-htlr2
TLR2	MAb-hTLR2-FITC	Monoclonal mouse IgG2a (TL2.1), FITC	Human TLR2	FC, IHC	100 μg	mab-htlr2f
TLR2	MAb-mTLR2	Monoclonal mouse IgG1 (T2.5)	Human/mouse TLR2	FC, IHC, Neutralization	100 μg	mab-mtlr2
TLR2	MAb-mTLR2-FITC	Monoclonal mouse IgG1 (T2.5), FITC	Human/mouse TLR2	FC	100 μg	mab-mtlr2f
TLR3	Anti-hTLR3-IgA	Monoclonal human IgA2	Human TLR3	FC	100 μg	maba-htlr3
TLR3	MAb-hTLR3	Monoclonal mouse IgG1 (TLR3.7)	Human TLR3	FC, WB	100 μg	mab-htlr3
TLR3	MAb-hTLR3-FITC	Monoclonal mouse IgG1 (TLR3.7), FITC	Human TLR3	FC, WB	100 μg	mab-htlr3f
TLR4	MAb-hTLR4	Monoclonal mouse IgG2a (HTA125)	Human/monkey TLR4	FC, IHC	100 μg	mab-htlr4
TLR4	MAb-hTLR4-FITC	Monoclonal mouse IgG2a (HTA125), FITC	Human/monkey TLR4	FC	100 μg	mab-htlr4f
TLR4	MAb-mTLR4/MD2	Monoclonal rat IgG2a (MTS510)	Mouse TLR4/MD2	FC, IHC	100 μg	mab-mtlr4md2
TLR4	MAb-mTLR4/MD2-FITC	Monoclonal rat IgG2a (MTS510), FITC	Mouse TLR4/MD2	FC	100 μg	mab-mtlr4md2f
TLR5	Anti-hTLR5-IgA	Monoclonal human IgA2	Human TLR5	FC, Neutralization	100 μg	maba2-htlr5
TLR9	MAb-mTLR9	Monoclonal mouse IgG2a (5G5)	Human/mouse TLR9	FC, IHC, WB	100 μg	mab-mtlr9
TLR9	MAb-mTLR9-FITC	Monoclonal mouse IgG2a (5G5), FITC	Human/mouse TLR9	FC	100 μg	mab-mtlr9f

Antibodies for Neutralization

TARGET	ANTIBODY	DESCRIPTION	SPECIFICITY	APPLICATIONS	QTY	CAT. CODE
CD14	Anti-hCD14-IgA	Monoclonal human IgA2	Human CD14	Neutralization, FC	100 µg	maba-hcd14
CD20	Anti-hCD20-hIgG1	Monoclonal human IgG1	Human CD20	Neutralization, FC	100 µg	hcd20-mab1
CD40L	Anti-hCD40L-hIgA2	Monoclonal human IgA2	Human CD40L	Neutralization	100 µg	maba-h40l
Dectin-1	MAb-mDectin-1	Monoclonal mouse IgG2b (clone 2A11)	Mouse Dectin-1	Neutralization, FC	100 µg	mab-mdect
IFN-α	Anti-hIFNα-IgA2	Monoclonal human IgA2	Human Interferon α	Neutralization	100 µg	maba-hifna
IFN-γ	Anti-hIFNγ-IgA2	Monoclonal human IgA2	Human Interferon γ	Neutralization	100 µg	maba-hifng
IL-1β	Anti-hIL-1β-IgA2	Monoclonal human IgA2	Human Interleukin 1β	Neutralization	100 µg	maba-hil1b
IL-4	Anti-hIL-4-IgA2	Monoclonal human IgA2	Human Interleukin 4	Neutralization	100 µg	maba-hil4
IL-6	Anti-hIL-6-IgA2	Monoclonal human IgA2	Human Interleukin 6	Neutralization	100 µg	maba-hil6
IL-13	Anti-hIL-13-IgA2	Monoclonal human IgA2	Human Interleukin 13	Neutralization	100 µg	maba-hil13
IL-18	Anti-hIL-18-IgA2	Monoclonal human IgA2	Human Interleukin 18	Neutralization	100 µg	maba-hil18
TGF-β	Anti-hTGFβ-IgA2	Monoclonal human IgA2	Human TGF-beta	Neutralization	100 µg	maba-htgfb
TLR1	Anti-hTLR1-IgG	Monoclonal mouse IgG1 (H2G2)	Human TLR1	Neutralization	100 µg	mabg-htlr1
TLR1	PAb-hTLR1	Polyclonal rat IgG	Human TLR1	Neutralization	200 µg	pab-hstlr1
TLR2	Anti-hTLR2-IgA	Monoclonal human IgA2	Human TLR2	Neutralization, FC	100 µg	maba2-htlr2
TLR2	Anti-mTLR2-IgG	Monoclonal mouse IgG2a (C9A12)	Mouse TLR2	Neutralization	100 µg	mabg-mtlr2
TLR2	MAb-mTLR2	Monoclonal mouse IgG1 (T2.5)	Human/mouse TLR2	Neutralization·FC, IHC	100 µg	mab-mtlr2
TLR2	PAb-hTLR2	Polyclonal rat IgG	Human TLR2	Neutralization	200 µg	pab-hstlr2
TLR4	Anti-hTLR4-IgG	Monoclonal mouse IgG1	Human TLR4	Neutralization	100 µg	mabg-htlr4
TLR4	PAb-hTLR4	Polyclonal rat IgG	Human TLR4	Neutralization	200 µg	pab-hstlr4
TLR5	Anti-hTLR5-IgA	Monoclonal human IgA2	Human TLR5	Neutralization, FC	100 µg	maba2-htlr5
TLR5	Anti-mTLR5-IgG	Monoclonal rat IgG2a (Q23D11)	Mouse TLR5	Neutralization	100 µg	mabg-mtlr5
TLR5	PAb-hTLR5	Polyclonal rat IgG	Human TLR5	Neutralization	200 µg	pab-hstlr5
TLR6	Anti-hTLR6-IgG	Monoclonal mouse IgG1 (C5C8)	Human TLR6	Neutralization	100 µg	mabg-htlr6
TLR6	PAb-hTLR6	Polyclonal rat IgG	Human TLR6	Neutralization	200 µg	pab-hstlr6
TNF-α	Anti-hTNF-α-hIgG1	Monoclonal human IgG1	Human TNF-alpha	Neutralization	100 µg	htnfa-mab1

Secondary Antibodies and Controls

TARGET	ANTIBODY	DESCRIPTION	APPLICATIONS	QTY	CAT. CODE
IgA	Goat F(ab')2 Anti-Human IgA - Biotin	Pepsin digest of goat anti-human IgA - biotin-labelled	ELISA, WB, IHC	500 µg	chiga-biot
IgA	Goat F(ab')2 Anti-Human IgA - FITC	Pepsin digest of goat anti-human IgA - FITC labelled	FC, IHC	500 µg	chiga-fitc
IgG	Goat F(ab')2 IgG Isotype Control - FITC	Pepsin digest of goat anti-human IgG - FITC labelled	FC, ELISA	100 tests	cgig-fitc
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

7 VACCINATION

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VACCINE ADJUVANTS

Adjuvants are essential for enhancing and directing the adaptative immune response to vaccine antigens. This response is mediated by two main types of lymphocytes, B and T cells. Upon activation by cytokines, B cells differentiate into memory B cells (long-lived antigen-specific B cells) or plasma cells (effector B cells that secrete large quantities of antibodies). Most antigens activate B cells using activated T helper (Th) cells, primarily Th1 and Th2 cells. Th1 cells secrete IFN-γ, which activates macrophages and induces the production of opsonizing antibodies by B cells. The Th1 response leads mainly to 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

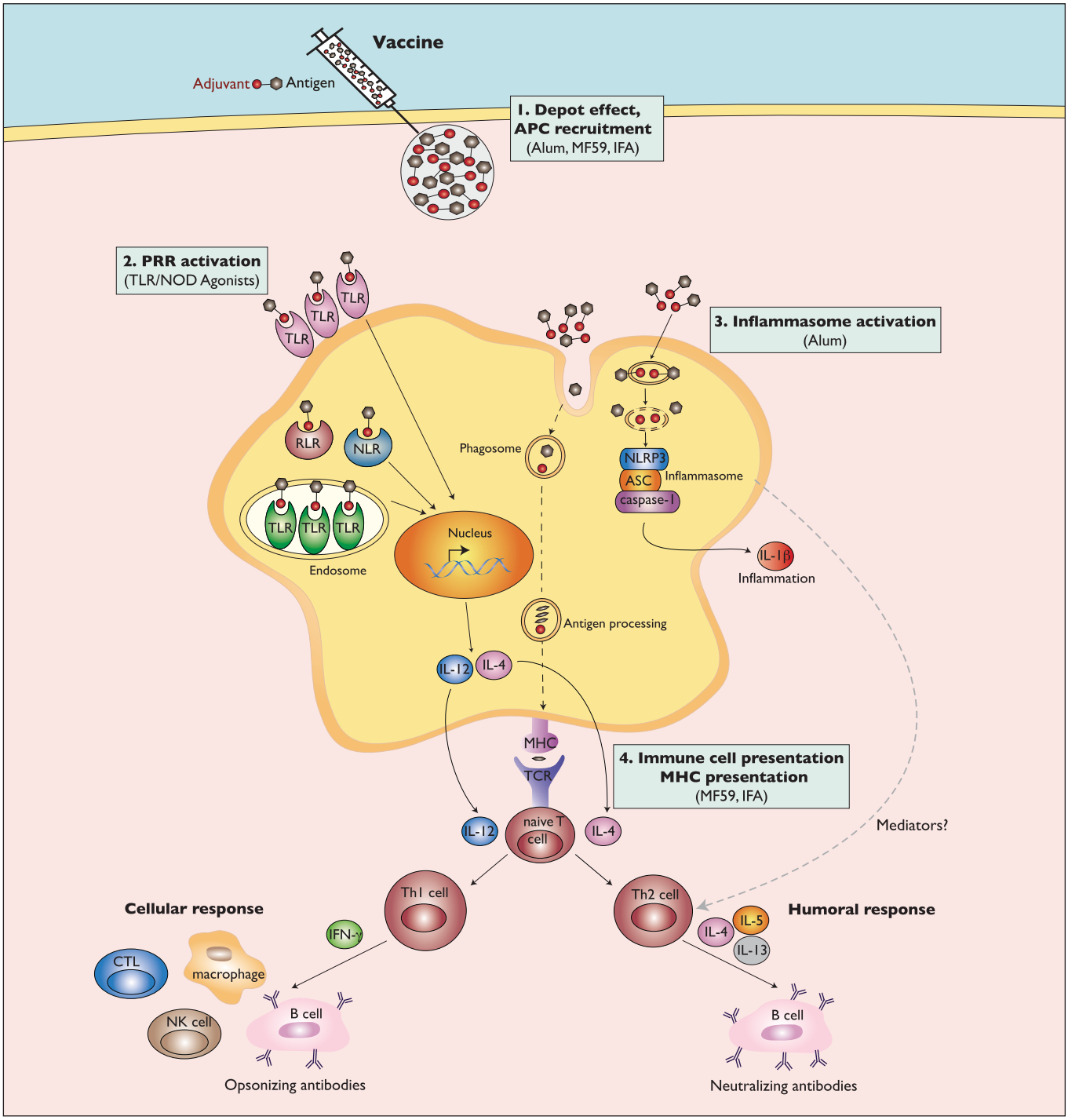
Double-stranded RNA (dsRNA), which is produced during the replication of most viruses, is a potent inducer of innate immunity. Synthetic analogs of dsRNA, such as poly(I:C), have been tested as adjuvants. They act through TLR3 and RIG-I/MDA-5, inducing IL-12 and type I IFNs production, facilitating antigen 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	Various
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)



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 compounds 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. Incorporation of TDB into cationic liposomes composed of DDA produce a potent adjuvant, known as CAF01. 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.

