INNATE IMMUNITY

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PATTERN RECOGNITION RECEPTORS

The innate immune system is an evolutionally conserved mechanism that provides an early and effective response against invading microbial pathogens. It relies on a limited set of pattern recognition receptors (PRRs) that recognize specific pathogen-associated molecular patterns (PAMPs) commonly present in microbes but not in host. Upon detection of PAMPs, the PRRs trigger an inflammatory response that recruits phagocytic cells, induce antimicrobial peptides and cytokine/chemokine secretion, leading to efficient destruction of the invading pathogens. Three main families of PRRs have been shown to initiate proinflammatory signaling pathways: the Toll-Like receptors (TLRs), the NOD-Like receptors (NLRs) and RIG-I-Like receptors (RLRs).

Toll-Like Receptors (TLRs)

TLRs are the first identified and best characterized receptors among the signaling PRRs. They initiate key inflammatory responses and also shape adaptative 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 region called the Toll/IL-1 receptor (TIR) domain. They 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. TLRs initiate shared and distinct signaling pathways by recruiting different combinations of four TIR-domain-containing adaptor molecules: MyD88, TIRAP (MAL), TRIF (TICAM1) and TRAM (TICAM2). 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 (IRFs) leading to the production of type I interferons.

Nod-Like Receptors (NLRs)

NOD-Like Receptors (NLRs, also known as CATERPILLERs) 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 signal, inducing immune responses. NLRs are characterized by a tripartite-domain organization with a conserved nucleotide binding oligomerization domain (NOD) and leucine-rich repeats (LRRs). The general domain structure consists of C-terminal LRRs involved in microbial sensing, a centrally located NOD domain and an N-terminal effector region comprising a protein-protein interaction domain such as the CARD, Pyrin or BIR domain. NLRs have been grouped into several subfamilies on the basis of their effector domains: NODs, NALPs, CIITA, IPAF, and NAIPs. NODs and IPAF contain CARD effector domains, whereas NALPs and NAIPs contain pyrin (PYD) effector domains and three BIR domains, respectively.

RIG-I-Like Receptors (RLRs)

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. 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.

C-Type Lectin Receptors (CLRs) and Other Pathogen Sensors

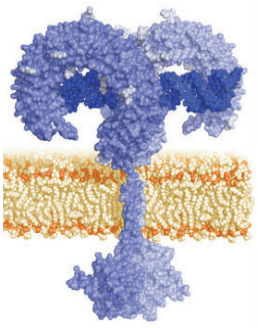
Besides TLRs, NLRs and RLRs, other receptors can also recognize molecules present on pathogenic organisms. Those receptors include members of the PGRP (peptidoglycan recognition proteins) and C-type lectin families. C-type lectins, also called C-type lectin receptors (CLRs), encompass a large family of proteins that act as phagocytic receptors that bind carbohydrate moieties of various pathogens. This family comprises MBL (mannose binding lectin), Dectin-1, DC-SIGN (dendritic cell-specific intercellular adhesion molecule-grabbing nonintegrin) and the structurally related receptors SIGNRs.

Toll-Like Receptors

Toll-Like Receptors (TLRs) play a critical role in the early innate immune response to invading pathogens by sensing microorganisms. These evolutionarily conserved receptors, homologues of the Drosophila Toll gene, recognize highly conserved structural motifs only expressed by microbial pathogens, called pathogen-associated microbial patterns (PAMPs). 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. Stimulation of TLRs by PAMPs initiates signaling cascades that involves a number of proteins, such as MyD88, TRIF and IRAK[1]. These signaling cascades lead to the activation of transcription factors, such as AP-1, NF-κB and IRFs inducing the secretion of 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 intracellularly[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 next 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 microbial antigens, TLRs induce the recruitment of MyD88 via its TIR domain which in turn recruits IRAK1 and IRAK4. IRAK4 then activates IRAK-1 by phosphorylation. Both IRAK-1 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, IRAK-2 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.

Differences between signaling pathways induced by individual TLRs are emerging. 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 (see Chapter "Cytokine Signaling"). They involve the interferon regulatory factors (IRFs), a growing 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].

Current knowledge of the TLRs indicates that these receptors are essential elements in host defense against pathogens by activating the innate immunity, a prerequisite to induction of adaptive immunity. The growing interest in TLRs should bring a more complete understanding of the role of TLR-mediated responses and increase our range of weapons to treat infectious and immune diseases.

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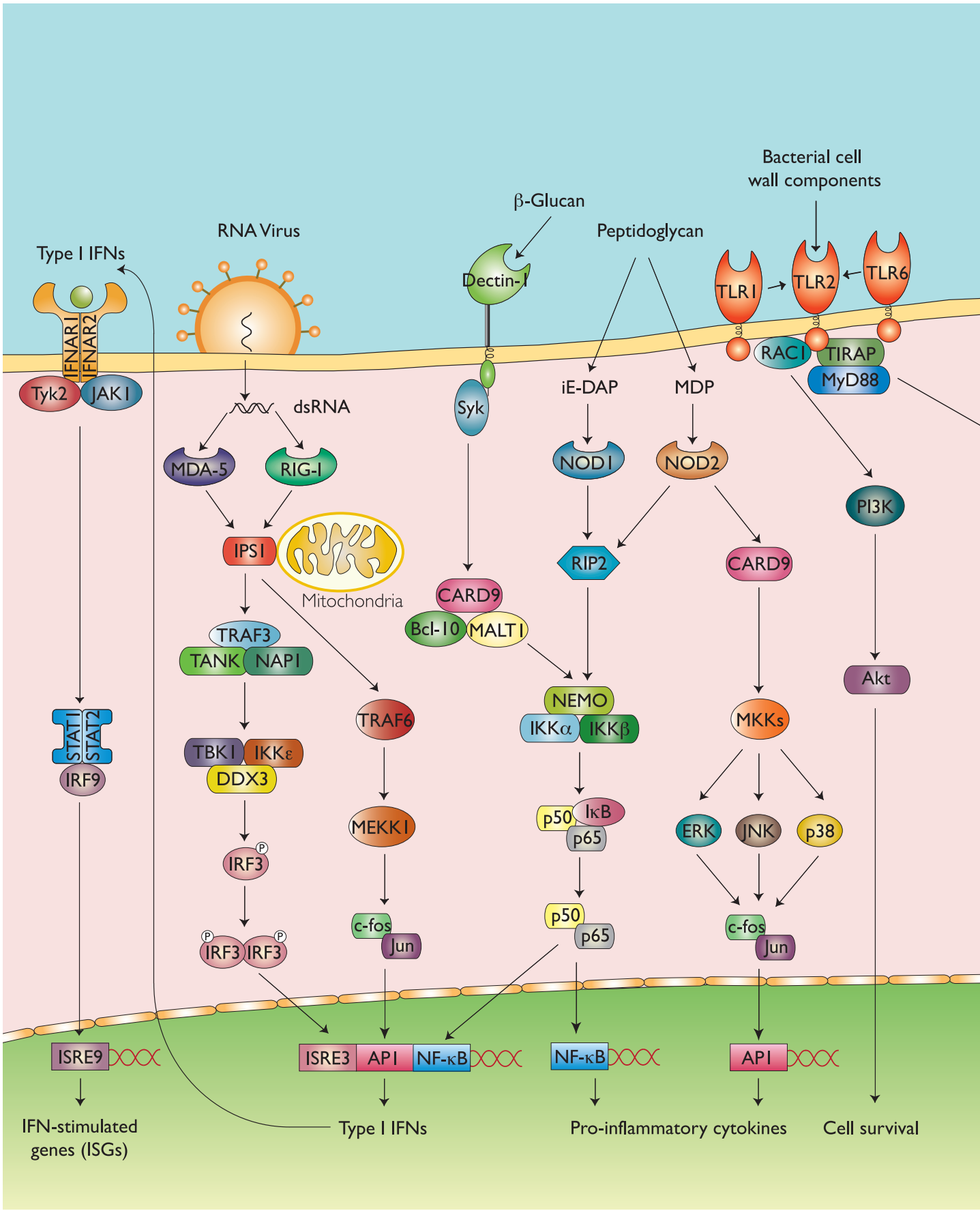
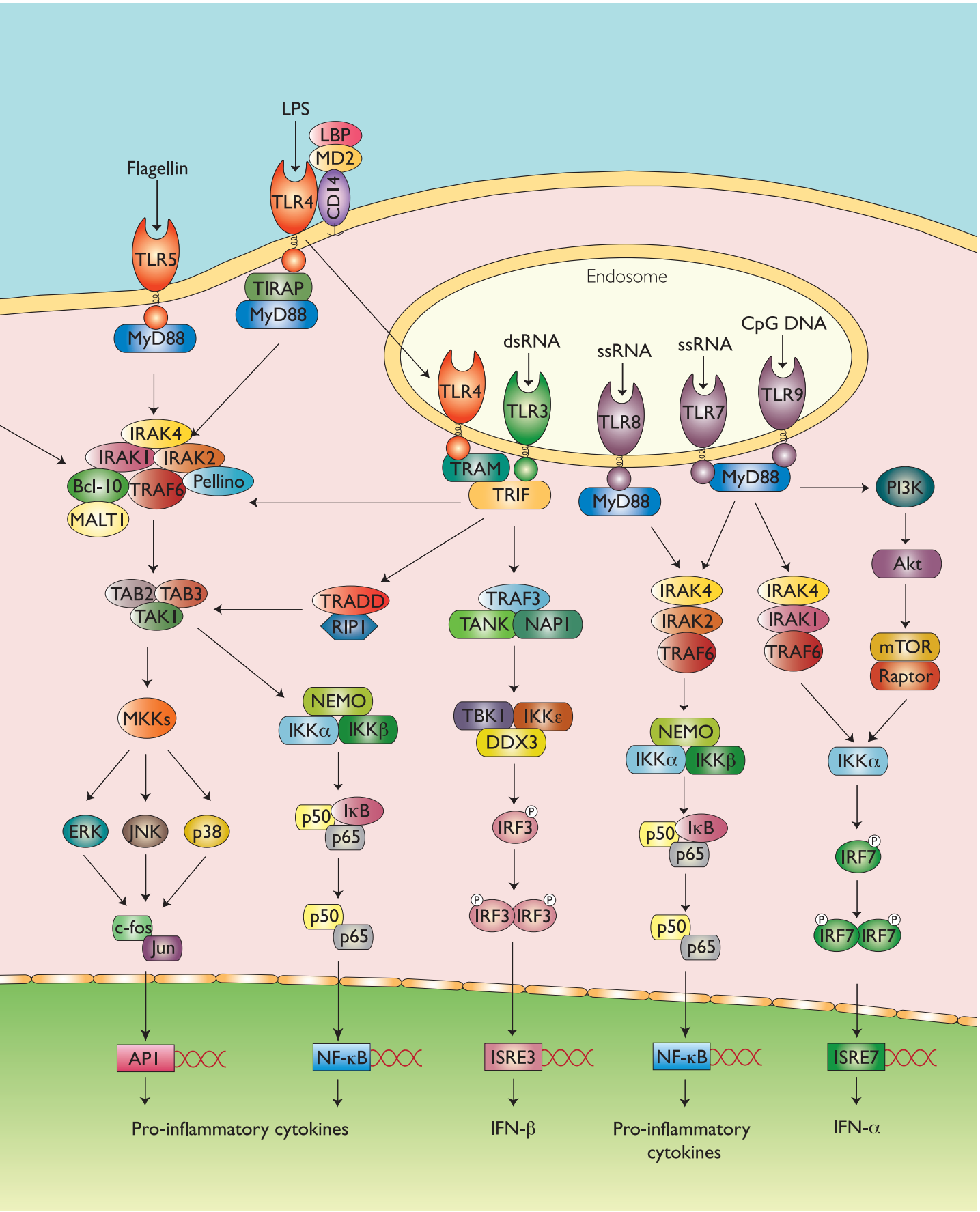

Type I IFNs
IFNAR1
IFNAR2
Tyk2
JAK1
STAT1
STAT2
IRF9
ISRE9
IFN-stimulated genes (ISGs)
RNA Virus
dsRNA
MDA-5
RIG-I
IPS1
Mitochondria
TRAF3
TANK
NAP1
TBK1
IKKε
DDX3
IRF3
IRF3
IRF3
TRAF6
MEKK1
c-fos
Jun
ISRE3
AP1
NF-κB
Type I IFNs
β-Glucan
Dectin-1
Syk
CARD9
Bcl-10
MALT1
Peptidoglycan
iE-DAP
MDP
NOD1
NOD2
RIP2
NEMO
IKKα
IKKβ
p50
IκB
p65
p50
p65
NF-κB
Pro-inflammatory cytokines
Bacterial cell wall components
TLR1
TLR2
TLR6
RAC1
TIRAP
MyD88
CARD9
MKKs
ERK
JNK
p38
c-fos
Jun
AP1
PI3K
Akt
Cell survival