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.

1. Leroux-Roels G., 2010. Unmet needs in modern vaccinology adjuvants to imporve 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.

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, Hookworm, Flu, 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

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

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™ (preclinical grade). They are prepared under strict aseptic conditions and tested for the presence of endotoxins. They are sterile and their endotoxin level is <1 EU/mg.

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					
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™ NEW	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™ NEW	CpG ODN, type A (murine) -TLR9 agonist	Th1	20 - 50 µg/mouse	1 mg	vac-1585-1
ODN 1826 VacciGrade™	CpG ODN, type B (murine) -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
Pam3CSK4 VacciGrade™ NEW	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

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.

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[1]. It has been demonstrated that flagellin can act as a potent adjuvant in flu vaccines[2, 3]. Furthermore, flagellin can also signal through the NLRC4 inflammasome[4], 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[5]. More specifically, R848 (Resiquimod) activates NF-κB and MAP kinase pathways in B cells, thereby promoting the production of antibodies[6]. R848 is a good inducer of IFN-related innate immunity pathways[7].

MPLA and 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 whilst maintaining the immunostimulatory activity[8]. Preclinical studies indicate that MPLA induces a strong Th1 response[9]. 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[10, 11].

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[12]. 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[13]. Furthermore MDP leads to the activation of the NLRP3 inflammasome[14].

ODN 1585, ODN 1826 and ODN 2006 VacciGrade™

Synthetic oligodeoxynucleotides containing unmethylated CpG motifs (CpG ODNs), such as ODN 2006 (also known as ODN 7909), have been extensively studied as adjuvants. CpG ODNs are recognized by TLR9, which is expressed exclusively on human B cells and plasmacytoid dendritic cells (pDCs), thereby inducing Th1-dominated immune responses. Pre-clinical studies conducted in rodents and non-human primates and human clinical trials have demonstrated that CpG ODNs can significantly improve vaccine-specific antibody responses[9]. Clinical data indicate that ODN 2006 is highly effective for enhancing antigen-specific antibody responses against a variety of antigens[15]. An increasing number of preclinical studies report the efficacy of ODN 1826, used alone or in combination with other adjuvants, to enhance the protective immunity of vaccination with diverse antigens[16,17]. 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[18].

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[7]. 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*[19]. 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[6]. 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[6]. Promising results have been obtained using poly(I:C) as an adjuvant in flu vaccine delivered intranasally[5].

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PRODUCT (QUANTITY)	CAT. CODE	PAGE
293/hMD2-CD14 (5-7 x 10^6 cells)	293-hmd2cd14	33
293/hNOD1 (5-7 x 10^6 cells)	293-hnod1	33
293/hNOD2 (5-7 x 10^6 cells)	293-hnod2	33
293/hTLR1-HA (5-7 x 10^6 cells)	293-htlr1ha	33
293/hTLR2 (5-7 x 10^6 cells)	293-htlr2	33
293/hTLR2-HA (5-7 x 10^6 cells)	293-htlr2ha	33
293/hTLR2/6 (5-7 x 10^6 cells)	293-htlr2/6	33
293/hTLR2-CD14 (5-7 x 10^6 cells)	293-htlr2cd14	33
293/hTLR3 (5-7 x 10^6 cells)	293-htlr3	33
293/hTLR3-HA (5-7 x 10^6 cells)	293-htlr3ha	33
293/hTLR4A (5-7 x 10^6 cells)	293-htlr4a	33
293/hTLR4-HA (5-7 x 10^6 cells)	293-htlr4ha	33
293/hTLR4A-MD2-CD14 (5-7 x 10^6 cells)	293-htlr4md2cd14	33
293/hTLR5 (5-7 x 10^6 cells)	293-htlr5	33
293/hTLR5-HA (5-7 x 10^6 cells)	293-htlr5ha	33
293/hTLR5-CD14 (5-7 x 10^6 cells)	293-htlr5cd14	33
293/hTLR6-HA (5-7 x 10^6 cells)	293-htlr6ha	33
293/hTLR10-HA (5-7 x 10^6 cells)	293-htlr10ha	33
293/LacZ (5-7 x 10^6 cells)	293-lacz	33
293/mNOD1 (5-7 x 10^6 cells)	293-mnod1	33
293/mNOD2 (5-7 x 10^6 cells)	293-mnod2	33
293/mTLR1 (5-7 x 10^6 cells)	293-mtlr1	33
293/mTLR1/2 (5-7 x 10^6 cells)	293-mtlr1/2	33
293/mTLR2 (5-7 x 10^6 cells)	293-mtlr2	33
293/mTLR2/6 (5-7 x 10^6 cells)	293-mtlr2/6	33
293/mTLR3 (5-7 x 10^6 cells)	293-mtlr3	33
293/mTLR4 (5-7 x 10^6 cells)	293-mtlr4	33
293/mTLR4-MD2-CD14 (5-7 x 10^6 cells)	293-mtlr4md2cd14	33
293/mTLR5 (5-7 x 10^6 cells)	293-mtlr5	33
293/mTLR6 (5-7 x 10^6 cells)	293-mtlr6	33
293/mTLR9 (5-7 x 10^6 cells)	293-mtlr9	33
293/null (5-7 x 10^6 cells)	293-null	33
293XL/hTLR7 (5-7 x 10^6 cells)	293xl-htlr7	33
293XL/hTLR7-HA (5-7 x 10^6 cells)	293xl-htlr7ha	33
293XL/hTLR8 (5-7 x 10^6 cells)	293xl-htlr8	33
293XL/hTLR8-HA (5-7 x 10^6 cells)	293xl-htlr8ha	33
293XL/hTLR9 (5-7 x 10^6 cells)	293xl-htlr9	33
293XL/hTLR9-HA (5-7 x 10^6 cells)	293xl-htlr9ha	33
293XL/mTLR7 (5-7 x 10^6 cells)	293xl-mtlr7	33
293XL/null (5-7 x 10^6 cells)	293xl-null	33
2-Aminopurine (250 mg)	tlrl-apr	90
3-Methyladenine (50 mg)	tlrl-3ma	90
5'ppp-dsRNA (25 µg)	tlrl-3prna	68
5'ppp-dsRNA (100 µg)	tlrl-3prna-100	68
5'ppp-dsRNA Control (25 µg)	tlrl-3prnac	68
5'ppp-dsRNA Control (100 µg)	tlrl-3prnac-100	68
AddaVax™ (2 ml)	vac-adx-2	104

PRODUCT (QUANTITY)	CAT. CODE	PAGE
AddaVax™ (5 x 2 ml)	vac-adx-10	104
AG490 (10 mg)	tlrl-ag4	90
Alhydrogel 2% (50 ml)	vac-alu-50	104
Alhydrogel 2% (250 ml)	vac-alu-250	104
Alum Crystals (1 g)	tlrl-alk	68
Anti-Flagellin FliC (100 µg)	mabg-flic	98
Anti-HA Tag (250 µl)	ab-hatag	98
Anti-hCD14-IgA (100 µg)	maba-hcd14	98
Anti-hCD20-hIgG1 (100 µg)	hcd20-mab1	98
Anti-hCD40L-IgA2 (100 µg)	maba-h40l	99
Anti-hIFN-α-IgA2 (100 µg)	maba-hifna	99
Anti-hIFN-γ-IgA2 (100 µg)	maba-hifng	99
Anti-hIL-1β-IgA2 (100 µg)	maba-hil1b	99
Anti-hIL-4-IgA2 (100 µg)	maba-hil4	99
Anti-hIL-6-IgA2 (100 µg)	maba-hil6	99
Anti-hIL-13-IgA2 (100 µg)	maba-hil13	99
Anti-hIL-18-IgA2 (100 µg)	maba-hil18	99
Anti-hTGFβ-IgA2 (100 µg)	maba-htgfb	99
Anti-hTLR1-IgG (100 µg)	mabg-htlr1	99
Anti-hTLR2-IgA (100 µg)	maba2-htlr2	99
Anti-hTLR3-IgA (100 µg)	maba-htlr3	99
Anti-hTLR4-IgG (100 µg)	mabg-htlr4	99
Anti-hTLR5-IgA (100 µg)	maba2-htlr5	99
Anti-hTLR6-IgG (100 µg)	mabg-htlr6	99
Anti-hTNF-α-hIgG1 (100 µg)	htnfa-mab1	99
Anti-Lucia-IgG (100 µg)	mabg-lucia	54
Anti-mTLR2-IgG (100 µg)	mabg-mtlr2	99
Anti-mTLR5-IgG (100 µg)	mabg-mtlr5	99
ATP (1 g)	tlrl-atp	68
B16-Blue™ IFNα/β Cells (5-7 x 10^6 cells)	bb-ifnab	46
Bafilomycin A1 (10 µg)	tlrl-baf	90
Bay11-7082 (10 mg)	tlrl-b82	90
Blasticidin (100 mg)	ant-bl-1	60
Blasticidin (500 mg)	ant-bl-5	60
Blasticidin (500 mg, bottle)	ant-bl-5b	60
Blasticidin (1 g powder)	ant-bl-10p	60
BX795 (5 mg)	tlrl-bx7	90
C12-iE-DAP (1 mg)	tlrl-c12dap	67
C3H/TLR4mut MEFs (5-7 x 10^6 cells)	mef-c3h4m	41
C3H/WT MEFs (5-7 x 10^6 cells)	mef-c3hwt	41
C57/WT MEFs (5-7 x 10^6 cells)	mef-c57wt	41
Celastrol (1 mg)	ant-cls	90
Chloroquine (250 mg)	tlrl-chq	90
CL075 (500 µg)	tlrl-c75	65
CL075 (5 mg)	tlrl-c75-5	65
CL097 (500 µg)	tlrl-c97	65
CL097 (5 mg)	tlrl-c97-5	65

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CL264 (500 µg)	tlrl-c264s	65
CL264 (5 mg)	tlrl-c264-5	65
CL264 Biotin (100 µg)	tlrl-bc264	65
CL264 FITC (100 µg)	tlrl-fc264	65
CL264 Rhodamine (100 µg)	tlrl-rc264	65
CLI-095 (1 mg)	tlrl-cli95	90
Compound Dose Response	tlrl-test-2	82
Compound Profiling	tlrl-test-1	82
CPPD crystals (5 mg)	tlrl-cppd	68
Curdlan (1 g)	tlrl-curd	68
Cyclosporin A (100 mg)	tlrl-cyca	90
Dexamethasone (100 mg)	tlrl-dex	90
***E. coli* DNA ef** (1 mg)	tlrl-ednaef	66
***E. coli* ssDNA / LyoVec** (200 µg)	tlrl-ssec	66
EndoFit™ Ovalbumin (10 mg)	vac-efova	103
FLA-BS (100 µg)	tlrl-bsfla	65
Flagellin FliC VacciGrade (50 µg)	vac-fla	104
FLA-ST (100 µg)	tlrl-stfla	65
FLA-ST Ultrapure (10 µg)	tlrl-pstfla	65
FLA-ST Ultrapure (50 µg)	tlrl-pstfla5	65
FLA-ST recombinant (1 µg)	tlrl-flic	65
FLA-ST recombinant (10 µg)	tlrl-flic-10	65
FSL-1 (100 µg)	tlrl-fsl	64
G418 (1 g)	ant-gn-1	60
G418 (5 g)	ant-gn-5	60
Gardiquimod (500 µg)	tlrl-gdqs	65
Gardiquimod (5 mg)	tlrl-gdq-5	65
Gardiquimod VacciGrade (5 mg)	vac-gdq	104
Gefitinib (10 mg)	tlrl-gef	90
Glybenclamide (1 g)	tlrl-gly	90
Goat F(ab')2 anti-human IgA - Biotin (0.5 mg)	chiga-biot	99
Goat F(ab')2 anti-human IgA - FITC (0.5 mg)	chiga-fitc	99
Goat F(ab')2 IgG isotype control - FITC (100 tests)	cgig-fitc	99
G-ODN (200 µg)	tlrl-godn	67
H-89 (5 mg)	tlrl-h89	90
HEK-Blue™ Detection (5 pouches)	hb-det2	56
HEK-Blue™ Detection (10 pouches)	hb-det3	56
HEK-Blue™ hMD2-CD14 Cells (5-7 x 10^6 cells)	hkb-hmdcd	34
HEK-Blue™ hNOD1 Cells (5-7 x 10^6 cells)	hkb-hnod1	34
HEK-Blue™ hNOD2 Cells (5-7 x 10^6 cells)	hkb-hnod2	34
HEK-Blue™ hTLR2 Cells (5-7 x 10^6 cells)	hkb-htlr2	34
HEK-Blue™ hTLR3 Cells (5-7 x 10^6 cells)	hkb-htlr3	34
HEK-Blue™ hTLR4 Cells (5-7 x 10^6 cells)	hkb-htlr4	34
HEK-Blue™ hTLR5 Cells (5-7 x 10^6 cells)	hkb-htlr5	34
HEK-Blue™ hTLR7 Cells (5-7 x 10^6 cells)	hkb-htlr7	34
HEK-Blue™ hTLR8 Cells (5-7 x 10^6 cells)	hkb-htlr8	34
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HEK-Blue™ IFN-γ Cells (5-7 x 10^6 cells)	hkb-ifng	47
HEK-Blue™ IL-1β Cells (5-7 x 10^6 cells)	hkb-il1b	43
HEK-Blue™ IL-4/IL-13 Cells (5-7 x 10^6 cells)	hkb-stat6	51
HEK-Blue™ IL-6 Cells (5-7 x 10^6 cells)	hkb-il6	51
HEK-Blue™ IL-18/IL-1β Cells (5-7 x 10^6 cells)	hkb-il18	50
HEK-Blue™ IL-33/IL-1β Cells (5-7 x 10^6 cells)	hkb-il33	50
HEK-Blue™ mNOD1 Cells (5-7 x 10^6 cells)	hkb-mnod1	34
HEK-Blue™ mNOD2 Cells (5-7 x 10^6 cells)	hkb-mnod2	34
HEK-Blue™ mTLR2 Cells (5-7 x 10^6 cells)	hkb-mtlr2	34
HEK-Blue™ mTLR3 Cells (5-7 x 10^6 cells)	hkb-mtlr3	34
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HEK-Blue™ Null1-v Cells (5-7 x 10^6 cells)	hkb-null1v	34
HEK-Blue™ Null2 Cells (5-7 x 10^6 cells)	hkb-null2	34
HEK-Blue™ Null2-k Cells (5-7 x 10^6 cells)	hkb-null2k	34
HEK-Blue™ TNF-α/IL-1β Cells (5-7 x 10^6 cells)	hkb-tnfil1	49
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HEK-Dual™ IFN-γ Cells (5-7 x 10^6 cells)	hkd-ifng	47
HEK-Dual™ TNF-α Cells (5-7 x 10^6 cells)	hkd-tnfa	48
Hemozoin (5 mg)	tlrl-hz	68
HKAL (10^9 cells)	tlrl-hkal	64
HKCA (10^9 cells)	tlrl-hkca	68
HKEB (10^{10} cells)	tlrl-hkeb	64
HKHP (10^9 cells)	tlrl-hkhp	64
HKLM (10^{10} cells)	tlrl-hklm	64
HKLP (10^9 cells)	tlrl-hklp	64
HKLR (10^{10} cells)	tlrl-hklr	64
HKMF (10^9 cells)	tlrl-hkmf	64
HKPA (10^{10} cells)	tlrl-hkpa	64
HKPG (10^{10} cells)	tlrl-hkpg	64
HKSA (10^{10} cells)	tlrl-hksa	64
HKSC (10^9 cells)	tlrl-hksc	68
HKSP (10^{10} cells)	tlrl-hksp	64
Human IgA2 Isotype Control (100 µg)	maba2-ctrl	99
Human MDA-5 RT-Primer Pair (2 x 2.5 nmol)	rtp-hmda5	85
Human NOD1 RT-Primer Pair (2 x 2.5 nmol)	rtp-hnod1	85
Human NOD2 RT-Primer Pair (2 x 2.5 nmol)	rtp-hnod2	85
Human RIG-I RT-Primer Pair (2 x 2.5 nmol)	rtp-hrigi	85
Human TLR1 RT-Primer Pair (2 x 2.5 nmol)	rtp-htlr1	85
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Human TLR3 RT-Primer Pair (2 x 2.5 nmol)	rtp-htlr3	85
Human TLR4 RT-Primer Pair (2 x 2.5 nmol)	rtp-htlr4	85
Human TLR5 RT-Primer Pair (2 x 2.5 nmol)	rtp-htlr5	85
Human TLR6 RT-Primer Pair (2 x 2.5 nmol)	rtp-htlr6	85
Human TLR7 RT-Primer Pair (2 x 2.5 nmol)	rtp-htlr7	85
Human TLR8 RT-Primer Pair (2 x 2.5 nmol)	rtp-htlr8	85
Human TLR9 RT-Primer Pair (2 x 2.5 nmol)	rtp-htlr9	85
HygroGold™ (1 g)	ant-hg-1	61
HygroGold™ (5 g)	ant-hg-5	61
HygroGold™ (10 g powder)	ant-hg-10p	61
Hygromycin B (1 g)	ant-hm-1	61
Hygromycin B (5 g)	ant-hm-5	61
iE-DAP (5 mg)	tlrl-dap	67
iE-Lys (5 mg)	tlrl-lys	67
IFA (10 ml)	vac-ifa-10	104
IFA (6 x 10 ml)	vac-ifa-60	104
Imiquimod (500 µg)	tlrl-imqs	65
Imiquimod (5 mg)	tlrl-imq	65
Imiquimod VacciGrade (5 mg)	vac-imq	104
Jurkat-Dual™ Cells (5-7 x 10^6 cells)	jktd-isnf	40
L18-MDP (1 mg)	tlrl-lmdp	67
LAM-MS (500 µg)	tlrl-lams	64
Leptomycin B (5 µg)	tlrl-lep	90
LL-37 (1 mg)	tlrl-l37	90
LM-MS (250 µg)	tlrl-lmms2	64
Loxoribine (50 mg)	tlrl-lox	65
LPS-EB (5 mg)	tlrl-eblps	64
LPS-EB Biotin (500 µg)	tlrl-bblps	64
LPS-EB Ultrapure (5 mg)	tlrl-3pelps	64
LPS-EK (5 mg)	tlrl-eklps	64
LPS-EK Ultrapure (1 mg)	tlrl-peklps	65
LPS-PG (1 mg)	tlrl-pglps	64
LPS-RS (5 mg)	tlrl-rslps	65
LPS-RS Ultrapure (1 mg)	tlrl-prslps	65
LPS-SM Ultrapure (5 mg)	tlrl-smlps	65
LTA-BS (5 mg)	tlrl-lta	64
LTA-SA (5 mg)	tlrl-slta	64
LTA-SA Purified (5 mg)	tlrl-pslta	64
LY294002 (5 mg)	tlrl-ly29	90
MAb-hTLR1 (100 µg)	mab-htlr1	98
MAb-hTLR1-FITC (100 µg)	mab-htlr1f	98
MAb-hTLR2 (100 µg)	mab-htlr2	98
MAb-hTLR2-FITC (100 µg)	mab-htlr2f	98
MAb-hTLR3 (100 µg)	mab-htlr3	98
MAb-hTLR3-FITC (100 µg)	mab-htlr3f	98

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MAb-hTLR4 (100 µg)	mab-htlr4	98
MAb-hTLR4-FITC (100 µg)	mab-htlr4f	98
MAb-mDectin-1 (100 µg)	mab-mdect	99
MAb-mTLR2 (100 µg)	mab-mtlr2	99
MAb-mTLR2-FITC (100 µg)	mab-mtlr2f	98
MAb-mTLR4/MD2 (100 µg)	mab-mtlr4md2	98
MAb-mTLR4/MD2-FITC (100 µg)	mab-mtlr4md2f	98
MAb-mTLR9 (100 µg)	mab-mtlr9	98
MAb-mTLR9-FITC (100 µg)	mab-mtlr9f	98
MDP (5 mg)	tlrl-mdp	67
MDP Biotin (500 µg)	tlrl-bmdp	67
MDP control (5 mg)	tlrl-mdpc	67
MDP FITC (500 µg)	tlrl-fmdp	67
MDP Rhodamine (500 µg)	tlrl-rmdp	67
MG-132 (5 mg)	tlrl-mg132	90
Mouse IgG1 Isotype Control (100 µg)	mabg1-ctrlm	99
Mouse IgG2a Isotype Control (100 µg)	mabg2a-ctrlm	99
Mouse IgG2b Isotype Control (100 µg)	mabg2b-ctrlm	99
Mouse MDA-5 RT-Primer Pair (2 x 2.5 nmol)	rtp-mmda5	85
Mouse NOD1 RT-Primer Pair (2 x 2.5 nmol)	rtp-mnod1	85
Mouse NOD2 RT-Primer Pair (2 x 2.5 nmol)	rtp-mnod2	85
Mouse RIG-I RT-Primer Pair (2 x 2.5 nmol)	rtp-mrigi	85
Mouse TLR1 RT-Primer Pair (2x2.5 nmol)	rtp-mtlr1	85
Mouse TLR1-9 RT-Primer Set (20 x 2.5 nmol)	rts-mtlrs	85
Mouse TLR2 RT-Primer Pair (2 x 2.5 nmol)	rtp-mtlr2	85
Mouse TLR3 RT-Primer Pair (2 x 2.5 nmol)	rtp-mtlr3	85
Mouse TLR4 RT-Primer Pair (2 x 2.5 nmol)	rtp-mtlr4	85
Mouse TLR5 RT-Primer Pair (2 x 2.5 nmol)	rtp-mtlr5	85
Mouse TLR6 RT-Primer Pair (2 x 2.5 nmol)	rtp-mtlr6	85
Mouse TLR7 RT-Primer Pair (2 x 2.5 nmol)	rtp-mtlr7	85
Mouse TLR8 RT-Primer Pair (2 x 2.5 nmol)	rtp-mtlr8	85
Mouse TLR9 RT-Primer Pair (2 x 2.5 nmol)	rtp-mtlr9	85
MPLA (1 mg)	tlrl-mpl	65
MPLAs (1 mg)	tlrl-mpls	65
MPLA VacciGrade (1 mg)	vac-mpl	104
MPLAs VacciGrade (1 mg)	vac-mpls	104
MSU Crystals (5 mg)	tlrl-msu	68
M-TriLYS (1 mg)	tlrl-mtl	68
M-TriLYS-D-ASN (1 mg)	tlrl-mtn	68
M-TriDAP (1 mg)	tlrl-mtd	68
Murabutide (5 mg)	tlrl-mbt	68
Murabutide control (5 mg)	tlrl-mbtc	68
Nano-SiO2 (10 mg)	tlrl-sio	68
N-Glycolyl-MDP (5 mg)	tlrl-gmdp	68
N-Glycolyl-MDP VacciGrade (5 mg)	vac-gmdp	104
Nigericin (10 mg)	tlrl-nig	68
Nigericin (50 mg)	tlrl-nig-5	68