LPS
LBP
MD2
Flagellin
TLR4
CD14
TLR5
TIRAP
MyD88
MyD88
Endosome
dsRNA
ssRNA
ssRNA
CpG DNA
TLR4
TLR3
TLR8
TLR7
TLR9
TRAM
TRIF
MyD88
MyD88
PI3K
IRAK4
IRAK1
IRAK2
Bcl-10
TRAF6
Pellino
MALT1
TAB2
TAB3
TAK1
TRADD
RIP1
TRAF3
TANK
NAP1
IRAK4
IRAK2
TRAF6
IRAK4
IRAK1
TRAF6
Akt
mTOR
Raptor
MKKs
NEMO
IKKα
IKKβ
TBK1
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DDX3
NEMO
IKKα
IKKβ
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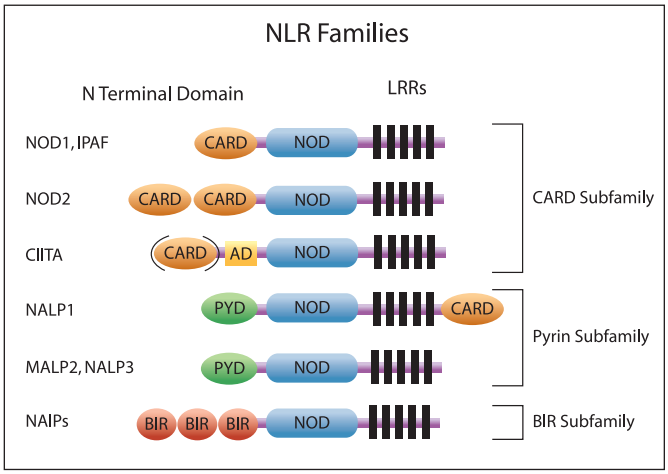
ERK
JNK
p38
p50
IκB
p65
IRF3
p50
IκB
p65
IRF7
c-fos
Jun
p50
p65
IRF3
IRF3
p50
p65
IRF7
IRF7
AP1
NF-κB
ISRE3
NF-κB
ISRE7
Pro-inflammatory cytokines
IFN-β
Pro-inflammatory cytokines
IFN-α

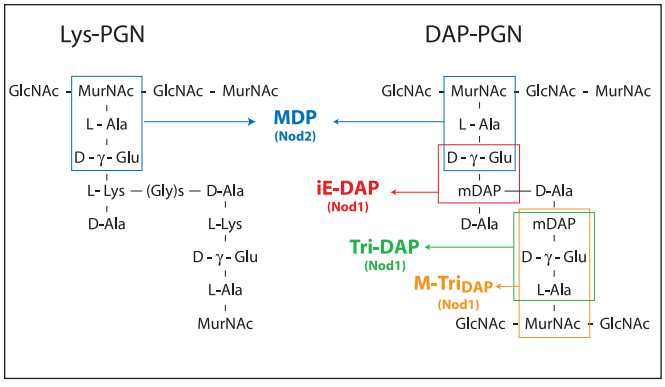
Nod-Like Receptors

NOD-Like Receptors (NLRs, also known as CATERPILLERs) 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 also called damage-associated molecular patterns (DAMPs), inducing immune responses. NLRs are characterized by a tripartite-domain organization with a conserved nucleotide binding oligomerization domain (NOD) and leucine-rich repeats (LRRs). The general domain structure consists of C-terminal LRRs involved in microbial sensing, a centrally located NOD domain and an N-terminal effector region comprising a protein-protein interaction domain such as the CARD, Pyrin or BIR domain. NLRs have been grouped into several subfamilies on the basis of their effector domains: NODs, NALPs, CIITA, IPAF, and NAIPs. NODs and IPAF contain CARD effector domains, whereas NALPs and NAIPs contain pyrin (PYD) effector domains and three BIR domains, respectively.



NOD1 and NOD2

The first mammalian NLRs reported to sense intracellular microbial PAMPs are NOD1 (CARD4) and NOD2 (CARD15), which contain one and two N-terminal CARD domains, respectively. They recognize peptidoglycan (PGN), an essential constituent of the bacterial cell wall. NOD1 and NOD2 detect specific motifs within the PGN. 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[3]. 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 to be detected by NOD1 and NOD2 respectively. NOD1 and NOD2 signal via the serine/threonine RIP2 (RICK,CARDIAK) kinase through CARD-CARD homophilic interactions[4]. Once activated, RIP2 mediates ubiquitination of NEMO/IKKγ leading to the activation of NF-κB and the production of inflammatory cytokines such as TNF-α and IL-6[5]. In addition to the NF-κB pathway, NOD1 and NOD2 stimulation induces the activation of MAPKs[6]. Recent work has implicated CARD9 in the selective control of NOD2-dependent p38 and JNK signaling[7]. The physiological importance of NOD1 and NOD2 in immune responses is evident from the linkage of their mutations with inflammatory diseases in humans. Genetic variation in NOD2 is associated with Crohn's disease, one of the major forms of inflammatory bowel diseases[8]. Several NOD1 polymorphisms are linked to the development of atopic eczema and asthma[9].

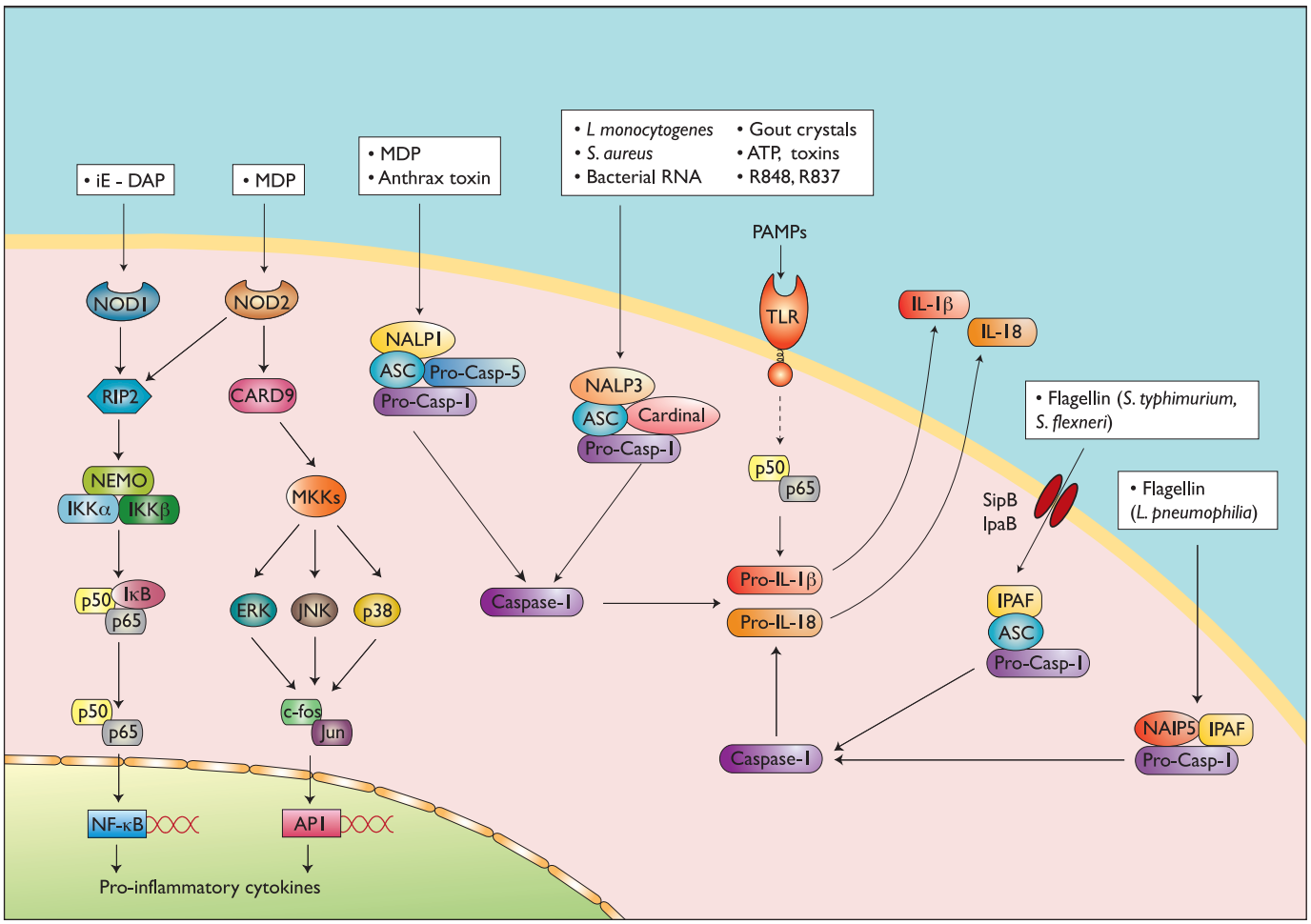


Schematic structure of Lys-PGN (found in Gram-positive bacteria) and DAP-PGN (found in Gram-negative bacteria)

NALPs

The NALP subfamily consists of 14 members that are characterized by the presence of PYD effector domains. Although the functions of many of the NALPs are largely unknown, several NALPs play a key role in the regulation of caspase-1 by forming a mutiprotein complex known as the 'inflammasome'. Caspase-1 participates in the processing and subsequent release of proinflammatory cytokines, such as IL-1β and IL-18. At least two types of NALP inflammasomes have been identified: the NALP1 inflammasome comprising NALP1 (NLRP1, CARD7), ASC, Caspase-1 and Caspase-5, and the NALP3 inflammasome containing NALP3 (NLRP3, cryopyrin, CIAS1), ASC, Cardinal and Caspase-1[10]. NALP1 and NALP3 recruit through their PYD domain the adaptor protein ASC which in turn interacts with Caspase-1 via a CARD-CARD interaction. NALP1 also recruits Caspase-5 via its additional CARD effector domain at the C terminus, whereas NALP3, lacking such a CARD, interacts with the CARD-containing adaptor Cardinal to recruit additional Caspase-1.

Two molecules have been recently described to activate NALP1: MDP and the anthrax toxin. *In vitro* reconstitution experiments revealed that NALP1 oligomerization is a two step mechanism requiring MDP and ATP[11]. *In vivo* studies with NALP1 knock-out mice are necessary to confirm that MDP is a true NALP1 ligand. A murine variant of NALP1 (NALP1b) was shown to respond to the anthrax toxin suggesting an engagement of the NALP1 inflammasome in the immune response to *Bacillus anthracis* infection[12]. NALP3 mediates caspase-1 activation in response to a wide variety of stimuli: whole bacteria (*Listeria monocytogenes*, *Staphylococcus aureus*), bacterial RNA, synthetic purine-like compounds (R848, R837), uric acid crystals, extracellular ATP and pore-forming toxins (nigericin, maitotoxin)[13-15]. Activation of Caspase-1 induced by NALP3 appears to be TLR-independent, whereas secretion of mature IL-1β seems to require two stimuli involving the TLRs and NALP3. The first stimulus, a TLR ligand such as LPS, triggers the generation of pro-IL-1β, while the second, a stimulus such as ATP, induces oligomerization and inflammasome assembly[16-17]. In addition, studies suggest that NALP3 does not sense ATP directly but rather intracellular potassium depletion resulting from ATP signaling. Bacterial toxins, such as nigericin and maitotoxin that cause a change in intracellular ion composition activate the NALP3 inflammasome. Thus, NALP3 appears to serve as an activator of the inflammasome in response to specific toxins, endogenous danger signals released by damaged cells or tissues (uric acid crystal, elevated ATP), or microbial pathogens. Mutations in NALP3 are the cause for several human diseases such as familial cold autoinflammatory syndrome and Muckle-Wells syndrome[18].



IPAF and NAIP5

IPAF (NLRC4, CLAN/CARD12) and NAIP5 constitute another set of NLRs. IPAF belongs to the CARD subfamily whereas NAIP5 is a member of the BIR subfamily. Both NLRs have been shown to respond to flagellin, the main component of the bacterial flagellum, restricting the proliferation of intracellular bacteria such as *Salmonella typhimurium*, *Shigella flexneri* and *Legionella pneumophila*. IPAF senses flagellin from *S. typhimurium* and *S. flexneri* secreted by the bacterial type III secretion system (TTSS). SipB in *S. typhimurium* and IpaB in *S. flexneri* are part of a TTSS and are required for Caspase-1 activation. These proteins participate in the translocation of flagellin in the cytosol by forming a pore in the host-cell membrane. Caspase-1 activation induced by cytosolic flagellin has been shown to be IPAF-dependent, but TLR5-independent[19]. Thus TLR5 and IPAF appear to be two different sensors that respond to extracellular and cytosolic flagellin, respectively[20]. The adaptor ASC seems to be involved in Caspase-1 activation in response to infection with *S. typhimurium* and *S. flexneri*[21]. Sensing of *L. pneumophila* flagellin which is secreted by the bacterial type IV secretion system is mediated by NAIP5. Mutations affecting the NAIP5 locus (also known as Birc1e) have been implicated in increased susceptibility of A/J macrophages to infection with *L. pneumophila*. NAIP5 appears to form an inflammasome complex containing IPAF (but not ASC) and Caspase-1[22]. NAIP5-mediated signaling pathway and IPAF-mediated Caspase-1 activation may act in concert to restrict *L. pneumophila* growth in macrophages[23,24].

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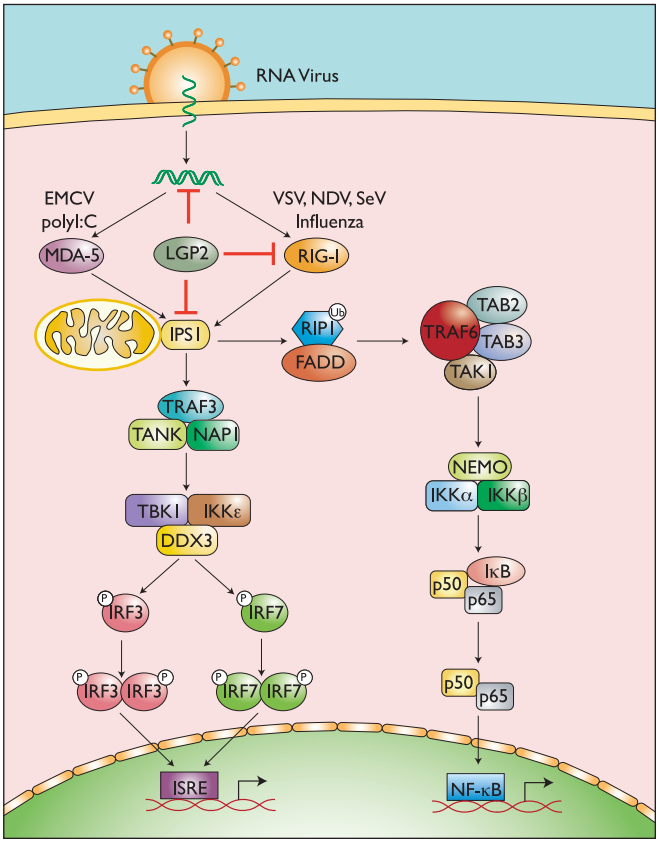
RIG-I-Like Receptors

RIG-I-like receptors (RLRs), also known as RIG-I-like helicases (RLHs) 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]. Mammalian RNA is either capped or contains base modifications suggesting that RIG-I is able to discriminate between self and non-self RNA. Recently Kato *et al.*, showed by using poly(I:C) treated with RNase III that RIG-I binds preferentially to short dsRNA while MDA-5 recognizes preferentially long dsRNA[5].



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[6]. 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)[6-8]. Recently, DDX3, a DEAD box helicase, was shown to interact with TBK1/IKKε[9]. 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[6-8, 10].

Because the production of cytokines is closely controlled, the RIG-I/MDA-5 pathway is also strictly regulated. 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[11-13]. 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. Furthermore, some viral proteases have been identified, such as NS3/NS4A of the hepatitis C virus, that inactivate RIG-I/MDA-5 signaling by targeting IPS-1 as effective means to evade innate immunity.

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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). CLRs include Dectin-1, macrophage-inducible C-type lectin (Mincle), the dendritic cell-specific ICAM3-grabbing nonintegrin (DC-SIGN), DC-SIGN related (DC-SIGNR) and the circulating mannose binding lectin (MBL). These CLRs share one or more CRD that were originally found in the mannose-binding lectin and are evolutionarily conserved. These receptors are involved in fungal recognition and the modulation of the innate immune response. CLRs are expressed by most cell types including macrophages and dendritic cells (DCs), which internalize various glycoproteins and microbes for the purposes of clearance and antigen presentation to T lymphocytes.

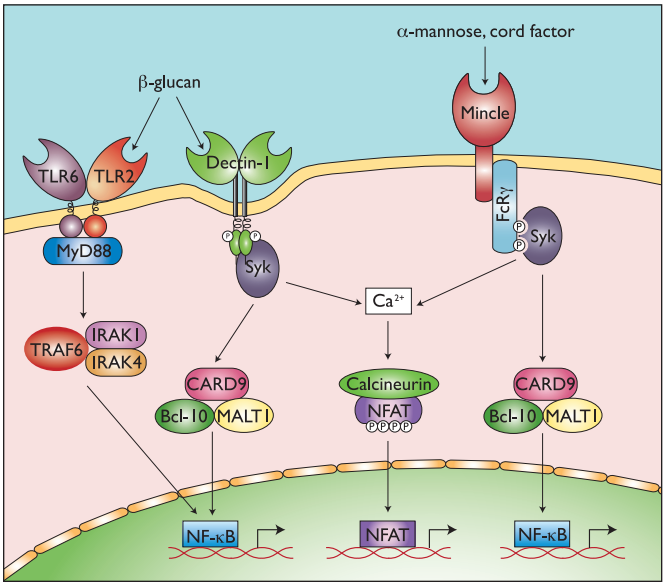
Dectin-1 plays an important role in antifungal innate immunity. Dectin-1, which is expressed on phagocytes, is a specific receptor for β-glucans[1]. β-Glucans are glucose polymers found in the cell walls of fungi, including the yeasts *Saccharomyces cerevisiae* and *Candida albicans*. Dectin-1 is a type II transmembrane protein with 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 subsequent secretion of proinflammatory cytokines[2, 3]. Dectin-1 signaling has been shown to collaborate with TLR2 signaling to enhance the responses triggered by each receptor[3, 4]. Furthermore, Dectin-1 can modulate cytokine expression by inducing NFAT through the Ca^{2+}-calcineurin-NFAT pathway[5].

Mincle is another CLR expressed in phagocytes which signaling leads to ITAM-dependent activation of NF-κB and NFAT. Mincle recognizes a variety of endogenous and exogenous stimuli, such as necrotic cells, mycobacteria and certain fungi, including *C. albicans* and *Malassezia species*[6]. Mincle senses damaged cells by recognizing spliceosome-associated protein 130 (SAP130), a soluble factor released by necrotic cells[7]. Mincle recognizes also fungal α-mannose, and the mycobacterial glycolipid, trehalose-6'6'-dimycolate (TDM, also known as cord factor), the most studied immunostimulatory component of *Mycobacterium tuberculosis*[8]. 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.

Human DC-SIGN is of special interest because it is involved in the interaction of certain DCs with several viruses (HIV-1, HCV, dengue virus, CMV, ebola virus) and other microbes, *Leishmania* and *Candida* species. This type II transmembrance protein has a single C-type lectin domain and is expressed on immature monocyte-derived DCs. Five mouse DC-SIGN homologues exist. Only one of these named mDC-SIGN is expressed in a restricted fashion in DCs, while the others have a different tissue distribution and are termed SIGNR1, SIGNR2, SIGNR3 and SIGNR4 for SIGN-related proteins[9]. Despite similarities in the carbohydrate recognition domains (CRDs) of the mouse homologues to the hDC-SIGN, the membrane proximal neck domains were much shorter.

A molecular signaling pathway induced by the C-type lectin DC-SIGN that modulates TLR signaling at the level of the transcription factor NF-κB has been identified. 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, but only after TLR-induced activation of NF-κB. Acetylation of p65 both prolonged and increased IL-10 transcription to enhance anti-inflammatory cytokine response. It has been demonstrated that different pathogens such as *Mycobacterium tuberculosis, M. leprae, Candida albicans*, measles virus, and HIV-1 interacted with DC-SIGN to activate the Raf-1-acetylation-dependent signaling pathway to modulate signaling by different TLRs[10]. Thus, this pathway is involved in regulation of adaptive immunity by DCs to bacterial, fungal, and viral pathogens.

MBL, a soluble C-type lectin, is an effector molecule of the innate immune system. MBL plays a crucial role in innate immunity against yeast by enhanced complement activation and enhanced uptake of polymorphonuclear cells[11]. 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[12]. Differences exist in polysaccharide recognition, endocytic capacities and microbe capture among CLRs. Furthermore, the ligands and physiological functions of many of the CLRs are still unknown. Mincle is one such orphan receptor. Mincle is the first example of a CLR that recognizes an endogenous nuclear ligand and necrotic cells[13].



1.Brown GD. *et al.* , 2003. Dectin-1 mediates the biological effects of beta glucans. J Exp Med. 197: 1119- 24. **2. Gross O. *et al.*, 2006.** Card9 controls a non-TLR signaling pathway for innate anti-fungal immunity. Nature. 442:651- 6. **3 Dennehy KM. & Brown GD., 2007.** The role of the beta-glucan receptor Dectin-1 in control of fungal infection . J Leukoc Biol.;82(2):253-8. **4. Gantner BN. *et al.*, 2003.** Collaborative induction of inflammatory responses by dectin-1 and Toll- like receptor 2. J Exp Med. 197: 1107-17. **5. 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. **6.Yamasaki S. *et al.*, 2009.** C-type lectin Mincle is an activating receptor for pathogenic fungus, Malassezia. PNAS 106(6): 1897–1902. **7. Yamasaki S. *et al.*, 2008.** Mincle is an ITAM-coupled activating receptor that senses damaged cells. Nat Immunol. 9(10):1179-88. **8. Ishikawa E. *et al.*, 2009.** Direct recognition of the mycobacterial glycolipid, trehalose dimycolate, by C-type lectin Mincle. J Exp Med. 206(13):2879-88. **9. Takahara K *et al.*, 2004.** Functional comparison of the mouse DC-SIGN, SIGNR1, SIGNR3 and Langerin C-type lectins. Int Immunol. 16:819-829. **10. den Dunnen J. *et al.*, 2008.** Innate signaling by C-type lectin DC-SIGN dictates immune responses. Cancer Immunol Immunother. 26:605-610. **11.Van Asbeck *et al.*, 2008.** Mannose binding lectin plays a crucila role in innate immunity against yeast by enhanced complment activation and enhanced uptake of polymorphonuclear cells. BMC Microbiol. 8:229. **12. Ji X. *et al.*, 2005.** Mannose-binding lectin binds to Ebola and Marburg envelope glycoproteins, resulting in blocking of virus interaction with DC-SIGN and complement-mediated virus neutralization. J Gen Virol. 86; 2535-2542. **13. Brown GD. 2008.** Sensing necrosis with Mincle. Nature Immunol. 9:1099-1100.

TLR, NLR, RLR & Related Genes

TLR, NLR, RLR & Related Genes is an expanding collection of genes encoding 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.

Native Genes (pUNO)

- Toll-Like receptor (TLR) & TLR co-receptor genes
- NOD-Like receptor (NLR) genes
- RIG-I-Like receptor (RLR) genes
- Other pathogen sensor genes
- Adaptor genes
- Signaling genes
- Signaling inhibitors
- Transcription factors

pUNO plasmids express native genes under the control of the strong and ubiquitous EF1α/HTLV composite promoter, comprised of the elongation factor 1 alpha (EF-1α) core promoter and the R-U5' of the human T cell leukemia virus (HTLV). pUNO plasmids are selectable with blasticidin allowing the selection of stable mammalian clones in only a few days.

HA-Tagged Genes (pUNO-HA)

- TLR genes
- Interferon regulatory factor (IRF) genes

pUNO-HA plasmids, which possess the same backbone as the pUNO plasmids, feature genes fused at their 3' end to the influenza hemaglutinine (HA) tag. This tag provides a simple and convenient method to detect genes by Western blot using the HA-tag antibody (see page 73).

Gene Associations (pDUO)

- TLR/TLR genes
- TLR/co-receptor genes
- Co-receptor/co-receptor genes

pDUO plasmids contain two transcription units allowing the co-expression of two TLR or TLR-related genes. They feature two strong composite promoters derived from the ferritin light chain (FerL) and heavy chain (FerH) core promoters. Most pDUO plasmids are selectable with blasticidin in both *E. coli* and mammalian cells. Some are selectable with hygromycin (pDUO2).