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NOD1/2 Agonist Kit (10 ligands)	tlrl-nodkit2	69
Normocin™ (500 mg)	ant-nr-1	59
Normocin™ (1 g)	ant-nr-2	59
ODN 1585 (200 µg)	tlrl-1585	66
ODN 1585 (1 mg)	tlrl-1585-1	66
ODN 1585 (5 mg)	tlrl-1585-5	66
ODN 1585 control (200 µg)	tlrl-1585c	66
ODN 1585 control (1 mg)	tlrl-1585c-1	66
ODN 1585 control (5 mg)	tlrl-1585c-5	66
ODN 1585 FITC (50 µg)	tlrl-1585f	66
ODN 1585 VacciGrade (1 mg)	vac-1585-1	104
ODN 1668 (200 µg)	tlrl-1668	66
ODN 1668 (1 mg)	tlrl-1668-1	66
ODN 1668 (5 mg)	tlrl-1668-5	66
ODN 1668 control (200 µg)	tlrl-1668c	66
ODN 1668 control (1 mg)	tlrl-1668c-1	66
ODN 1668 control 5 mg)	tlrl-1668c-5	66
ODN 1668 FITC (50 µg)	tlrl-1668f	66
ODN 1826 (200 µg)	tlrl-1826	66
ODN 1826 (1 mg)	tlrl-1826-1	66
ODN 1826 (5 mg)	tlrl-1826-5	66
ODN 1826 Biotin (50 µg)	tlrl-1826b	66
ODN 1826 control (200 µg)	tlrl-1826c	66
ODN 1826 control (1 mg)	tlrl-1826c-1	66
ODN 1826 control (5 mg)	tlrl-1826c-5	66
ODN 1826 FITC (50 µg)	tlrl-1826f	66
ODN 1826 VacciGrade (1 mg)	vac-1826-1	104
ODN 2006 (200 µg)	tlrl-2006	66
ODN 2006 (1 mg)	tlrl-2006-1	66
ODN 2006 (5 mg)	tlrl-2006-5	66
ODN 2006 Biotin (50 µg)	tlrl-2006b	66
ODN 2006 control (200 µg)	tlrl-2006c	66
ODN 2006 control (1 mg)	tlrl-2006c-1	66
ODN 2006 control (5 mg)	tlrl-2006c-5	66
ODN 2006 FITC (50 µg)	tlrl-2006f	66
ODN 2006-G5 (200 µg)	tlrl-2006g5	66
ODN 2006-G5 (1 mg)	tlrl-2006g5-1	66
ODN 2006-G5 (5 mg)	tlrl-2006g5-5	66
ODN 2006 VacciGrade (1 mg)	vac-2006-1	104
ODN 2007 (200 µg)	tlrl-2007	66
ODN 2007 (1 mg)	tlrl-2007-1	66
ODN 2007 (5 mg)	tlrl-2007-5	66
ODN 2007 Control (200 µg)	tlrl-2007c	66
ODN 2007 Control (1 mg)	tlrl-2007c-1	66
ODN 2007 Control (5 mg)	tlrl-2007c-5	66
ODN 2088 (200 µg)	tlrl-2088	67
ODN 2088 (1 mg)	tlrl-2088-1	67

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ODN 2088 control (200 µg)	tlrl-2088c	67
ODN 2088 control (1 mg)	tlrl-2088c-1	67
ODN 2216 (200 µg)	tlrl-2216	66
ODN 2216 (1 mg)	tlrl-2216-1	66
ODN 2216 (5 mg)	tlrl-2216-5	66
ODN 2216 Biotin (50 µg)	tlrl-2216b	66
ODN 2216 control (200 µg)	tlrl-2216c	66
ODN 2216 control (1 mg)	tlrl-2216c-1	66
ODN 2216 control (5 mg)	tlrl-2216c-5	66
ODN 2216 FITC (50 µg)	tlrl-2216f	66
ODN 2336 (200 µg)	tlrl-2336	67
ODN 2336 (1 mg)	tlrl-2336-1	67
ODN 2336 (5 mg)	tlrl-2336-5	67
ODN 2336 control (200 µg)	tlrl-2336c	67
ODN 2336 control (1 mg)	tlrl-2336c-1	67
ODN 2336 control (5 mg)	tlrl-2336c-5	67
ODN 2336 FITC (50 µg)	tlrl-2336f	67
ODN 2395 (200 µg)	tlrl-2395	67
ODN 2395 (1 mg)	tlrl-2395-1	67
ODN 2395 (5 mg)	tlrl-2395-5	67
ODN 2395 control (200 µg)	tlrl-2395c	67
ODN 2395 control (1 mg)	tlrl-2395c-1	67
ODN 2395 control (5 mg)	tlrl-2395c-5	67
ODN 2395 FITC (50 µg)	tlrl-2395f	67
ODN 4084-F (200 µg)	tlrl-4084	67
ODN INH-1 (200 µg)	tlrl-inh1	67
ODN INH-47 (200 µg)	tlrl-inh47	67
ODN M362 (200 µg)	tlrl-m362	67
ODN M362 (1 mg)	tlrl-m362-1	67
ODN M362 (5 mg)	tlrl-m362-5	67
ODN M362 control (200 µg)	tlrl-m362c	67
ODN M362 control (1 mg)	tlrl-m362c-1	67
ODN M362 control (5 mg)	tlrl-m362c-5	67
ODN M362 FITC (50 µg)	tlrl-m362f	67
ODN TTAGGG (200 µg)	tlrl-ttag	67
ODN TTAGGG (1 mg)	tlrl-ttag-1	67
ODN TTAGGG control (200 µg)	tlrl-ttagc	67
ODN TTAGGG control (1 mg)	tlrl-ttagc-1	67
ORN02 / LyoVec (4 x 25 µg)	tlrl-orn2	65
ORN06 / LyoVec (4 x 25 µg)	tlrl-orn6	65
OVA 257-264 (1 mg)	vac-sin	103
OVA 323-339 (1 mg)	vac-isq	103
Ovalbumin (1 g)	vac-ova	103
Ovalbumin EndoFit™ (10 mg)	vac-efova	103
OxPAPC (1 mg)	tlrl-oxp1	90
PAb Control (200 µg)	pab-sctr	99
PAb-hTLR1 (200 µg)	pab-hstlr1	99

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PRODUCT (QUANTITY)	CAT. CODE	PAGE
PAb-hTLR2 (200 µg)	pab-hstlr2	99
PAb-hTLR4 (200 µg)	pab-hstlr4	99
PAb-hTLR5 (200 µg)	pab-hstlr5	99
PAb-hTLR6 (200 µg)	pab-hstlr6	99
Pam2CSK4 (100 µg)	tlrl-pm2s	64
Pam2CSK4 (1 mg)	tlrl-pm2s-1	64
Pam2CSK4 Biotin (50 µg)	tlrl-bpam2	64
Pam2CSK4 Rhodamine (50 µg)	tlrl-rpam2	64
Pam3CSK4 (1 mg)	tlrl-pms	64
Pam3CSK4 Biotin (50 µg)	tlrl-bpms	64
Pam3CSK4 Rhodamine (50 µg)	tlrl-rpms	64
Pam3CSK4 VacciGrade (1 mg)	vac-pms	104
pCpG-Giant (1 mg)	tlrl-cpgg	67
PD98059 (10 mg)	tlrl-pd98	90
pDeNy-<Gene> (*E.coli* disk)	pdn-<gene>	30
pDUO-<Genes> (*E.coli* disk)	pduo-<genes>	27
pDUO2-<Genes> (*E.coli* disk)	pduo2-<genes>	27
Pepinh-Control (2 mg)	tlrl-pictrl	90
Pepinh-MYD (2 mg)	tlrl-pimyd	90
Pepinh-TRAM (2 mg)	tlrl-pitram	90
Pepinh-TRIF (2 mg)	tlrl-pitrif	90
PGN-BS (5 mg)	tlrl-pgnbs	64
PGN-EB (1 mg)	tlrl-pgnec	64
PGN-ECndi ultrapure, insoluble (5 mg)	tlrl-kipgn	68
PGN-ECndss ultrapure, soluble (1 mg)	tlrl-ksspgn	68
PGN-EK (1 mg)	tlrl-pgnek	64
PGN-SA (5 mg)	tlrl-pgnsa	64
PGN-SAndi ultrapure, insoluble (5 mg)	tlrl-sipgn	68
Piceatannol (5 mg)	tlrl-pct	90
Plasmocin™ prophylactic (25 mg)	ant-mpp	59
Plasmocin™ treatment (50 mg)	ant-mpt	59
PlasmoTest™ (kit)	rep-pt2	58
PlasmoTest™ Controls (200 tests)	pt-ctr2	58
PlasmoTest™ Reagent Kit (500 samples)	rep-ptrk	58
PMA (5 mg)	tlrl-pma	90
pNiFty-Luc (*E.coli* disk)	pnifty-luc	87
pNiFty-SEAP (*E.coli* disk)	pnifty-seap	87
pNiFty2-56K-SEAP (*E.coli* disk)	pnf2-56ksp	87
pNiFty2-Luc (*E.coli* disk)	pnifty2-luc	87
pNiFty2-SEAP (*E.coli* disk)	pnifty2-seap	87
pNiFty3-Lucia (*E.coli* disk)	pnf3-lc1	87
pNiFty3-SEAP (*E.coli* disk)	pnf3-sp1	87
pNiFty3-A-Lucia (*E.coli* disk)	pnf3-lc3	87
pNiFty3-A-SEAP (*E.coli* disk)	pnf3-sp3	87
pNiFty3-AN-Lucia (*E.coli* disk)	pnf3-lc6	87
pNiFty3-AN-SEAP (*E.coli* disk)	pnf3-sp6	87
pNiFty3-I-Lucia (*E.coli* disk)	pnf3-lc4	87

PRODUCT (QUANTITY)	CAT. CODE	PAGE
pNiFty3-I-SEAP (*E.coli* disk)	pnf3-sp4	87
pNiFty3-IAN-Lucia (*E.coli* disk)	pnf3-lc7	87
pNiFty3-IAN-SEAP (*E.coli* disk)	pnf3-sp7	87
pNiFty3-N-Lucia (*E.coli* disk)	pnf3-lc2	87
pNiFty3-N-SEAP (*E.coli* disk)	pnf3-sp2	87
pNiFty3-T-Lucia (*E.coli* disk)	pnf3-lc5	87
pNiFty3-T-SEAP (*E.coli* disk)	pnf3-sp5	87
pNiFty3-TAN-Lucia (*E.coli* disk)	pnf3-lc8	87
pNiFty3-TAN-SEAP (*E.coli* disk)	pnf3-sp8	87
Poly(A:U) (10 mg)	tlrl-pau	64
Poly(dA:dT) / LyoVec (100 µg)	tlrl-patc	68
Poly(dA:dT) Naked (200 µg)	tlrl-patn	68
Poly(dA:dT) Naked (1 mg)	tlrl-patn-1	68
Poly(dG:dC) / LyoVec (100 µg)	tlrl-pgcc	68
Poly(dG:dC) Naked (200 µg)	tlrl-pgcn	68
Poly(dT) (100 nmol)	tlrl-pt17	65
Poly(I:C) (HMW) (10 mg)	tlrl-pic	64
Poly(I:C) (HMW) (50 mg)	tlrl-pic-5	64
Poly(I:C) (HMW) / LyoVec (100 µg)	tlrl-piclv	68
Poly(I:C) (HMW) / LyoVec (1 mg)	tlrl-piclv-10	68
Poly(I:C) (HMW) Fluorescein (10 µg)	tlrl-picf	64
Poly(I:C) (HMW) Rhodamine (10 µg)	tlrl-picr	64
Poly(I:C) (HMW) VacciGrade (10 mg)	vac-pic	104
Poly(I:C) (LMW) (25 mg)	tlrl-picw	64
Poly(I:C) (LMW) (250 mg)	tlrl-picw-250	64
Poly(I:C) (LMW) / LyoVec (100 µg)	tlrl-picwlv	68
Poly(I:C) (LMW) / LyoVec (1 mg)	tlrl-picwlv-10	68
Poly(I:C) (LMW) Rhodamine (10 µg)	tlrl-piwr	64
Polymyxin B (100 mg)	tlrl-pmb	90
pORF-<Gene> (*E.coli* disk)	porf-<gene>	26
pSELECT-GFP-LC3 (20 µg)	psetz-gfplc3	30
pSELECT-NGFP-zeo (20 µg)	psetz-ngfp	30
pSELECT-zeo-Lucia (20 µg)	psetz-lucia	54
pSELECT-zeo-seap (20 µg)	psetz-seap	57
pUNO-<Gene> (*E.coli* disk)	puno-<gene>	23
pUNO-<TLR Gene>-HA (*E.coli* disk)	punoha-<gene>	28
pUNO-hTLR1-GFP (*E.coli* disk)	phtlr1-gfp	28
pUNO-hTLR2-GFP (*E.coli* disk)	phtlr2-gfp	28
pUNO-hTLR3-GFP (*E.coli* disk)	phtlr3-gfp	28
pUNO-hTLR4-GFP (*E.coli* disk)	phtlr4-gfp	28
pUNO-hTLR5-GFP (*E.coli* disk)	phtlr5-gfp	28
pUNO-hTLR6-GFP (*E.coli* disk)	phtlr6-gfp	28
pUNO1-<Gene> (*E.coli* disk)	puno1-<gene>	23
pUNO2-<Gene> (*E.coli* disk)	puno2-<gene>	23
pUNO3-<Gene> (*E.coli* disk)	puno3-<gene>	23
Puromycin (100 mg)	ant-pr-1	61
Puromycin (500 mg)	ant-pr-5	61