Dominant Negative Variants (pZERO-TLR & pDeNy)

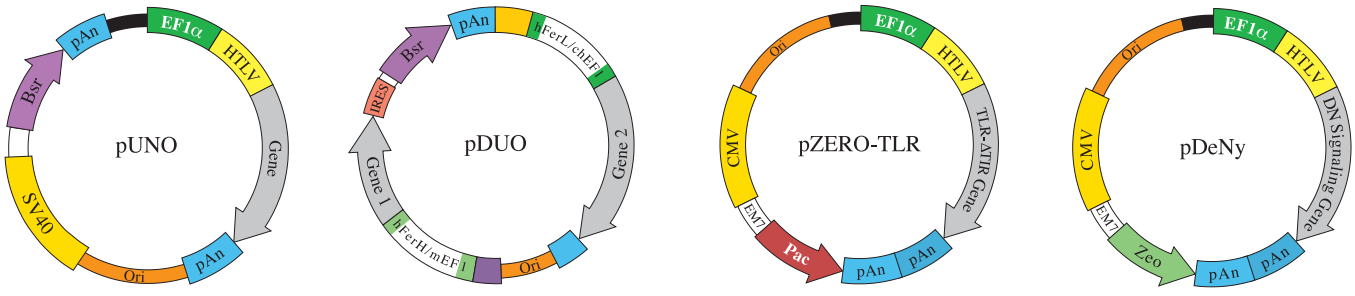
- TLR, NLR, RLR genes
- Adaptor and signaling genes

pZERO-TLR and pDeNy plasmids express dominant negative variants created by insertion of a mutation and/or deletion of a key region such as the TIR domain of the TLR genes. The variants are cloned under the control of the EF1α/HTLV composite promoter. pZERO-TLR and pDeNy are selectable with puromycin and Zeocin™, respectively.

HA-Tagged Dominant Negative Variants (pZERO-TLR-HA)

- TLR genes

pZERO-TLR-HA plasmids express HA-tagged TIR-deleted TLR genes that act as dominant negative variants. They can be detected by Western blot using the HA-tag antibody (see page 73).



	pUNO	pUNO-HA	pDUO	pZERO/pDeNy	pZERO-TLR-HA
Toll-Like Receptors (TLRs)					
TLR1	h, m, p	h, m	h, m	h, m	h, m
TLR2	h, m, p, b	h, m	h, m	h, m	h, m
TLR3	h, m	h, m	-	h, m	h, m
TLR4	h, m	h, m	h, m	h, m	h, m
TLR5	h, m, p, b	h, m	-	h, m	h, m
TLR6	h, m, p, b	h, m	h, m	h, m	h, m
TLR7	h, m, p, b	h, m	-	h, m	h, m
TLR8	h, m	h, m	-	h, m	h, m
TLR9	h, m, p, b	h, m	-	h, m	h, m
TLR10	h, p	h	-	h	h
TLR11	m	-	-	-	-
TLR11/12	m	m	-	-	-
TLR13	m	-	-	-	-
NOD-Like Receptors (NLRs)					
IPAF / CARD12	h	-	-	-	-
NAIP5 / BIRC1E	m	-	-	-	-
NALP1	h	-	-	-	-
NALP2	h	-	-	-	-
NALP3 / NLRP3	h	-	-	-	-
NALP12 / Monarch-1	h	-	-	-	-
NOD1 / CARD4	h, m	-	-	h	-
NOD2 / CARD15	h, m	-	-	h	-
NOD9 / NLRX1	h, m	-	-		-
RIG-I-Like Receptors (RLRs)					
LGP2	h, m	-	-	-	-
MDA-5	h	-	-	-	-
RIG-I / DDX58	h, m	-	-	h	-
Other Pathogen Sensors					
AIM2 / IFI210	h, m	-	-	-	-
CLEC9A	h	-	-	-	-
DAI / ZBP	h, m	-	-	-	-
DC-SIGN / CD209	h, m	-	-	-	-
Dectin-1	h, m	-	-	-	-
IFI16	h, m	-	-	-	-
L-SIGN	h	-	-	-	-
MBL1, 2	h, m	-	-	-	-
Mincle / CLEC4E	h, m	-	-	-	-
PGRP-L, -S, -Iα	h, m	-	-	-	-
SIGNR1, 2, 3, 4	m	-	-	-	-
Adaptors					
ASC / PYCARD / CARD5	h, m	-	-	-	-
Cardinal / CARD8	h	-	-	-	-
IPS1 / MAVS / VISA	h, m	-	-	-	-
MyD88	h, m	-	-	h, m	-
RAC1	m	-	-	-	-
SARM1	h, m	-	-	-	-
TIRAP / Mal	h, m	-	-	h	-
TRAM / TICAM2	h, m	-	-	h	-
TRIF / TICAM1	h, m	-	-	h	-
Co-receptors					
CD14	h, m	-	h, m	-	-
sCD14 (soluble)	h	-	-	-	-
CD36	h, m	-	-	-	-
LBP	h, m	-	-	-	-
MD2 / Ly96	h, m	-	h, m	-	-
PRAT4A, 4B	h, m	-	-	-	-

	pUNO	pUNO-HA	pDUO	pZERO/pDeNy	pZERO-TLR-HA
Signaling Effectors					
BCL10 / CLAP	h, m	-	-	-	-
CARD9	h, m	-	-	-	-
DDX3	m	-	-	-	-
FADD / MORT1	h, m	-	-	-	-
IKKα, β	h, m	-	-	-	-
IKKε	h, m	-	-	-	-
IRAK-1, -4	h, m	-	-	-	-
ITCH	h, m	-	-	-	-
MAPK1	h	-	-	-	-
MAPK2	h, m	-	-	-	-
NAP1 / AZI2	h, m	-	-	-	-
NEMO / IKKγ	h, m	-	-	-	-
Pellino1, 2, 3	h, m	-	-	-	-
PRKD1	h	-	-	-	-
PKR	h, m	-	-	h	-
PRKRA / PACT	h, m	-	-	-	-
RIP1 / RIPK1	h, m	-	-	-	-
RIP2 / RIPK2	h, m	-	-	-	-
RIP3 / RIPK3	h, m	-	-	-	-
STING	h, m	-	-	-	-
SUGT1	h, m	-	-	-	-
Syk	h, m	-	-	-	-
TAB1, 2, 3	h	-	-	-	-
TAK1 / MAP3K7	h, m	-	-	-	-
TANK	h, m	-	-	-	-
TBK1	h, m	-	-	-	-
TIFA	h, m	-	-	-	-
TRADD	h, m	-	-	-	-
TRAF3	h, m	-	-	-	-
TRAF6	h, m	-	-	-	-
UNC93B1	h, m	-	-	-	-
Signaling Inhibitors					
ABIN1, 2, 3	h, m	-	-	-	-
ATF3	h, m	-	-	-	-
AXL / UFO	h, m	-	-	-	-
Bcl-3	h, m	-	-	-	-
DAK	h, m	-	-	-	-
DUBA / OTUD5	m	-	-	-	-
FLII / Fliih	h, m	-	-	-	-
IRAK-M	h, m	-	-	-	-
MD-1	h, m	-	h, m	-	-
MFN2	h, m	-	-	-	-
MULAN / Dublin	h, m	-	-	-	-
PIN1 / DOB	h, m	-	-	-	-
PPP3CA, CB	h, m	-	-	-	-
PPP3R1	h, m				
RNF125 / TRAC-1	h, m	-	-	-	-
RP105	h, m	-	h, m	-	-
SHP-1 / PTPN6	h, m	-	-	-	-
SIGIRR / TIR8	h, m	-	-	-	-
SIKE	h, m	-	-	-	-
T1 / ST2 / IL1RL1	h	-	-	-	-
TNFAIP3 / A20	h, m	-	-	-	-
Tollip / IL-1RAcPIP	h, m	-	-	-	-
TRAFD1 / FLN29	h, m	-	-	-	-
TRIAD3A	h, m	-	-	-	-
Tyro3 / BYK	h, m	-	-	-	-

pUNO - Native Genes

Features and Benefits

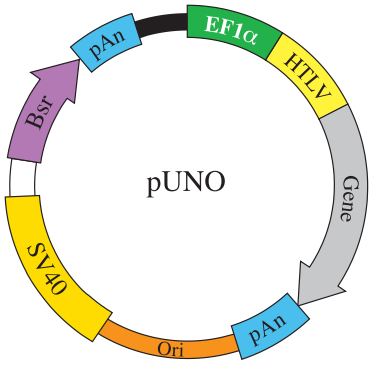
Numerous genes encoding pattern recognition receptors (PRRs), co-receptors, adaptors, signaling effectors and signaling inhibitors are available in the pUNO plasmid. The genes are cloned from the ATG to the Stop codon, excluding introns and untranslated regions. All genes are fully sequenced, the sequences 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 is comprised of 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 17
Hygromycin B, HygroGold™, page 18
Fast-Media® Blas, page 45
Fast-Media® Hygro, page 45

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
- pUNO3, selectable with hygromycin

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 **NEW**	-
TLR2	Toll-like Receptor 2	puno-htlr2	puno3-htlr2	puno-mtlr2	puno1-ptlr2 **NEW**	puno1-btlr2 **NEW**
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 **NEW**	puno1-btlr5 **NEW**
TLR6	Toll-like Receptor 6	puno-htlr6	puno3-htlr6	puno-mtlr6	puno1-ptlr6 **NEW**	puno1-btlr6 **NEW**
TLR7	Toll-like Receptor 7	puno-htlr7	puno3-htlr7	puno-mtlr7	puno1-ptlr7 **NEW**	puno1-btlr7 **NEW**
TLR8	Toll-like Receptor 8	puno1-htlr8b	puno3-htlr8b	puno-mtlr8	-	
TLR9	Toll-like Receptor 9	puno1-htlr9a	puno3-htlr9a	puno-mtlr9	puno1-ptlr9 **NEW**	puno1-btlr9 **NEW**
TLR10	Toll-like Receptor 10	puno-htlr10	puno3-htlr10	-	puno1-ptlr10 **NEW**	
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)			
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
Other Pathogen Sensors			
AIM2 / IFI210	Cytoplasmic DNA receptor - Caspase-1-activating protein	puno1-haim2	puno1-maim2
CLEC9A	C-type lectin-like receptor - Ligand unknown	puno1-hclec9a	puno1-mclec9a
DAI / ZBP1	B-DNA binding protein	puno1-hzbp1	puno1-mzbp1a
DC-SIGN / CD209	Mannose-binding C-type lectin	puno-hdcsign1a	puno-mdcsign
Dectin-1	Beta-glucan receptor	puno-hdectin1b	puno-mdectin1
IFI16 **NEW**	Cytosolic DNA sensor	puno1-hifi16	puno1-mifi16
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
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
IRAK-4	Signal transduction mediator for TLR signaling	puno-hirak4	puno-mirak4

GENE NAME/ALIASES	DESCRIPTION	CAT. CODE (HUMAN)	CAT. CODE (MOUSE)
Signaling Effectors			
ITCH NEW	p38 and JNK activator and NF-κB inhibitor	puno1-hitch	puno1-mitch
MAPK1 NEW	p38 and JNK activator	puno1-hmapk1	-
MAPK2 NEW	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	-
PKD1 / PKRD	Protein kinase D1	puno1-hprkd1	-
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	Signal transducer for TLRs and NODs	puno-hrick	puno-mrick
RIP3 / RIPK3	Modulator of NF-κB activation	puno-hripk3	puno-mripk3
STING NEW	Stimulator of interferon genes	puno1-hsting	puno1-msting
SUGT1 NEW	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
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 NEW	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 NEW	MyD88 and TRIF inhibitor - Calcineurin A (NFAT pathway)	puno1-hppp3cab	puno1-mppp3ca
PPP3CB NEW	MyD88 and TRIF inhibitor - Calcineurin B (NFAT pathway)	puno1-hppp3cb	puno1-mppp3cb
PPP3R1 NEW	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

pDUO - Gene Associations

Features and Benefits

TLR Associations in a Single Plasmid
pDUO is an expression vector containing two transcription units allowing the co-expression of two TLR or TLR-related genes.

Strong & Similar Levels of Expression of Both 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, an ubiquitous protein, therefore eliminating potential transcriptional interferences.

Rapid Selection of Stable Transfectants
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

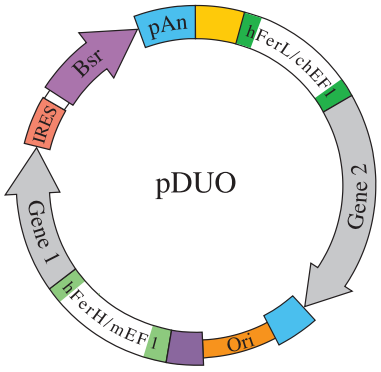
Each pDUO plasmid is provided as a lyophilized transformed *E. coli* strain on a paper disk. Transformed strains are shipped at room temperature and should be stored at -20°C. Lyophilized *E. coli* cells are stable for at least 1 year when properly stored.
Each pDUO plasmid is provided with 4 pouches of the appropriate *E.coli* Fast-Media® (2 TB and 2 Agar).

PRODUCT	QTY	CAT. CODE*
pDUO-<Genes> (Blasti)	*E. coli* disk	pduo-<genes>
pDUO2-<Genes> (Hygro)	*E. coli* disk	pduo2-<genes>

*See table

Related Products

Blasticidin, page 17
Fast-Media® Blas, page 45
Hygromycin B, page 18
Fast-Media® Hygro, page 45



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

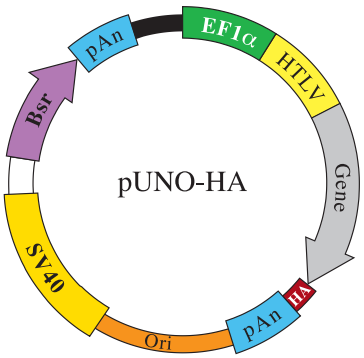
GENES	PLASMID	DESCRIPTION	CAT. CODE (human)	CAT. CODE (mouse)
CD14/TLR2	pDUO-CD14-TLR2	CD14 with Toll-like receptor 2	pduo-hcd14tlr2	pduo2-mcd14tlr2
CD14/TLR4	pDUO-CD14-TLR4	CD14 with Toll-like receptor 4	pduo-hcd14tlr4	pduo-mcd14tlr4
MD2/CD14	pDUO2-MD2-CD14 (hygro)	MD2 with CD14	pduo2-hmd2cd14	pduo2-mmd2cd14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

pUNO-HA - TLR-HA Genes

Features and Benefits

pUNO-HA is a family of expression vectors featuring HA-tagged TLR and IRF (Interferon Regulatory Factor) genes. The genes have been fused at the 3'end to the influenza hemaglutinine (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 73).

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



GENE	PLASMID	QTY	CAT. CODE (human)	CAT. CODE (mouse)
TLR1-HA	pUNO-TLR1-HA	*E. coli* disk	punoha-htlr1	punoha-mtlr1
TLR2-HA	pUNO-TLR2-HA	*E. coli* disk	punoha-htlr2	punoha-mtlr2
TLR3-HA	pUNO-TLR3-HA	*E. coli* disk	punoha-htlr3	punoha-mtlr3
TLR4-HA	pUNO-TLR4-HA	*E. coli* disk	punoha-htlr4a	punoha-mtlr4
TLR5-HA	pUNO-TLR5-HA	*E. coli* disk	punoha-htlr5	punoha-mtlr5
TLR6-HA	pUNO-TLR6-HA	*E. coli* disk	punoha-htlr6	punoha-mtlr6
TLR7-HA	pUNO-TLR7-HA	*E. coli* disk	punoha-htlr7	punoha-mtlr7
TLR8-HA	pUNO-TLR8-HA	*E. coli* disk	punoha-htlr8a	punoha-mtlr8
TLR9-HA	pUNO-TLR8-HA	*E. coli* disk	punoha-htlr9a	punoha-mtlr9
TLR10-HA	pUNO-TLR10-HA	*E. coli* disk	punoha-htlr10	-
TLR11-HA	pUNO-TLR11-HA	*E. coli* disk	-	punoha-mtlr11

Contents and Storage

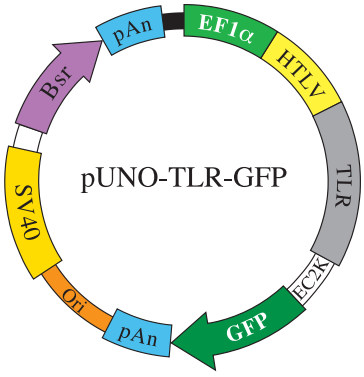
Each pUNO-HA plasmid is provided as a lyophilized transformed *E. coli* strain on a paper disk. Transformed strains are shipped at room temperature and should be stored at -20°C. Lyophilized *E. coli* cells are stable for at least 1 year when properly stored.
Each plasmid is provided with 4 pouches of *E.coli* Fast-Media® Blas (2 TB and 2 Agar). For more information about Fast-Media®, see pages 44-45.

pUNO-TLR-GFP - TLR-GFP Fusion Genes

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.

Features and Benefits

TLR-GFP fusion genes were generated by fusing the GFP gene to the C terminus of various human TLR genes (TLR1 to TLR6). The TLR-GFP fusion genes are cloned in the pUNO plasmid under the control of the strong and ubiquitous mammalian promoter EF1α/HTLV. The pUNO plasmid is 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.



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 pages 44-45).

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

pZERO-TLR & pDeNy - Dominant Negative TLR & TLR Signaling Genes

Features and Benefits

DN TLR Genes

Dominant negative (DN) TLR 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 (TLR-ΔTIR genes). These truncated genes are still able to recognize their ligands but are unable to induce the signaling cascade.
DN TLR genes are provided in the pZERO plasmid. pZERO-TLR plasmids are selectable with puromycin.

HA-Tagged DN TLR Genes

DN TLR genes are available with the influenza hemaglutinine (HA) tag fused at their 3' end to facilitate their detection by Western blot. The HA-tagged DN TLR proteins can be detected using the anti-HAtag antibody (see page 73).
HA-tagged DN TLR genes are also provided in the pZERO plasmid. pZERO-TLR-HA plasmids are selectable with puromycin.

DN TLR Signaling Genes

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.
DN TLR signaling genes are provided in the pDeNy plasmid which is selectable with Zeocin™.

pZERO or pDeNy plasmids can be cotransfected with a pUNO plasmid (see page 58). Selection of stable clones expressing both plasmids, pZERO/pUNO or pDeNy/pUNO, is achieved with addition of puromycin and blasticidin or Zeocin™ and blasticidin, respectively.

Contents and Storage

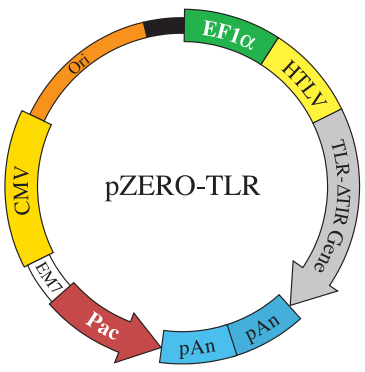
pZERO-TLR, pZERO-TLR-HA and pDeNy plasmids are provided as lyophilized transformed *E. coli* strains on paper disk. Transformed strains are shipped at room temperature and should be stored at -20°C. Lyophilized *E. coli* cells are stable for at least 1 year when properly stored.
pZERO-TLR and pZERO-TLR-HA plasmids are provided with 4 pouches of *E. coli* Fast-Media® Puro, while pDeNy plasmids are provided with 4 pouches of *E.coli* Fast-Media® Zeo (2 TB and 2 Agar).

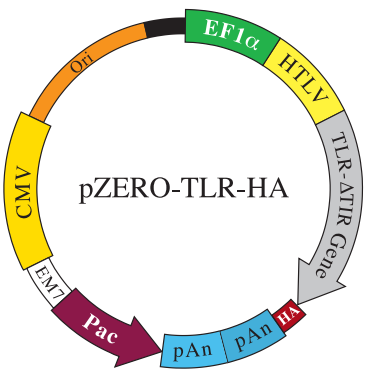
Related Products

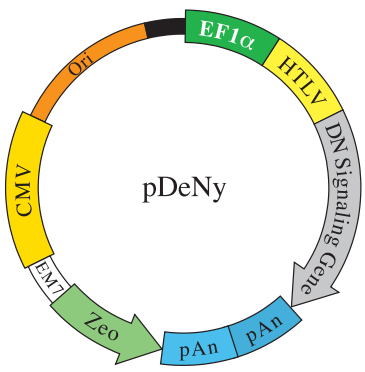
Puromycin, page 17
Anti-HAtag antibody, page 73
Zeocin™, page 18
Fast-Media®, page 45

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	-

PLASMID	SPECIES	MUTATION/ DELETION	CAT. CODE
DN TLR Signaling Genes			
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







Reporter Cell Lines

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 human or murine TLR or NOD genes.

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

Immune Reporter Cells

InvivoGen provides THP1-XBlue™, Ramos-Blue™ and RAW-Blue™ Cells, derived from a human monocytic cell line, a human B lymphocyte and murine macrophages, respectively. They naturally express a large repertoire of different classes of pattern recognition receptors (PRRs), such as the TLRs, NLRs and RLRs and stably express an NF-κB-inducible SEAP reporter gene to facilitate the study of these PRRs.

- THP1-XBlue™ Cells
- Ramos-Blue™ Cells
- RAW-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

Expression of TLR, NOD1/2 and RIG-I/MDA-5 mRNAs and their activity in InvivoGen's Reporter Cell Lines*

REPORTER CELL LINES	TLR1	TLR2	TLR3	TLR4	TLR5	TLR6	TLR7	TLR8	TLR9	TLR10	NOD1	NOD2	RIG-I	MDA-5
HEK293-Null cells	+/-	-	+/-	-	+/-	+/-	-	-	-	-	+/-	-	-	-
	+	-	+	-	+	+	-	-	-	-	+	-	-	-
THP1-XBlue Cells	+	+	+/-	+	+	+	+	+	+	+	+	+	+	+
	+	+	-	+	+	+	-	+	-	ND	+	?	ND	ND
Ramos-Blue Cells	+	+	+	+	+	+	+	+	+	+	+	-	**+**	**+**
	+/-	+/-	+	-	-	+/-	+	-	+	+	+	-	ND	ND
RAW-Blue Cells	+	+	+	+	+/-	+	+	+	+	-	+/-	+	+	+
	+	+	+/-	+	-	+	+	ND	+	-	-	+	ND	ND
C3H MEFs	+	+	+	+	-	+	-	-	-	-	+	+	+	+
	+	+	+	+	-	+	-	-	-	-	ND	ND	ND	ND
C57 MEFs	+	+	+	+	-	+	+	+	+	-	+	+	+	+
	ND	ND	ND	ND	-	ND	ND	ND	ND	-	ND	ND	+	+

Rows with no background correspond to mRNA expression, rows with a grey background correspond to the activity

B16-Blue™ IFN-α/β Cells

InvivoGen provides B16-Blue™ IFN-α/β, a cell line that in addition to detect bioactive murine type I IFNs can also be used to study RIG-I/MDA-5 agonists, such as transfected poly(I:C) or 5'ppp-dsRNA (see page 106).

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 74).

293/TLR-HA Clones

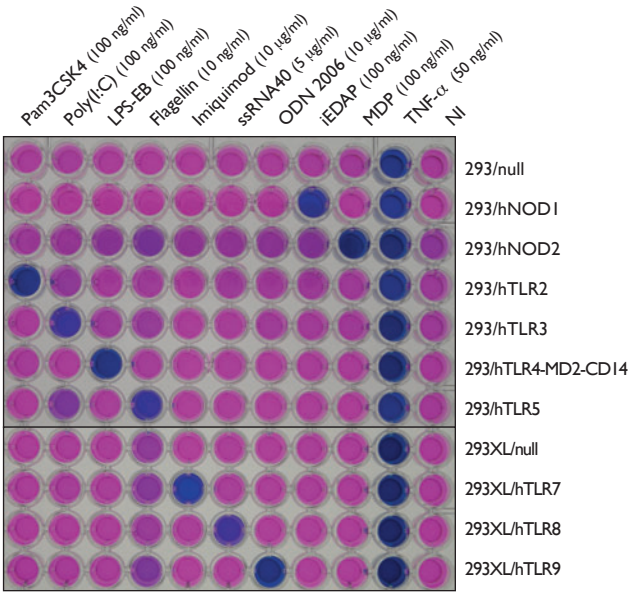
293/hTLR-HA clones were obtained by stably transfecting HEK293 cells with a pUNO-TLR-HA plasmid. pUNO-TLR-HA plasmids express TLR genes that have been fused at the 3'end to the influenza hemaglutinine (HA) tag. Addition of this tag has no deleterious effect on the expression and function of the TLR genes. The HA tag is the epitope of a very efficient and specific monoclonal antibody (see page 73).

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).



TLR and NOD induction profile of 293 clones : 293/TLR, NOD and control clones were transfected transiently with pNiFty-SEAP and stimulated with TLR and NOD ligands. After 16 hour stimulation, NF-κB-induced SEAP activity was assessed using QUANTI-Blue™, a SEAP-detection medium.

Note: 293 clones and in particular 293XL clones express endogenous levels of TLR3 and TLR5, as well as TLR1, TLR6 and NOD1.

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.*

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.

Related Products

Blasticidin, page 17
pNiFty, see page 74
TLR Ligands, pages 77-80
NOD Ligands, see page 80

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 QUANTI-Blue™ or HEK-Blue™ Detection.

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™ Null cells are the parental cell lines used to generate HEK-Blue™ TLR and HEK-Blue™ NOD cells.
• **HEK-Blue™ Null1 cells** express the SEAP reporter gene under the control of the IFN-β minimal promoter fused to five NF-κB binding sites. This cell line is the parental cell line of HEK-Blue™ hTLR2, hTLR3, hTLR5, hTLR8, h/mTLR9 and h/mNOD1 cells.
• **HEK-Blue™ Null1-k cells** express the same reporter system than HEK-Blue™ Null1 cells but are slightly different genotypically. This cell line is the parental cell line of HEK-Blue™ mTLR3 and hTLR7 cells.
• **HEK-Blue™ Null1-v cells** express the same reporter system than HEK-Blue™ Null1 cells but are slightly different genotypically. This cell line is the parental cell line of HEK-Blue™ mTLR4 and mTLR8 cells.
• **HEK-Blue™ Null2 cells** express the SEAP reporter gene under the control of the IL-12 p40 minimal promoter fused to five NF-κB binding sites. This cell line is the parental cell line of HEK-Blue™ mTLR2, hTLR4 and h/mNOD2 cells.
• **HEK-Blue™ Null2-k cells** express the same reporter system than HEK-Blue™ Null2 cells but are slightly different genotypically. This cell line is the parental cell line of HEK-Blue™ mTLR5 and mTLR7 cells.