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pZERO-<TLR Gene> (*E.coli* disk)	pzero-<gene>	29
pZERO-<TLR Gene>-HA (*E.coli* disk)	pzero-<gene>-ha	29
QUANTI-Blue™ (5 pouches)	rep-qb1	56
QUANTI-Blue™ (10 pouches)	rep-qb2	56
QUANTI-Luc™ (2 pouches)	rep-qlc1	55
QUANTI-Luc™ (5 pouches)	rep-qlc2	55
R848 (500 μg)	tlrl-r848	65
R848 (5 mg)	tlrl-r848-5	65
R848 VacciGrade (5 mg)	vac-r848	104
Ramos-Blue™ Cells (5-7 x 10^6 cells)	rms-sp	40
Rapamycin (5 mg)	tlrl-rap	90
RAW-Blue™ Cells (5-7 x 10^6 cells)	raw-sp	39
RAW-Blue™ ISG Cells (5-7 x 10^6 cells)	raw-isg	46
Ready-Made psiRNA plasmid (20 μg)	psirna42-<gene>	94
Ready-Made psiRNA kit	ksirna42-<gene>	94
RecFLA-ST (1 μg)	tlrl-flic	65
RecFLA-ST (10 μg)	tlrl-flic-10	65
Recombinant human CD40L (10 μg)	rhcd-40L	96
Recombinant human IFN-γ (20 μg)	rhifn-g	96
Recombinant human IL-1β (10 μg)	rhil-1b	96
Recombinant human IL-4 (10 μg)	rhil-4	96
Recombinant human IL-6 (10 μg)	rhil-6	96
Recombinant human IL-13 (10 μg)	rhil-13	96
Recombinant human IL-18 (10 μg)	rhil-18	96
Recombinant human IL-33 (10 μg)	rhil-33	96
Recombinant human TNF-α (20 μg)	rhtnf-a	96
Recombinant Lucia Protein (1 μg)	rec-lucia	54
Recombinant SEAP Protein (10 μg)	rec-hseap	57
Resveratrol (100 mg)	tlrl-resv	90
Salmon sperm DNA (50 mg)	tlrl-sdef	67
SB202190 (5 mg)	tlrl-sb90	90
SB203580 (5 mg)	tlrl-sb20	90
SP600125 (10 mg)	tlrl-sp60	90
ssPolyU / LyoVec (100 μg)	tlrl-lpu	65
ssPolyU Naked (10 mg)	tlrl-sspu	65
ssRNA40 / LyoVec (100 μg)	tlrl-lrna40	65
ssRNA41 / LyoVec (100 μg)	tlrl-lrna41	65
ssRNA-DR / LyoVec (100 μg)	tlrl-ssdr	65
Tamoxifen (200 mg)	tlrl-txf	90
TDB (1 mg)	tlrl-tdb	68
THP1-Blue™ ISG Cells (5-7 x 10^6 cells)	thp-isg	37
THP1-Blue™ NF-κB Cells (5-7 x 10^6 cells)	thp-nfkb	37
THP1-defASC Cells (5-7 x 10^6 cells)	thp-dasc	42
THP1-defNLRP3 Cells (5-7 x 10^6 cells)	thp-dnlp	42
THP1-Dual™ (NF-kB, ISG) Cells (5-7 x 10^6 cells)	thpd-nfist	38
THP1-Lucia™ NF-κB Cells (5-7 x 10^6 cells)	thpl-nfkb	38
THP1-Null Cells (5-7 x 10^6 cells)	thp-null	42
THP1-XBlue™ Cells (5-7 x 10^6 cells)	thpx-sp	36
THP1-XBlue™-defMyD Cells (5-7 x 10^6 cells)	thpx-dmyd	36
THP1-XBlue™-MD2-CD14 Cells (5-7 x 10^6 cells)	thpx-mdcdsp	36
TLR1-9 Agonist Kit-Human (10 ligands)	tlrl-kit1hw	69
TLR1-9 Agonist Kit-Mouse (9 ligands)	tlrl-kit1mw	69
TLR2 Agonist Kit (7 ligands)	tlrl-kit2hm	69
TLR3/7/8/9 Agonist Kit (14 ligands)	tlrl-kit3hw3	69
Tri-DAP (1 mg)	tlrl-tdap	67
Tri-Lys (1 mg)	tlrl-tlys	67
Triptolide (1 mg)	ant-tpl	90
U0126 (5 mg)	tlrl-u0126	90
WGP Dispersable (50 mg)	tlrl-wgp	68
WGP Soluble (50 mg)	tlrl-wgps	68
Wortmannin (5 mg)	tlrl-wtm	90
Zeocin™ (1 g)	ant-zn-1	61
Zeocin™ (1 g powder)	ant-zn-1p	61
Zeocin™ (5 g)	ant-zn-5	61
Zeocin™ (5 g, bottle)	ant-zn-5b	61
Zeocin™ (5 g powder)	ant-zn-5p	61
Z-VAD-FMK (1 mg)	tlrl-vad	90
Zymosan (100 mg)	tlrl-zyn	68
Zymosan Depleted (10 mg)	tlrl-dzn	68

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CAT. CODE	PRODUCT (QUANTITY)	PAGE
293-hmd2cd14	293/hMD2-CD14 (5-7 x 10^6 cells)	33
293-hnod1	293/hNOD1 (5-7 x 10^6 cells)	33
293-hnod2	293/hNOD2 (5-7 x 10^6 cells)	33
293-htlr1ha	293/hTLR1-HA (5-7 x 10^6 cells)	33
293-htlr2	293/hTLR2 (5-7 x 10^6 cells)	33
293-htlr2ha	293/hTLR2-HA (5-7 x 10^6 cells)	33
293-htlr2/6	293/hTLR2/6 (5-7 x 10^6 cells)	33
293-htlr2cd14	293/hTLR2-CD14 (5-7 x 10^6 cells)	33
293-htlr3	293/hTLR3 (5-7 x 10^6 cells)	33
293-htlr3ha	293/hTLR3-HA (5-7 x 10^6 cells)	33
293-htlr4a	293/hTLR4A (5-7 x 10^6 cells)	33
293-htlr4ha	293/hTLR4-HA (5-7 x 10^6 cells)	33
293-htlr4md2cd14	293/hTLR4A-MD2-CD14 (5-7 x 10^6 cells)	33
293-htlr5	293/hTLR5 (5-7 x 10^6 cells)	33
293-htlr5ha	293/hTLR5-HA (5-7 x 10^6 cells)	33
293-htlr5cd14	293/hTLR5-CD14 (5-7 x 10^6 cells)	33
293-htlr6ha	293/hTLR6-HA (5-7 x 10^6 cells)	33
293-htlr10ha	293/hTLR10-HA (5-7 x 10^6 cells)	33
293-lacz	293/LacZ (5-7 x 10^6 cells)	33
293-mnod1	293/mNOD1 (5-7 x 10^6 cells)	33
293-mnod2	293/mNOD2 (5-7 x 10^6 cells)	33
293-mtlr1	293/mTLR1 (5-7 x 10^6 cells)	33
293-mtlr1/2	293/mTLR1/2 (5-7 x 10^6 cells)	33
293-mtlr2	293/mTLR2 (5-7 x 10^6 cells)	33
293-mtlr2/6	293/mTLR2/6 (5-7 x 10^6 cells)	33
293-mtlr3	293/mTLR3 (5-7 x 10^6 cells)	33
293-mtlr4	293/mTLR4 (5-7 x 10^6 cells)	33
293-mtlr4md2cd14	293/mTLR4-MD2-CD14 (5-7 x 10^6 cells)	33
293-mtlr5	293/mTLR5 (5-7 x 10^6 cells)	33
293-mtlr6	293/mTLR6 (5-7 x 10^6 cells)	33
293-mtlr9	293/mTLR9 (5-7 x 10^6 cells)	33
293-null	293/null (5-7 x 10^6 cells)	33
293xl-htlr7	293XL/hTLR7 (5-7 x 10^6 cells)	33
293xl-htlr7ha	293XL/hTLR7-HA (5-7 x 10^6 cells)	33
293xl-htlr8	293XL/hTLR8 (5-7 x 10^6 cells)	33
293xl-htlr8ha	293XL/hTLR8-HA (5-7 x 10^6 cells)	33
293xl-htlr9	293XL/hTLR9 (5-7 x 10^6 cells)	33
293xl-htlr9ha	293XL/hTLR9-HA (5-7 x 10^6 cells)	33
293xl-mtlr7	293XL/mTLR7 (5-7 x 10^6 cells)	33
293xl-null	293XL/null (5-7 x 10^6 cells)	33
ab-hatag	Anti-HA Tag (250 µl)	98
ant-bl-1	Blasticidin (100 mg)	60
ant-bl-5	Blasticidin (500 mg)	60
ant-bl-5b	Blasticidin (500 mg, bottle)	60
ant-bl-10p	Blasticidin (1 g powder)	60
ant-cls	Celastrol (1 mg)	90
ant-gn-1	G418 (1 g)	60