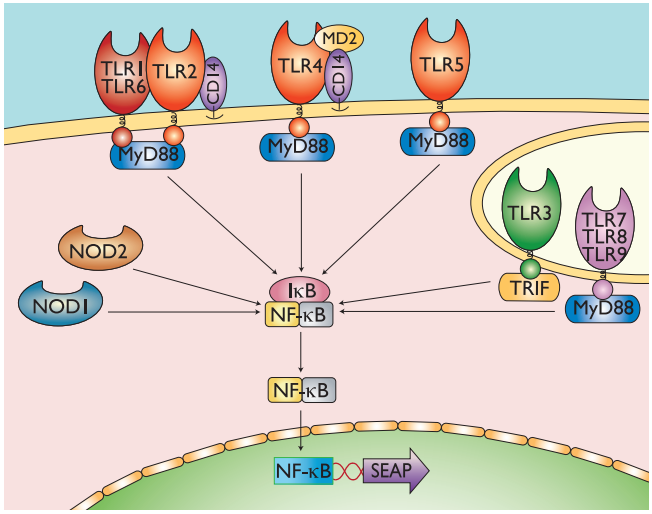
HEK-Blue™ Null cells are resistant to Zeocin™.

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 (figure 1). SEAP 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.

Quality Control

Expression of exogenous TLRs, NOD1/2, CD14 and/or MD2 was confirmed by RT-PCR. The functionality of each cell line is validated through stimulation assays performed with a selection of TLR and NOD agonists.
Note: HEK-Blue™ TLR, HEK-Blue™ NOD and HEK-Blue™ Null cells express endogenous levels of TLR3, TLR5 and NOD1.

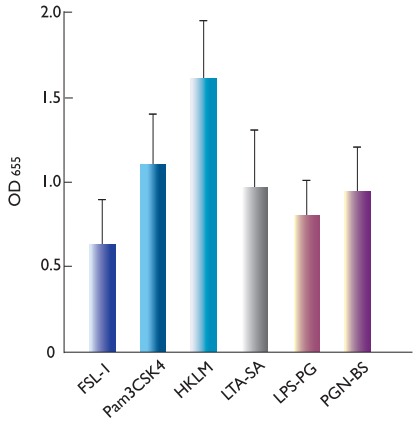


TLR- and NOD-induced NF-κB signaling pathway

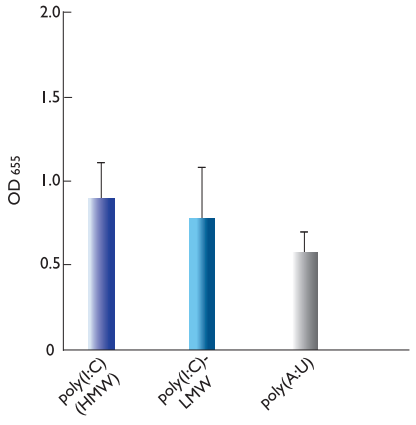
PRODUCT	CAT. CODE (human)	CAT. CODE (mouse)
HEK-Blue™ TLR Cells		
HEK-Blue™ MD2-CD14 Cells	hkb-hmdcd **NEW**	-
HEK-Blue™ TLR2 Cells	hkb-htlr2	hkb-mtlr2 **NEW**
HEK-Blue™ TLR3 Cells	hkb-htlr3	hkb-mtlr3 **NEW**
HEK-Blue™ TLR4 Cells	hkb-htlr4	hkb-mtlr4 **NEW**
HEK-Blue™ TLR5 Cells	hkb-htlr5	hkb-mtlr5 **NEW**
HEK-Blue™ TLR7 Cells	hkb-htlr7	hkb-mtlr7 **NEW**
HEK-Blue™ TLR8 Cells	hkb-htlr8	hkb-mtlr8 **NEW**
HEK-Blue™ TLR9 Cells	hkb-htlr9	hkb-mtlr9 **NEW**
HEK-Blue™ NOD Cells		
HEK-Blue™ NOD1 Cells	hkb-hnod1	hkb-mnod1 **NEW**
HEK-Blue™ NOD2 Cells	hkb-hnod2	hkb-mnod2 **NEW**
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	-

Contents and Storage

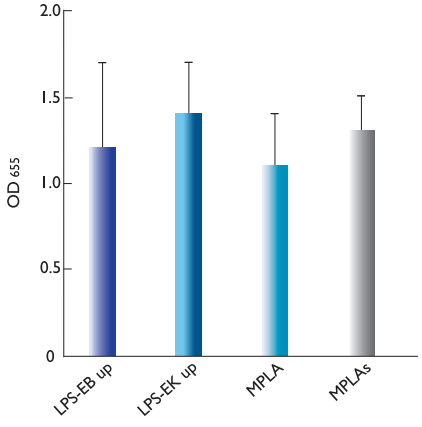
HEK-Blue™ TLR, HEK-Blue™ NOD and HEK-Blue™ Null cells are grown in DMEM medium, 2mM L-glutamine, 10% FBS and supplemented with 100 µg/ml Zeocin™, 30 µg/ml blasticidin and/or 200 µg/ml HygroGold™ (ultra-pure hygromycin) depending on the cell line. Cells are provided frozen in a cryotube containing 5-7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



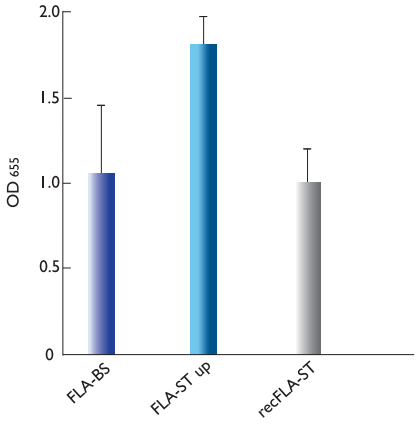
Response of HEK-Blue™ hTLR2 cells to TLR2 agonists. Cells were incubated in HEK-Blue™ Detection medium and stimulated with 0.1 ng/ml FSL-1 (TLR2/6), 0.1 ng/ml Pam3CSK4(TLR1/2), 10^7 cells/ml HKLM, 1 µg/ml LTA-SA, 1 ng/ml LPS-PG or 1 µg/ml PGN-BS. After 24h incubation, the levels of NF-κB-induced SEAP were determined by reading the OD at 655 nm.



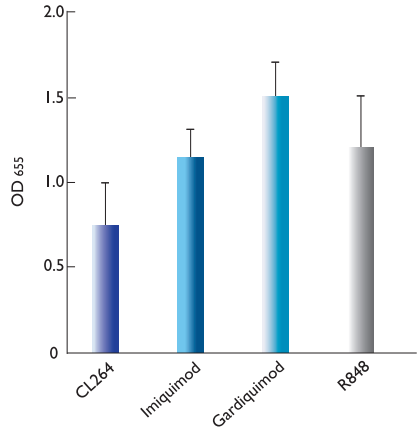
Response of HEK-Blue™ hTLR3 cells to TLR3 agonists. Cells were stimulated with 100 ng/ml poly(I:C) (HMW), 100 ng/ml poly(I:C)-LMW or 1 µg/ml poly(A:U). After 24h incubation, the levels of NF-κB-induced SEAP were determined using QUANTI-Blue™ by reading the OD at 655 nm.



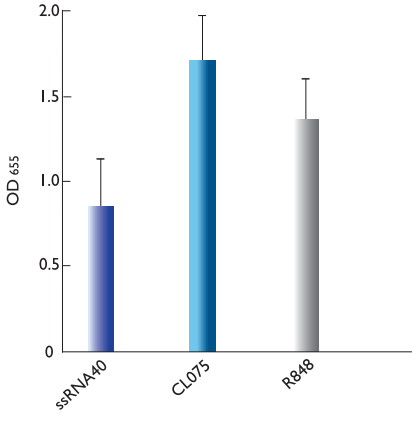
Response of HEK-Blue™ hTLR4 cells to TLR4 agonists. Cells were incubated in HEK-Blue™ Detection medium and stimulated with 10 ng/ml LPS-EB ultrapure, 10 ng/ml LPS-EK ultrapure, 100 ng/ml MPLA or 100 ng/ml MPLAs. After 24h incubation, the levels of NF-κB-induced SEAP were determined by reading the OD at 655 nm.



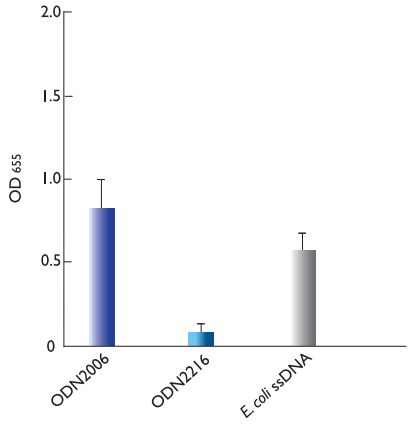
Response of HEK-Blue™ hTLR5 cells to TLR5 agonists. Cells were incubated in HEK-Blue™ Detection medium and stimulated with 100 ng/ml FLA-BS, 10 ng/ml FLA-ST ultrapure or 10 ng/ml recFLA-ST. After 24h incubation, the levels of NF-κB-induced SEAP were determined by reading the OD at 655 nm.



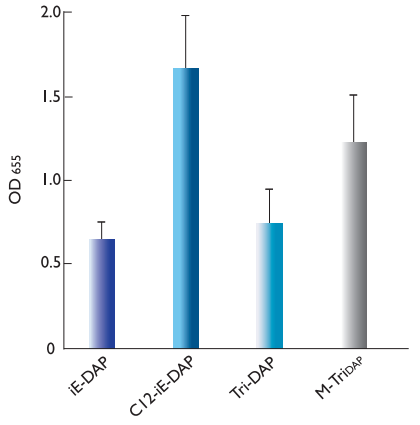
Response of HEK-Blue™ hTLR7 cells to TLR7/8 agonists. Cells were incubated in HEK-Blue™ Detection medium and stimulated with 100 ng/ml CL264, 5 µg/ml imiquimod, 1 µg/ml gardiquimod or 100 ng/ml R848. After 24h incubation, the levels of NF-κB-induced SEAP were determined by reading the OD at 655 nm.



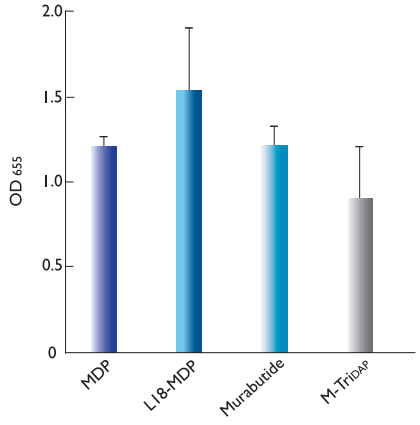
Response of HEK-Blue™ hTLR8 cells to TLR7/8 agonists. Cells were incubated in HEK-Blue™ Detection medium and stimulated with 5 µg/ml ssRNA40, 1 µg/ml CL075 or1 µg/ml R848. After 24h incubation, the levels of NF-κB-induced SEAP were determined by reading the OD at 655 nm.



Response of HEK-Blue™ hTLR9 cells to TLR9 agonists. Cells were stimulated with 1 µg/ml ODN2006 (type B), 1 µg/ml ODN2216 (type A) or 5 µg/ml *E. coli* ssDNA. After 24h incubation, the levels of NF-κB-induced SEAP were determined using QUANTI-Blue™ by reading the OD at 655 nm.



Response of HEK-Blue™ hNOD1 cells to NOD1/2 agonists. Cells were incubated in HEK-Blue™ Detection medium and stimulated with 1 µg/ml iE-DAP, 100 ng/ml C12-iE-DAP, 1 µg/ml TRI-DAP or 1 µg/ml M-TriDAP. After 24h incubation, the levels of NF-κB-induced SEAP were determined by reading the OD at 655 nm.



Response of HEK-Blue™ hNOD2 cells to NOD1/2 agonists. Cells were incubated in HEK-Blue™ Detection medium and stimulated with 100 ng/ml MDP, 10 ng/ml L18-MDP 100 ng/ml murabutide or 100 ng/ml M-TriDAP. After 24h incubation, the levels of NF-κB-induced SEAP were determined by reading the OD at 655 nm.

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

THP1-XBlue™ 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™-CD14, which overexpresses the cell surface protein 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 14). THP1-XBlue™ cells are resistant to the selectable marker Zeocin™.