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ant-gn-5	G418 (5 g)	60
ant-hg-1	HygroGold™ (1 g)	61
ant-hg-5	HygroGold™ (5 g)	61
ant-hg-10p	HygroGold™ (10 g powder)	61
ant-hm-1	Hygromycin B (1 g)	61
ant-hm-5	Hygromycin B (5 g)	61
ant-mpp	Plasmocin™ prophylactic (25 mg)	59
ant-mpt	Plasmocin™ treatment (50 mg)	59
ant-nr-1	Normocin™ (500 mg)	59
ant-nr-2	Normocin™ (1 g)	59
ant-pr-1	Puromycin (100 mg)	61
ant-pr-5	Puromycin (500 mg)	61
ant-tpl	Triptolide (1 mg)	90
ant-zn-1	Zeocin™ (1 g)	61
ant-zn-1p	Zeocin™ (1 g powder)	61
ant-zn-5	Zeocin™ (5 g)	61
ant-zn-5b	Zeocin™ (5 g, bottle)	61
ant-zn-5p	Zeocin™ (5 g powder)	61
bb-ifnab	B16-Blue™ IFNα/β Cells (5-7 x 106 cells)	46
chiga-biot	Goat F(ab')2 anti-human IgA - Biotin (0.5 mg)	99
chiga-fitc	Goat F(ab')2 anti-human IgA - FITC (0.5 mg)	99
cgig-fitc	Goat F(ab')2 IgG isotype control - FITC (100 tests)	99
hcd20-mab1	Anti-hCD20-hIgG1 (100 µg)	98
hb-det2	HEK-Blue™ Detection (5 pouches)	56
hb-det3	HEK-Blue™ Detection (10 pouches)	56
hb-sel	HEK-Blue™ Selection (5 x 2 ml)	84
hkb-hmdcd	HEK-Blue™ hMD2-CD14 Cells (5-7 x 10^6 cells)	34
hkb-hnod1	HEK-Blue™ hNOD1 Cells (5-7 x 10^6 cells)	34
hkb-hnod2	HEK-Blue™ hNOD2 Cells (5-7 x 10^6 cells)	34
hkb-htlr2	HEK-Blue™ hTLR2 Cells (5-7 x 10^6 cells)	34
hkb-htlr3	HEK-Blue™ hTLR3 Cells (5-7 x 10^6 cells)	34
hkb-htlr4	HEK-Blue™ hTLR4 Cells (5-7 x 10^6 cells)	34
hkb-htlr5	HEK-Blue™ hTLR5 Cells (5-7 x 10^6 cells)	34
hkb-htlr7	HEK-Blue™ hTLR7 Cells (5-7 x 10^6 cells)	34
hkb-htlr8	HEK-Blue™ hTLR8 Cells (5-7 x 10^6 cells)	34
hkb-htlr9	HEK-Blue™ hTLR9 Cells (5-7 x 10^6 cells)	34
hkb-ifnab	HEK-Blue™ IFN-α/β Cells (5-7 x 10^6 cells)	45
hkb-ifng	HEK-Blue™ IFN-γ Cells (5-7 x 10^6 cells)	47
hkb-il1b	HEK-Blue™ IL-1β Cells (5-7 x 10^6 cells)	43
hkb-il6	HEK-Blue™ IL-6 Cells (5-7 x 10^6 cells)	51
hkb-il18	HEK-Blue™ IL-18/IL-1β Cells (5-7 x 10^6 cells)	50
hkb-il33	HEK-Blue™ IL-33/IL-1β Cells (5-7 x 10^6 cells)	50
hkb-mnod1	HEK-Blue™ mNOD1 Cells (5-7 x 10^6 cells)	34
hkb-mnod2	HEK-Blue™ mNOD2 Cells (5-7 x 10^6 cells)	34
hkb-mtlr2	HEK-Blue™ mTLR2 Cells (5-7 x 10^6 cells)	34
hkb-mtlr3	HEK-Blue™ mTLR3 Cells (5-7 x 10^6 cells)	34
hkb-mtlr4	HEK-Blue™ mTLR4 Cells (5-7 x 10^6 cells)	34

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hkb-mtlr5	HEK-Blue™ mTLR5 Cells (5-7 x 10^6 cells)	34
hkb-mtlr7	HEK-Blue™ mTLR7 Cells (5-7 x 10^6 cells)	34
hkb-mtlr8	HEK-Blue™ mTLR8 Cells (5-7 x 10^6 cells)	34
hkb-mtlr9	HEK-Blue™ mTLR9 Cells (5-7 x 10^6 cells)	34
hkb-null1	HEK-Blue™ Null1 Cells (5-7 x 10^6 cells)	34
hkb-null1k	HEK-Blue™ Null1-k Cells (5-7 x 10^6 cells)	34
hkb-null1v	HEK-Blue™ Null1-v Cells (5-7 x 10^6 cells)	34
hkb-null2	HEK-Blue™ Null2 Cells (5-7 x 10^6 cells)	34
hkb-null2k	HEK-Blue™ Null2-k Cells (5-7 x 10^6 cells)	34
hkb-stat6	HEK-Blue™ IL-4/IL-13 Cells (5-7 x 10^6 cells)	51
hkb-tnfil1	HEK-Blue™ TNF-α/IL-1β Cells (5-7 x 10^6 cells)	49
hkd-ifng	HEK-Dual™ IFN-γ Cells (5-7 x 10^6 cells)	47
hkd-tnfa	HEK-Dual™ TNF-α Cells (5-7 x 10^6 cells)	48
htnfa-mab1	Anti-hTNF-α-hIgG1 (100 µg)	99
jktd-isnf	Jurkat-Dual™ Cells (5-7 x 10^6 cells)	40
ksirna42-<gene>	Ready-Made psiRNA kit	94
mab-htlr1	MAb-hTLR1 (100 µg)	98
mab-htlr1f	MAb-hTLR1-FITC (100 µg)	98
mab-htlr2	MAb-hTLR2 (100 µg)	98
mab-htlr2f	MAb-hTLR2-FITC (100 µg)	98
mab-htlr3	MAb-hTLR3 (100 µg)	98
mab-htlr3f	MAb-hTLR3-FITC (100 µg)	98
mab-htlr4	MAb-hTLR4 (100 µg)	98
mab-htlr4f	MAb-hTLR4-FITC (100 µg)	98
mab-mdect	MAb-mDectin-1 (100 µg)	99
mab-mtlr2	MAb-mTLR2 (100 µg)	99
mab-mtlr2f	MAb-mTLR2-FITC (100 µg)	98
mab-mtlr4md2	MAb-mTLR4/MD2 (100 µg)	98
mab-mtlr4md2f	MAb-mTLR4/MD2-FITC (100 µg)	98
mab-mtlr9	MAb-mTLR9 (100 µg)	98
mab-mtlr9f	MAb-mTLR9-FITC (100 µg)	98
maba-h40l	Anti-hCD40L-IgA2 (100 µg)	99
maba-hcd14	Anti-hCD14-IgA (100 µg)	98
maba-hifna	Anti-hIFN-α-IgA2 (100 µg)	99
maba-hifng	Anti-hIFN-γ-IgA2 (100 µg)	99
maba-hil1b	Anti-hIL-1β-IgA2 (100 µg)	99
maba-hil4	Anti-hIL-4-IgA2 (100 µg)	99
maba-hil6	Anti-hIL-6-IgA2 (100 µg)	99
maba-hil13	Anti-hIL-13-IgA2 (100 µg)	99
maba-hil18	Anti-hIL-18-IgA2 (100 µg)	99
maba-htgfb	Anti-hTGFβ-IgA2 (100 µg)	99
maba-htlr3	Anti-hTLR3-IgA (100 µg)	98
maba2-ctrl	Human IgA2 Isotype Control (100 µg)	99
maba2-htlr2	Anti-hTLR2-IgA (100 µg)	99
maba2-htlr5	Anti-hTLR5-IgA (100 µg)	99
mabg-flic	Anti-Flagellin FliC (100 µg)	98
mabg-htlr1	Anti-hTLR1-IgG (100 µg)	99

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mabg-htlr4	Anti-hTLR4-IgG (100 µg)	99
mabg-htlr6	Anti-hTLR6-IgG (100 µg)	99
mabg-lucia	Anti-Lucia-IgG (100 µg)	54
mabg-mtlr2	Anti-mTLR2-IgG (100 µg)	99
mabg-mtlr5	Anti-mTLR5-IgG (100 µg)	99
mabg1-ctrlm	Mouse IgG1 Isotype Control (100 µg)	99
mabg2a-ctrlm	Mouse IgG2a Isotype Control (100 µg)	99
mabg2b-ctrlm	Mouse IgG2b Isotype Control (100 µg)	99
mef-c3h4m	C3H/TLR4mut MEFs (5-7 x 106 cells)	41
mef-c3hwt	C3H/WT MEFs (5-7 x 106 cells)	41
mef-c57wt	C57/WT MEFs (5-7 x 106 cells)	41
pab-hstlr1	PAb-hTLR1 (200 µg)	99
pab-hstlr2	PAb-hTLR2 (200 µg)	99
pab-hstlr4	PAb-hTLR4 (200 µg)	99
pab-hstlr5	PAb-hTLR5 (200 µg)	99
pab-hstlr6	PAb-hTLR6 (200 µg)	99
pab-sctr	PAb Control (200 µg)	99
pdn-<gene>	pDeNy-<Gene> (E.coli disk)	30
pduo-<genes>	pDUO-<Genes> (E.coli disk)	27
pduo2-<genes>	pDUO2-<Genes> (E.coli disk)	27
phtlr1-gfp	pUNO-hTLR1-GFP (E.coli disk)	28
phtlr2-gfp	pUNO-hTLR2-GFP (E.coli disk)	28
phtlr3-gfp	pUNO-hTLR3-GFP (E.coli disk)	28
phtlr4-gfp	pUNO-hTLR4-GFP (E.coli disk)	28
phtlr5-gfp	pUNO-hTLR5-GFP (E.coli disk)	28
phtlr6-gfp	pUNO-hTLR6-GFP (E.coli disk)	28
pnifty-luc	pNiFty-Luc (E.coli disk)	87
pnifty-seap	pNiFty-SEAP (E.coli disk)	87
pnf2-56ksp	pNiFty2-56K-SEAP (E.coli disk)	87
pnifty2-luc	pNiFty2-Luc (E.coli disk)	87
pnifty2-seap	pNiFty2-SEAP (E.coli disk)	87
pnf3-lc1	pNiFty3-Lucia (E.coli disk)	87
pnf3-lc2	pNiFty3-N-Lucia (E.coli disk)	87
pnf3-lc3	pNiFty3-A-Lucia (E.coli disk)	87
pnf3-lc4	pNiFty3-I-Lucia (E.coli disk)	87
pnf3-lc5	pNiFty3-T-Lucia (E.coli disk)	87
pnf3-lc6	pNiFty3-AN-Lucia (E.coli disk)	87
pnf3-lc7	pNiFty3-IAN-Lucia (E.coli disk)	87
pnf3-lc8	pNiFty3-TAN-Lucia (E.coli disk)	87
pnf3-sp1	pNiFty3-SEAP (E.coli disk)	87
pnf3-sp2	pNiFty3-N-SEAP (E.coli disk)	87
pnf3-sp3	pNiFty3-A-SEAP (E.coli disk)	87
pnf3-sp4	pNiFty3-I-SEAP (E.coli disk)	87
pnf3-sp5	pNiFty3-T-SEAP (E.coli disk)	87
pnf3-sp6	pNiFty3-AN-SEAP (E.coli disk)	87
pnf3-sp7	pNiFty3-IAN-SEAP (E.coli disk)	87
pnf3-sp8	pNiFty3-TAN-SEAP (E.coli disk)	87

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porf-<gene>	pORF-<Gene> (E.coli disk)	26
psetz-gfplc3	pSELECT-GFP-LC3 (20 µg)	30
psetz-lucia	pSELECT-zeo-Lucia (20 µg)	54
psetz-ngfp	pSELECT-NGFP-zeo (20 µg)	30
psetz-seap	pSELECT-zeo-seap (20 µg)	57
psirna42-<gene>	Ready-Made psiRNA plasmid (20 µg)	94
pt-ctr2	PlasmoTest™ Controls (200 tests)	58
puno-<gene>	pUNO-<Gene> (E.coli disk)	23
puno1-<gene>	pUNO1-<Gene> (E.coli disk)	23
puno2-<gene>	pUNO2-<Gene> (E. coli disk)	23
puno3-<gene>	pUNO3-<Gene> (E.coli disk)	23
punoha-<tlr gene>	pUNO-<TLR Gene>-HA (E.coli disk)	28
pzero-<tlr gene>	pZERO-<TLR Gene> (E.coli disk)	29
pzero-<tlr gene>-ha	pZERO-<TLR Gene>-HA (E.coli disk)	29
raw-isg	RAW-Blue™ IGS Cells (5-7 x 106 cells)	46
raw-sp	RAW-Blue™ Cells (5-7 x 106 cells)	39
rec-lucia	Recombinant Lucia Protein (1 µg)	54
rec-hseap	Recombinant SEAP Protein (10 µg)	57
rep-lps	HEK-Blue™ LPS Detection Kit	84
rep-pt2	PlasmoTest™ (kit)	58
rep-ptrk	PlasmoTest™ Reagent Kit (500 samples)	58
rep-qb1	QUANTI-Blue™ (5 pouches)	56
rep-qb2	QUANTI-Blue™ (10 pouches)	56
rep-qlc1	QUANTI-Luc™ (2 pouches)	55
rep-qlc2	QUANTI-Luc™ (5 pouches)	55
rhcd-40L	Recombinant human CD40L (10 µg)	96
rhifn-g	Recombinant human IFN-γ (20 µg)	96
rhil-1b	Recombinant human IL-1β (10 µg)	96
rhil-4	Recombinant human IL-4 (10 µg)	96
rhil-6	Recombinant human IL-6 (10 µg)	96
rhil-13	Recombinant human IL-13 (10 µg)	96
rhil-18	Recombinant human IL-18 (10 µg)	96
rhil-33	Recombinant human IL-33 (10 µg)	96
rhtnf-a	Recombinant human TNF-α (20 µg)	96
rms-sp	Ramos-Blue™ Cells (5-7 x 10^6 cells)	40
rtp-hmda5	Human MDA-5 Primer Pair (2 x 2.5 nmol)	85
rtp-hnod1	Human NOD1 Primer Pair (2 x 2.5 nmol)	85
rtp-hnod2	Human NOD2 Primer Pair (2 x 2.5 nmol)	85
rtp-hrigi	Human RIG-I Primer Pair (2 x 2.5 nmol)	85
rtp-htlr1	Human TLR1 Primer Pair (2 x 2.5 nmol)	85
rtp-htlr2	Human TLR2 Primer Pair (2 x 2.5 nmol)	85
rtp-htlr3	Human TLR3 Primer Pair (2 x 2.5 nmol)	85
rtp-htlr4	Human TLR4 Primer Pair (2 x 2.5 nmol)	85
rtp-htlr5	Human TLR5 Primer Pair (2 x 2.5 nmol)	85
rtp-htlr6	Human TLR6 Primer Pair (2 x 2.5 nmol)	85
rtp-htlr7	Human TLR7 Primer Pair (2 x 2.5 nmol)	85
rtp-htlr8	Human TLR8 Primer Pair (2 x 2.5 nmol)	85

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rtp-htlr9	Human TLR9 Primer Pair (2 x 2.5 nmol)	85
rtp-htlr10	Human TLR10 Primer Pair (2 x 2.5 nmol)	85
rtp-mmda5	Mouse MDA-5 Primer Pair (2 x 2.5 nmol)	85
rtp-mnod1	Mouse NOD1 Primer Pair (2 x 2.5 nmol)	85
rtp-mnod2	Mouse NOD2 Primer Pair (2 x 2.5 nmol)	85
rtp-mrigi	Mouse RIG-I Primer Pair (2 x 2.5 nmol)	85
rtp-mtlr1	Mouse TLR1 Primer Pair (2 x 2.5 nmol)	85
rtp-mtlr2	Mouse TLR2 Primer Pair (2 x 2.5 nmol)	85
rtp-mtlr3	Mouse TLR3 Primer Pair (2 x 2.5 nmol)	85
rtp-mtlr4	Mouse TLR4 Primer Pair (2 x 2.5 nmol)	85
rtp-mtlr5	Mouse TLR5 Primer Pair (2 x 2.5 nmol)	85
rtp-mtlr6	Mouse TLR6 Primer Pair (2 x 2.5 nmol)	85
rtp-mtlr7	Mouse TLR7 Primer Pair (2 x 2.5 nmol)	85
rtp-mtlr8	Mouse TLR8 Primer Pair (2 x 2.5 nmol)	85
rtp-mtlr9	Mouse TLR9 Primer Pair (2 x 2.5 nmol)	85
rts-htlrs	Human TLR1-9 Primer Set (25 x 2.5 nmol)	85
rts-mtlrs	Mouse TLR1-9 Primer Set (25 x 2.5 nmol)	85
thp-dasc	THP1-defASC Cells (5-7 x 10^6 cells)	42
thp-dnlp	THP1-defNLRP3 Cells (5-7 x 10^6 cells)	42
thp-isg	THP1-Blue™ ISG Cells (5-7 x 10^6 cells)	37
thp-nfkb	THP1-Blue™ NF-κB Cells (5-7 x 10^6 cells)	37
thp-null	THP1-Null Cells (5-7 x 10^6 cells)	42
thpd-nfist	THP1-Dual™ (NF-kB, ISG) Cells (5-7 x 10^6 cells)	38
thpl-nfkb	THP1-Lucia™ NF-κB Cells (5-7 x 10^6 cells)	38
thpx-sp	THP1-XBlue™ Cells (5-7 x 106 cells)	36
thpx-dmyd	THP1-XBlue™-defMyD Cells (5-7 x 106 cells)	36
thpx-mdcdsp	THP1-XBlue™-MD2-CD14 Cells (5-7 x 106 cells)	36
tlrl-1585	ODN 1585 (200 µg)	66
tlrl-1585-1	ODN 1585 (1 mg)	66
tlrl-1585-5	ODN 1585 (5 mg)	66
tlrl-1585c	ODN 1585 control (200 µg)	66
tlrl-1585c-1	ODN 1585 control (1 mg)	66
tlrl-1585c-5	ODN 1585 control (5 mg)	66
tlrl-1585f	ODN 1585 FITC (50 µg)	66
tlrl-1668	ODN 1668 (200 µg)	66
tlrl-1668-1	ODN 1668 (1 mg)	66
tlrl-1668-5	ODN 1668 (5 mg)	66
tlrl-1668c	ODN 1668 control (200 µg)	66
tlrl-1668c-1	ODN 1668 control (1 mg)	66
tlrl-1668c-5	ODN 1668 control 5 mg)	66
tlrl-1668f	ODN 1668 FITC (50 µg)	66
tlrl-1826	ODN 1826 (200 µg)	66
tlrl-1826-1	ODN 1826 (1 mg)	66
tlrl-1826-5	ODN 1826 (5 mg)	66
tlrl-1826b	ODN 1826 Biotin (50 µg)	66
tlrl-1826c	ODN 1826 control (200 µg)	66
tlrl-1826c-1	ODN 1826 control (1 mg)	66

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tlrl-1826c-5	ODN 1826 control (5 mg)	66
tlrl-1826f	ODN 1826 FITC (50 µg)	66
tlrl-2006	ODN 2006 (200 µg)	66
tlrl-2006-1	ODN 2006 (1 mg)	66
tlrl-2006-5	ODN 2006 (5 mg)	66
tlrl-2006b	ODN 2006 Biotin (50 µg)	66
tlrl-2006c	ODN 2006 control (200 µg)	66
tlrl-2006c-1	ODN 2006 control (1 mg)	66
tlrl-2006c-5	ODN 2006 control (5 mg)	66
tlrl-2006f	ODN 2006 FITC (50 µg)	66
tlrl-2006g5	ODN 2006-G5 (200 µg)	66
tlrl-2006g5-1	ODN 2006-G5 (1 mg)	66
tlrl-2006g5-5	ODN 2006-G5 (5 mg)	66
tlrl-2007	ODN 2007 (200 µg)	66
tlrl-2007-1	ODN 2007 (1 mg)	66
tlrl-2007-5	ODN 2007 (5 mg)	66
tlrl-2007c	ODN 2007 Control (200 µg)	66
tlrl-2007c-1	ODN 2007 Control (1 mg)	66
tlrl-2007c-5	ODN 2007 Control (5 mg)	66
tlrl-2088	ODN 2088 (200 µg)	67
tlrl-2088-1	ODN 2088 (1 mg)	67
tlrl-2088c	ODN 2088 control (200 µg)	67
tlrl-2088c-1	ODN 2088 control (1 mg)	67
tlrl-2216	ODN 2216 (200 µg)	66
tlrl-2216-1	ODN 2216 (1 mg)	66
tlrl-2216-5	ODN 2216 (5 mg)	66
tlrl-2216b	ODN 2216 Biotin (50 µg)	66
tlrl-2216c	ODN 2216 control (200 µg)	66
tlrl-2216c-1	ODN 2216 control (1 mg)	66
tlrl-2216c-5	ODN 2216 control (5 mg)	66
tlrl-2216f	ODN 2216 FITC (50 µg)	66
tlrl-2336	ODN 2336 (200 µg)	67
tlrl-2336-1	ODN 2336 (1 mg)	67
tlrl-2336-5	ODN 2336 (5 mg)	67
tlrl-2336c	ODN 2336 control (200 µg)	67
tlrl-2336c-1	ODN 2336 control (1 mg)	67
tlrl-2336c-5	ODN 2336 control (5 mg)	67
tlrl-2336f	ODN 2336 FITC (50 µg)	67
tlrl-2395	ODN 2395 (200 µg)	67
tlrl-2395-1	ODN 2395 (1 mg)	67
tlrl-2395-5	ODN 2395 (5 mg)	67
tlrl-2395c	ODN 2395 control (200 µg)	67
tlrl-2395c-1	ODN 2395 control (1 mg)	67
tlrl-2395c-5	ODN 2395 control (5 mg)	67
tlrl-2395f	ODN 2395 FITC (50 µg)	67
tlrl-3ma	3-Methyladenine (50 mg)	90
tlrl-3pelps	LPS-EB Ultrapure (5 mg)	64

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tlrl-3prna	5'ppp-dsRNA (25 µg)	68
tlrl-3prna-100	5'ppp-dsRNA (100 µg)	68
tlrl-3prnac	5'ppp-dsRNA Control (25 µg)	68
tlrl-3prnac-100	5'ppp-dsRNA Control (100 µg)	68
tlrl-4084	ODN 4084-F (200 µg)	67
tlrl-ag4	AG490 (10 mg)	90
tlrl-alk	Alum Crystals (1 g)	68
tlrl-apr	2-Aminopurine (250 mg)	90
tlrl-atp	ATP (1 g)	68
tlrl-b82	Bay11-7082 (10 mg)	90
tlrl-baf	Bafilomycin A1 (10 µg)	90
tlrl-bblps	LPS-EB Biotin (500 µg)	64
tlrl-bc264	CL264 Biotin (100 µg)	65
tlrl-bmdp	MDP Biotin (500 µg)	67
tlrl-bpam2	Pam2CSK4 Biotin (50 µg)	64
tlrl-bpms	Pam3CSK4 Biotin (50 µg)	64
tlrl-bsfla	FLA-BS (100 µg)	65
tlrl-bx7	BX795 (5 mg)	90
tlrl-c12dap	C12-iE-DAP (1 mg)	67
tlrl-c264s	CL264 (500 µg)	65
tlrl-c264-5	CL264 (5 mg)	65
tlrl-c75	CL075 (500 µg)	65
tlrl-c75-5	CL075 (5 mg)	65
tlrl-c97	CL097 (500 µg)	65
tlrl-c97-5	CL097 (5 mg)	65
tlrl-chq	Chloroquine (250 mg)	90
tlrl-cli95	CLI-095 (1 mg)	90
tlrl-cpgg	pCpG-Giant (1 mg)	67
tlrl-cppd	CPPD crystals (5 mg)	68
tlrl-curd	Curdlan (1 g)	68
tlrl-cyca	Cyclosporin A (100 mg)	90
tlrl-dap	iE-DAP (5 mg)	67
tlrl-dex	Dexamethasone (100 mg)	90
tlrl-dzn	Zymosan Depleted (10 mg)	68
tlrl-eblps	LPS-EB (5 mg)	64
tlrl-ednaef	E. coli DNA ef (1 mg)	66
tlrl-eklps	LPS-EK (5 mg)	64
tlrl-fc264	CL264 FITC (100 µg)	65
tlrl-flic	FLA-ST recombinant (1 µg)	65
tlrl-flic-10	FLA-ST recombinant (10 µg)	65
tlrl-fmdp	MDP FITC (500 µg)	67
tlrl-fsl	FSL-1 (100 µg)	64
tlrl-gdqs	Gardiquimod (500 µg)	65
tlrl-gdq-5	Gardiquimod (5 mg)	65
tlrl-gef	Gefitinib (10 mg)	90
tlrl-gly	Glybenclamide (1 g)	90
tlrl-gmdp	N-Glycolyl-MDP (5 mg)	68