THP1-XBlue™-MD2-CD14 NEW

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™-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 which signaling is dependent on MyD88, such as TLR2, TLR4 and IL-1Rs. They remain responsive to MyD88-independent receptors, such as NOD1 and TNFR (Figure 2).

Quality Control

Expression of the TLR (TLR1 to TLR10), MD2 and CD14 genes was determined by RT-PCR in THP1-XBlue™, THP1-XBlue™-MD2-CD14 and THP1-XBlue™-defMyD cells. All TLR mRNAs were detected but the cells do not respond to all TLR agonists (Figure 1). Considering the concentration of ligands used to stimulate these cells, TLR2, TLR2/1and TLR2/6 responses are considered to be very strong. TLR4, TLR5, TLR8, NOD1 and NOD2 responses are robust. TLR7 response is weak (a very high concentration of its cognate ligands is needed to detect a response). TLR3 and TLR9 responses are not detectable even when high concentrations of the ligands are used.

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 is 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. The cells are guaranteed mycoplasma-free.

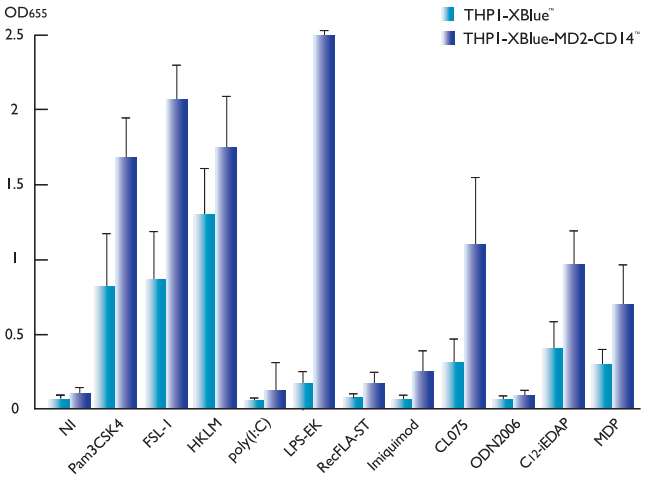


Figure 1: TLR and NOD stimulation profile in THP1-XBlue™ and THP1-XBlue™-MD2-CD14. Cells were incubated with 1 ng/ml Pam3CSK4(TLR1/2), 10 ng/ml FSL-1 (TLR2/6), 10^7 cells/ml HKLM (TLR2), 10 µg/ml poly(I:C) (TLR3), 10 ng/ml LPS-EK (TLR4), 10 ng/ml RecFLA-ST (TLR5), 5 µg/ml imiquimod (TLR7), 5 µg/ml CL075 (TLR8), 10 µg/ml ODN2006 (TLR9), 100 ng/ml C12-iEDAP (NOD1) or 10 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™.

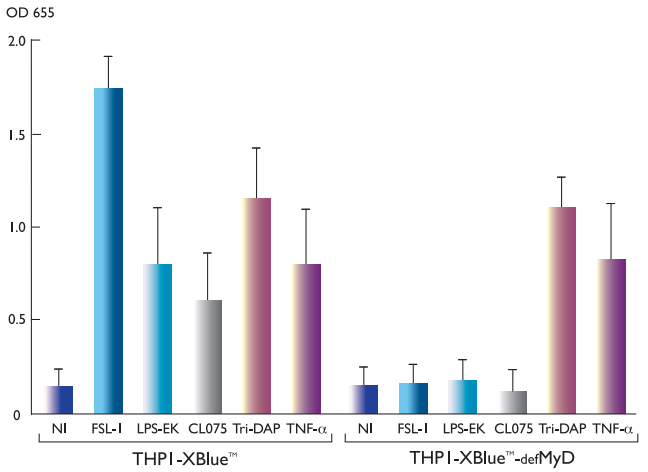


Figure 2: MyD88-dependent and -independent responses in THP1-XBlue™ and THP1-XBlue™-defMyD. Cells were incubated with 1 µg/ml FSL-1 (TLR2/6), 1 µg/ml LPS-EK (TLR4), 5 µg/ml CL075 (TLR8), 10 µg/mlTri-DAP (NOD1) and 50 ng/ml TNF-α. After 24h incubation, NF-κB activation was determined using QUANTI-Blue™.

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

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

B lymphocytes are key players in the adaptative immune system but are also prominent in the innate immune response. Consistent with their dual role, they express Toll-like receptors (TLRs) and other pattern recognition receptors (PRRs) that allow them to discriminate among a wide spectrum of pathogen-associated molecules (PAMPs). Upon PRR stimulation by PAMPs, various signaling pathways are induced leading to the activation of transcription factors, such as NF-κB and AP-1, and the subsequent production of inflammatory cytokines.

Ramos-Blue™ Cells

Ramos-Blue™ is a B lymphocyte cell line 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 NF-κB inducers, such as TNF-α and TLR agonists. When stimulated, they produce SEAP in the supernatant that can be readily monitored using QUANTI-Blue™. QUANTI-Blue™ is a SEAP detection medium that turns blue in the presence of SEAP (see page 14). Levels of SEAP can be determined qualitatively with the naked eye or quantitatively using a spectrophotometer at 620-655 nm.

Ramos-Blue™ cells are resistant to Zeocin™.

Ramos-Blue™-defMyD

Ramos-Blue™-defMyD are MyD88-deficient cells. Due to their deficiency in MyD88 activity, these cells are unable to respond to the activation of receptors which signaling is dependent on MyD88, such as TLR7 and TLR9. However, they are still responsive to TLR3 which signals through TRIF independently of MyD88 (Figure 2).

Quality Control

Ramos-Blue™ cells express all TLRs and NOD1 mRNAs as detected by RT-PCR. However, activation of NF-κB/AP-1 was only observed following stimulation with TLR2, TLR3, TLR7, TLR9 and NOD1 agonists (Figure 1). Considering the concentration of agonists used to stimulate these cells, TLR3, TLR7, TLR9 and NOD1 responses are considered to be strong. TLR2 response is detectable only at high concentrations of the ligands. Responses to TLR4, TLR5 and NOD2 agonists are not detectable.
MyD88 deficiency in Ramos-Blue™-defMyD cells was confirmed by qRT-PCR.

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. The cells are guaranteed mycoplasma-free.

Related Products

Zeocin™, page 18
QUANTI-Blue™ page 14
TLR Ligands , pages 77-80
NOD Ligands, page 80

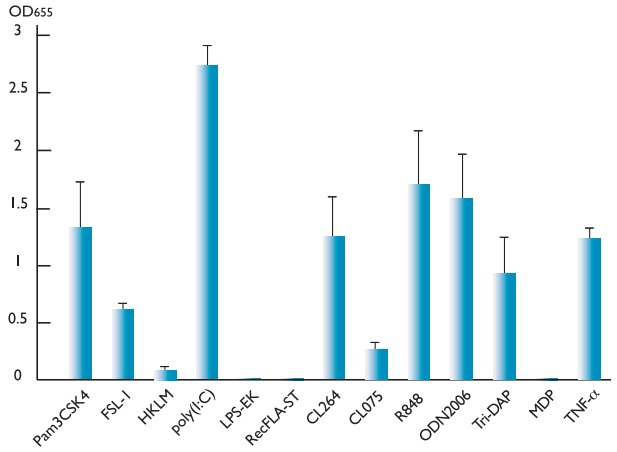


Figure 1: NF-κB/AP-1 activation in Ramos-Blue™ cells induced by various activators. Cells were incubated with 10 µg/ml each of Pam3CSK4 (TLR1/2), FSL-1 (TLR2/6), HKLM (TLR2, 1.10^9 cells/ml), poly(I:C) (TLR3), LPS-EK (TLR4), recFLA-ST (TLR5), imiquimod and CL264 (TLR7), CL075 (TLR8), R848 (TLR7/8), ODN2006 and ODN2216 (TLR9), TriDAP (NOD1), MDP (NOD2) and TNF-α. After 24h incubation, NF-κB/AP-1 activation was assessed by measuring the levels of SEAP in the supernatant using QUANTI-Blue™.

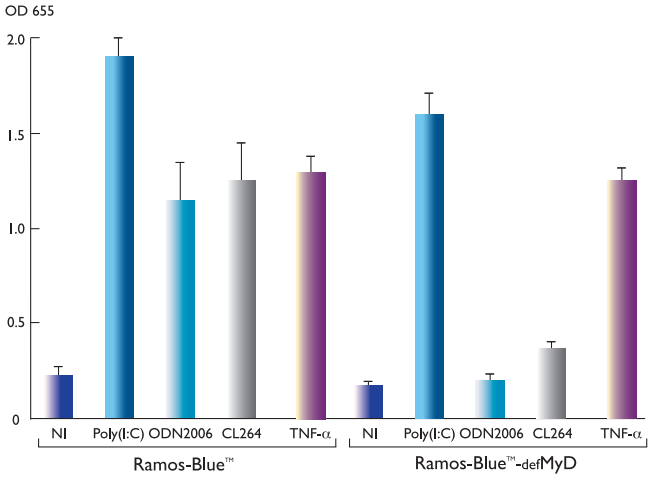


Figure 2: MyD88-dependent and -independent responses in Ramos-Blue™ and Ramos-Blue™-defMyD. Cells were incubated with 1 µg/ml poly(I:C) (TLR3), 10 µg/ml CL264 (TLR7), 2 µg/ml ODN2006 (TLR9) and 100 ng/ml TNF-α. After 24h incubation, NF-κB activation was determined using QUANTI-Blue™.

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

RAW-Blue™ Cells

RAW-Blue™ Cells

TLR & NOD 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™.
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™.

Dectin-1 Reporter Macrophages

RAW 264.7 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 (see graph).

1. Brown GD. *et al.*, 2003, Dectin-1 Mediates the Biological Effects of ß-Glucans. J. Exp. Med., 197: 1119.

Quality Control

Expression of TLRs (TLR1 to TLR9), RLRs (RIG-I and MDA-5), NODs (NOD1 and NOD2) was determined by RT-PCR (see figure 1). Dectin-1 expression was also confirmed by RT-PCR. RT-PCR on TLR and mRNAs were performed using InvivoGen's Mouse TLR RT-Primer Set and RLR RT-Primers.

The cells are guaranteed mycoplasma-free.

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.

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

Related Products

Blasticidin, page 17
TLR Ligands, page 77-80
NOD Ligands, see page 80
Dectin Ligands, see page 81
MAb mDectin-1, see page 72
QUANTI-Blue™, see page 14

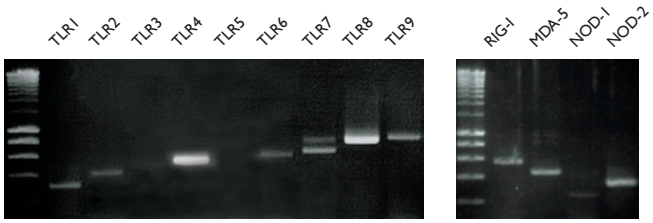
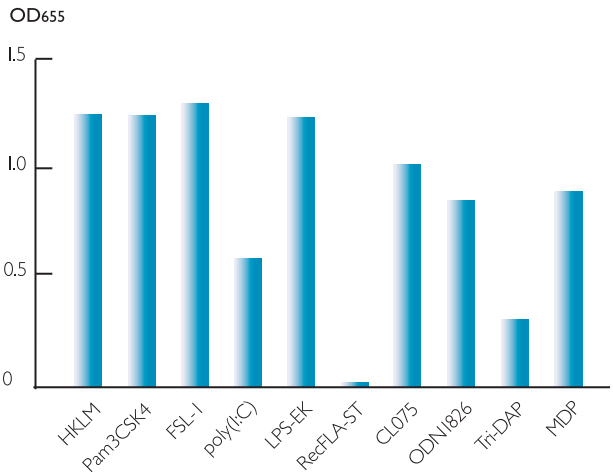
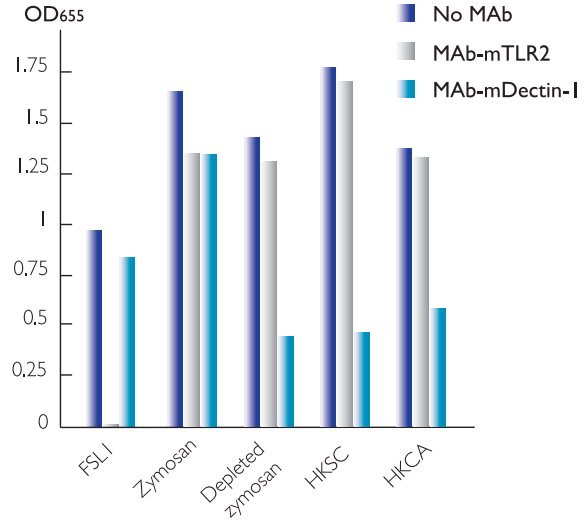


Figure 1: Expression of TLR, RLR and NOD mRNAs in RAW-Blue™ cells determined by RT-PCR. RT-PCRs were performed using InvivoGen's Mouse TLR RT-PCR Primer Set, NOD and RLR RT-Primer Pairs.