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tlrl-godn	G-ODN (200 µg)	67
tlrl-h89	H-89 (5 mg)	90
tlrl-hkal	HKAL (10^9 cells)	64
tlrl-hkca	HKCA (10^9 cells)	68
tlrl-hkeb	HKEB (10^{10} cells)	64
tlrl-hkhp	HKHP (10^9 cells)	64
tlrl-hklm	HKLM (10^{10} cells)	64
tlrl-hklp	HKLP (10^9 cells)	64
tlrl-hklr	HKLR (10^{10} cells)	64
tlrl-hkmf	HKMF (10^9 cells)	64
tlrl-hkpa	HKPA (10^{10} cells)	64
tlrl-hkpg	HKPG (10^{10} cells)	64
tlrl-hksa	HKSA (10^{10} cells)	64
tlrl-hksc	HKSC (10^9 cells)	68
tlrl-hksp	HKSP (10^{10} cells)	64
tlrl-hz	Hemozoin (5 mg)	68
tlrl-imqs	Imiquimod (500 µg)	65
tlrl-imq	Imiquimod (5 mg)	65
tlrl-inh1	ODN INH-1 (200 µg)	67
tlrl-inh47	ODN INH-47 (200 µg)	67
tlrl-kipgn	PGN-ECndi ultrapure, insoluble (5 mg)	68
tlrl-kit1hw	TLR1-9 Agonist Kit-Human (10 ligands)	69
tlrl-kit1mw	TLR1-9 Agonist Kit-Mouse (9 ligands)	69
tlrl-kit2hm	TLR2 Agonist Kit (7 ligands)	69
tlrl-kit3hw3	TLR3/7/8/9 Agonist Kit (14 ligands)	69
tlrl-ksspgn	PGN-ECndss ultrapure, soluble (1 mg)	68
tlrl-l37	LL-37 (1 mg)	90
tlrl-lams	LAM-MS (500 µg)	64
tlrl-lep	Leptomycin B (5 µg)	90
tlrl-lmdp	L18-MDP (1 mg)	67
tlrl-lmms2	LM-MS (250 µg)	64
tlrl-lox	Loxoribine (50 mg)	65
tlrl-lpu	ssPolyU / LyoVec (100 µg)	65
tlrl-lrna40	ssRNA40 / LyoVec (100 µg)	65
tlrl-lrna41	ssRNA41 / LyoVec (100 µg)	65
tlrl-lta	LTA-BS (5 mg)	64
tlrl-ly29	LY294002 (5 mg)	90
tlrl-lys	iE-Lys (5 mg)	67
tlrl-m362	ODN M362 (200 µg)	67
tlrl-m362-1	ODN M362 (1 mg)	67
tlrl-m362-5	ODN M362 (5 mg)	67
tlrl-m362c	ODN M362 control (200 µg)	67
tlrl-m362c-1	ODN M362 control (1 mg)	67
tlrl-m362c-5	ODN M362 control (5 mg)	67
tlrl-m362f	ODN M362 FITC (50 µg)	67
tlrl-mbt	Murabutide (5 mg)	68
tlrl-mbtc	Murabutide control (5 mg)	68

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tlrl-mdp	MDP (5 mg)	67
tlrl-mdpc	MDP control (5 mg)	67
tlrl-mg132	MG-132 (5 mg)	90
tlrl-mpl	MPLA (1 mg)	65
tlrl-mpls	MPLAs (1 mg)	65
tlrl-msu	MSU Crystals (5 mg)	68
tlrl-mtd	M-TriDAP (1 mg)	68
tlrl-mtl	M-TriLYS (1 mg)	68
tlrl-mtn	M-TriLYS-D-ASN (1 mg)	68
tlrl-nig	Nigericin (10 mg)	68
tlrl-nig-5	Nigericin (50 mg)	68
tlrl-nodkit2	NOD1/2 Agonist Kit (10 ligands)	69
tlrl-orn2	ORN02 / LyoVec (4 x 25 µg)	67
tlrl-orn6	ORN06 / LyoVec (4 x 25 µg)	67
tlrl-oxp1	OxPAPC (1 mg)	90
tlrl-patc	Poly(dA:dT) / LyoVec (100 µg)	68
tlrl-patn	Poly(dA:dT) Naked (200 µg)	68
tlrl-patn-1	Poly(dA:dT) Naked (1 mg)	68
tlrl-pau	Poly(A:U) (10 mg)	64
tlrl-pct	Piceatannol (5 mg)	90
tlrl-pd98	PD98059 (10 mg)	90
tlrl-pgcc	Poly(dG:dC) / LyoVec (100 µg)	68
tlrl-pgcn	Poly(dG:dC) Naked (200 µg)	68
tlrl-peklps	LPS-EK Ultrapure (1 mg)	65
tlrl-pglps	LPS-PG (1 mg)	64
tlrl-pgnbs	PGN-BS (5 mg)	64
tlrl-pgnec	PGN-EB (1 mg)	64
tlrl-pgnek	PGN-EK (1 mg)	64
tlrl-pgnsa	PGN-SA (5 mg)	64
tlrl-pic	Poly(I:C) (HMW) (10 mg)	64
tlrl-pic-5	Poly(I:C) (HMW) (50 mg)	64
tlrl-picf	Poly(I:C) (HMW) Fluorescein (10 µg)	64
tlrl-piclv	Poly(I:C) (HMW) / LyoVec (100 µg)	68
tlrl-piclv-10	Poly(I:C) (HMW) / LyoVec (1 mg)	68
tlrl-picr	Poly(I:C) (HMW) Rhodamine (10 µg)	64
tlrl-pictrl	Pepinh-Control (2 mg)	90
tlrl-picw	Poly(I:C) (LMW) (25 mg)	64
tlrl-picw-250	Poly(I:C) (LMW) (250 mg)	64
tlrl-picwlv	Poly(I:C) (LMW) / LyoVec (100 µg)	68
tlrl-picwlv-10	Poly(I:C) (LMW) / LyoVec (1 mg)	68
tlrl-pimyd	Pepinh-MYD (2 mg)	90
tlrl-pitram	Pepinh-TRAM (2 mg)	90
tlrl-pitrif	Pepinh-TRIF (2 mg)	90
tlrl-piwr	Poly(I:C) (LMW) Rhodamine (10 µg)	64
tlrl-pm2s	Pam2CSK4 (100 µg)	64
tlrl-pm2s-1	Pam2CSK4 (1 mg)	64
tlrl-pma	PMA (5 mg)	90

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tlrl-pmb	Polymyxin B (100 mg)	90
tlrl-pms	Pam3CSK4 (1 mg)	64
tlrl-prslps	LPS-RS Ultrapure (1 mg)	65
tlrl-pslta	LTA-SA Purified (5 mg)	64
tlrl-pstfla	FLA-ST Ultrapure (10 µg)	65
tlrl-pstfla-5	FLA-ST Ultrapure (50 µg)	65
tlrl-pt17	Poly(dT) (100 nmol)	65
tlrl-r848	R848 (500 µg)	65
tlrl-r848-5	R848 (5 mg)	65
tlrl-rap	Rapamycin (5 mg)	90
tlrl-rc264	CL264 Rhodamine (100 µg)	65
tlrl-resv	Resveratrol (100 mg)	90
tlrl-rmdp	MDP Rhodamine (500 µg)	67
tlrl-rpam2	Pam2CSK4 Rhodamine (50 µg)	64
tlrl-rpms	Pam3CSK4 Rhodamine (50 µg)	64
tlrl-rslps	LPS-RS (5 mg)	65
tlrl-sb20	SB203580 (5 mg)	90
tlrl-sb90	SB202190 (5 mg)	90
tlrl-sdef	Salmon sperm DNA (50 mg)	67
tlrl-sio	Nano-SiO2 (10 mg)	68
tlrl-sipgn	PGN-SAndi ultrapure, insoluble (5 mg)	68
tlrl-slta	LTA-SA (5 mg)	64
tlrl-smlps	LPS-SM Ultrapure (5 mg)	65
tlrl-sp60	SP600125 (10 mg)	90
tlrl-ssec	E. coli ssDNA / LyoVec (200 µg)	66
tlrl-ssdr	ssRNA-DR / LyoVec (100 µg)	65
tlrl-sspu	ssPolyU Naked (10 mg)	65
tlrl-stfla	FLA-ST (100 µg)	65
tlrl-tdap	Tri-DAP (1 mg)	67
tlrl-tdb	TDB (1 mg)	68
tlrl-test-1	Compound Dose Response	82
tlrl-test-2	Compound Profiling	82
tlrl-tlys	Tri-Lys (1 mg)	67
tlrl-ttag	ODN TTAGGG (200 µg)	67
tlrl-ttag-1	ODN TTAGGG (1 mg)	67
tlrl-ttagc	ODN TTAGGG control (200 µg)	67
tlrl-ttagc-1	ODN TTAGGG control (1 mg)	67
tlrl-txf	Tamoxifen (200 mg)	90
tlrl-u0126	U0126 (5 mg)	90
tlrl-vad	Z-VAD-FMK (1 mg)	90
tlrl-wgp	WGP Dispersable (50 mg)	68
tlrl-wgps	WGP Soluble (50 mg)	68
tlrl-wtm	Wortmannin (5 mg)	90
tlrl-zyn	Zymosan (100 mg)	68
vac-1585-1	ODN 1585 VacciGrade (1 mg)	104
vac-1826-1	ODN 1826 VacciGrade (1 mg)	104
vac-2006-1	ODN 2006 VacciGrade (1 mg)	104

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vac-adx-2	AddaVax™ (2 ml)	104
vac-adx-10	AddaVax™ (5 x 2 ml)	104
vac-alu-50	Alhydrogel 2% (50 ml)	104
vac-alu-250	Alhydrogel 2% (250 ml)	104
vac-efova	EndoFit™ Ovalbumin (10 mg)	103
vac-fla	Flagellin FliC VacciGrade (50 µg)	104
vac-gdq	Gardiquimod VacciGrade (5 mg)	104
vac-gmdp	N-Glycolyl-MDP VacciGrade (5 mg)	104
vac-ifa-10	IFA (10 ml)	104
vac-ifa-60	IFA (6 x 10 ml)	104
vac-imq	Imiquimod VacciGrade (5 mg)	104
vac-isq	OVA 323-339 (1 mg)	103
vac-mpl	MPLA VacciGrade (1 mg)	104
vac-mpls	MPLAs VacciGrade (1 mg)	104
vac-ova	Ovalbumin (1 g)	103
vac-pic	Poly(I:C) VacciGrade (10 mg)	104
vac-pms	Pam3CSK4 VacciGrade (1 mg)	104
vac-r848	R848 VacciGrade (5 mg)	104
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