TLR and NOD stimulation profile in RAW-Blue™ Cells. RAW-Blue™ Cells were incubated with TLR or NOD agonists: TLR2 (HKLM, 1.10^8 cells/ml), TLR1/2 (Pam3CSK4, 100 ng/ml), TLR2/6 (FSL-1, 100 ng/ml), TLR3 (poly(I:C), 10 µg/ml), TLR4 (LPS-EK, 1 µg/ml), TLR5 (RecFLA-ST, 1 µg/ml), TLR7 (CL075, 300 ng/ml), TLR9 (ODN1826, 10 µg/ml), NOD1 (Tri-DAP, 10 µg/ml), NOD2 (MDP, 10 µg/ml). After 24h incubation, TLR and NOD stimulation was assessed by measuring the levels of SEAP using QUANTI-Blue™.



Response of RAW-Blue™ cells to Dectin-1 and TLR2 agonists. RAW-Blue™ cells were stimulated with FSL-1 (10 ng/ml), zymosan (10 µg/ml), depleted zymosan (100 µg/ml), HKSC (10⁸ cells/ml) or HKCA (10⁸ cells/ml) in the presence or absence of 10 µg/ml of an anti-mTLR2 (clone T2.5) or anti-mDectin-1 monoclonal antibody. After 24h incubation, NF-κB activation was assessed by measuring the levels of SEAP in the supernatant by using QUANTI-Blue™.

MEF Cells

C3H/TLR4mut & C3H/WT MEFs

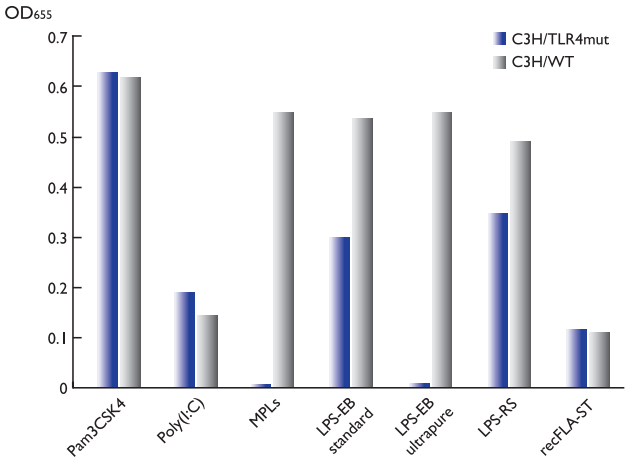
TLR4 Reporter Murine Embryonic Fibroblasts

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 graph, LPS-EB standard versus LPS-EB ultrapure).

Contents

C3H/TLR4mut and C3H/WT MEF cell lines are grown in DMEM medium with 2mM L-glutamine, 10% FBS supplemented with 10 μg/ml blasticidin. Each vial contains 5-7 x 10^6 cells and is supplied with 1 mg blasticidin. Cells are shipped on dry ice. The cells are guaranteed mycoplasma-free.



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-RS, 10 μg/ml (TLR2), 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™.

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

RLR Reporter Murine Embryonic Fibroblasts

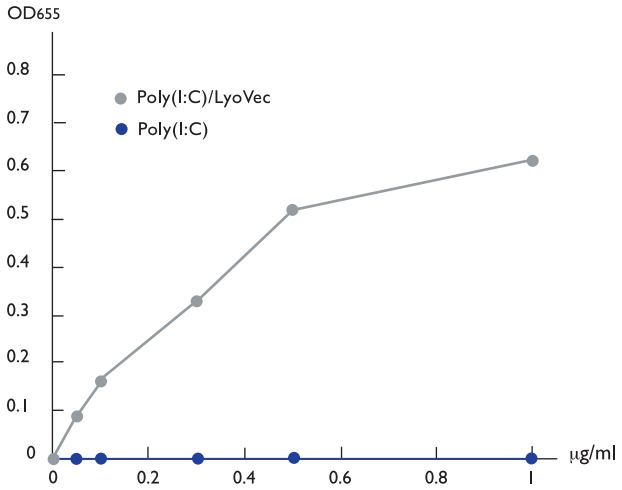
MEFs produce IFN-β in response to viral infection in a RLR-dependent manner[1]. Thus, these cells are commonly used to study the RLR pathway. C57/WT MEFs were isolated from embryos under C57BL/6 background and immortalized with the SV40 large antigen. They stably express a SEAP reporter gene inducible 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). 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].

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

C57/WT MEFs are grown in DMEM medium with 10% FBS, 2 mM L-glutamine and supplemented with 100 μg/ml Zeocin™ and 3 μg/ml blasticidin. Each vial contains 5-7 x 10^6 cells and is supplied with 10 mg Zeocin™ and 1 mg blasticidin. Cells are shipped on dry ice. The cells are guaranteed mycoplasma-free.



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™, a SEAP detection medium.

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

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 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.

Antibodies for Neutralization

ANTIBODY	DESCRIPTION	SPECIFICITY	APPLICATIONS	QTY	CAT. CODE
Anti-CD14					
Anti-hCD14-IgA	Monoclonal human IgA2	Human CD14	Neutralization (TLR2,TLR4), FC	100 µg	maba-hcd14
Anti-Dectin-1					
MAb-mDectin-1	Monoclonal mouse IgG2b (clone 2A11)	Mouse Dectin-1	Neutralization, FC	100 µg	mab-mdect
Anti-TLR1					
Anti-hTLR1-IgG	Monoclonal mouse IgG1 (H2G2)	Human TLR1	Neutralization	100 µg	mabg-htlr1
PAb-hTLR1	Polyclonal rat IgG	Human TLR1	Neutralization	200 µg	pab-hstlr1
Anti-TLR2					
Anti-hTLR2-IgA	Monoclonal human IgA2	Human TLR2	Neutralization, FC	100 µg	maba2-htlr2
Anti-mTLR2-IgG	Monoclonal mouse IgG2a (C9A12)	Mouse TLR2	Neutralization	100 µg	mabg-mtlr2
MAb-mTLR2	Monoclonal mouse IgG1 (T2.5)	Human/mouse TLR2	Neutralization·FC, IHC	100 µg	mab-mtlr2
PAb-hTLR2	Polyclonal rat IgG	Human TLR2	Neutralization	200 µg	pab-hstlr2
Anti-TLR4					
Anti-hTLR4-IgA	Monoclonal human IgA2	Human TLR4	Neutralization	100 µg	maba2-htlr4
PAb-hTLR4	Polyclonal rat IgG	Human TLR4	Neutralization	200 µg	pab-hstlr4
Anti-TLR5					
Anti-hTLR5-IgA	Monoclonal human IgA2	Human TLR5	Neutralization, FC	100 µg	maba2-htlr5
Anti-mTLR5-IgG	Monoclonal rat IgG2a (Q23D11)	Mouse TLR5	Neutralization	100 µg	mabg-mtlr5
PAb-hTLR5	Polyclonal rat IgG	Human TLR5	Neutralization	200 µg	pab-hstlr5
Anti-TLR6					
Anti-hTLR6-IgG	Monoclonal mouse IgG1 (C5C8)	Human TLR6	Neutralization	100 µg	mabg-htlr6
PAb-hTLR6	Polyclonal rat IgG	Human TLR6	Neutralization	200 µg	pab-hstlr6

* FC: flowcytometry; IHC: immunohistochemistry; WB: Western blot

Antibodies for Detection

ANTIBODY	DESCRIPTION	SPECIFICITY	APPLICATIONS*	QTY	CAT. CODE
Anti-Flagellin					
Anti-Flagellin FliC	Monoclonal mouse IgG1	*S. typhimurium* flagellin	WB	100 µg	mabg-flic
Anti-TLR1					
MAb-hTLR1	Monoclonal mouse IgG1 (GD2.F4)	Human TLR1	FC	100 µg	mab-htlr1
MAb-hTLR1-FITC	Monoclonal mouse IgG1 (GD2.F4), FITC	Human TLR1	FC	100 µg	mab-htlr1f
Anti-TLR2					
MAb-hTLR2	Monoclonal mouse IgG2a (TL2.1)	Human TLR2	FC, IHC, WB	100 µg	mab-htlr2
MAb-hTLR2-FITC	Monoclonal mouse IgG2a (TL2.1), FITC	Human TLR2	FC, IHC	100 µg	mab-htlr2f
MAb-mTLR2-FITC	Monoclonal mouse IgG1 (T2.5), FITC	Human/mouse TLR2	FC	100 µg	mab-mtlr2f
Anti-TLR3					
Anti-hTLR3-IgA	Monoclonal human IgA2	Human TLR3	FC	100 µg	maba-htlr3
MAb-hTLR3	Monoclonal mouse IgG1 (TLR3.7)	Human TLR3	FC, WB	100 µg	mab-htlr3
MAb-hTLR3-FITC	Monoclonal mouse IgG1 (TLR3.7), FITC	Human TLR3	FC, WB	100 µg	mab-htlr3f
Anti-TLR4					
MAb-hTLR4	Monoclonal mouse IgG2a (HTA125)	Human/monkey TLR4	FC, IHC	100 µg	mab-htlr4
MAb-hTLR4-FITC	Monoclonal mouse IgG2a (HTA125), FITC	Human/monkey TLR4	FC	100 µg	mab-htlr4f
MAb-mTLR4/MD2	Monoclonal rat IgG2a (MTS510)	Mouse TLR4/MD2	FC, IHC	100 µg	mab-mtlr4md2
MAb-mTLR4/MD2-FITC	Monoclonal rat IgG2a (MTS510), FITC	Mouse TLR4/MD2	FC	100 µg	mab-mtlr4md2f
Anti-TLR9					
MAb-mTLR9	Monoclonal mouse IgG2a (5G5)	Human/mouse TLR9	FC, IHC, WB	100 µg	mab-mtlr9
MAb-mTLR9-FITC	Monoclonal mouse IgG2a (5G5), FITC	Human/mouse TLR9	FC	100 µg	mab-mtlr9f

* FC: flowcytometry; IHC: immunohistochemistry; WB: Western blot

Contents and Storage

MAb-TLR-IgA and **MAb-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.

Anti-HA Tag Antibody

The monoclonal antibody Anti-HAtag recognizes the influenza hemagglutinin epitope (YPYDVPDYA) which has been used extensively as a general epitope tag in expression vectors. The specificity of this antibody allows for unambiguous identification of the tagged protein.

Applications: detection of HA-tagged proteins by Western Blot or immunoprecipitation.

Contents and Storage

Anti-HA tag antibody is provided as 250 µl Raw Ascites Fluid. All antibodies are shipped at room temperature. Store at -20°C.

PRODUCT	QTY	CAT. CODE
Anti-HA Tag	250 µl	ab-hatag

Anti-Human IgA Secondary Antibodies

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

Contents and Storage

Secondary anti-human IgA antibodies are supplied in 1 ml PBS/NaN3. Store at 4°C.

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

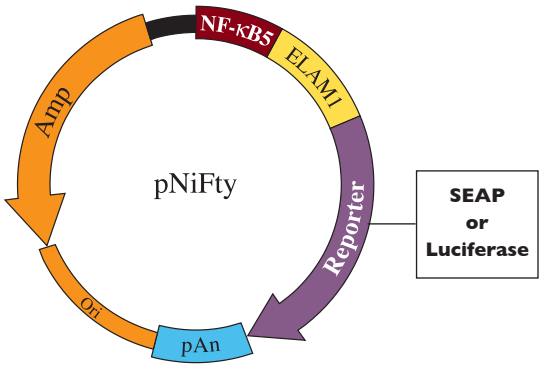
pNiFty - PRR Signaling Reporter Plasmids

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

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	pnf2-56sp
pNiFty3-SEAP NEW	None	IFN-β	Zeocin™	SEAP	pnf3-sp1
pNiFty3-N-SEAP NEW	NF-κB (x5)	IFN-β	Zeocin™	SEAP	pnf3-sp2
pNiFty3-A-SEAP NEW	AP-1 (x5)	IFN-β	Zeocin™	SEAP	pnf3-sp3
pNiFty3-I-SEAP NEW	ISRE (x5)	IFN-β	Zeocin™	SEAP	pnf3-sp4
pNiFty3-T-SEAP NEW	NFAT (x5)	IFN-β	Zeocin™	SEAP	pnf3-sp5
pNiFty3-AN-SEAP NEW	AP-1 (x5) NF-κB (x5)	IFN-β	Zeocin™	SEAP	pnf3-sp6
pNiFty3-IAN-SEAP NEW	ISRE (x5) AP-1 (x5) NF-κB (x5)	IFN-β	Zeocin™	SEAP	pnf3-sp7
pNiFty3-TAN-SEAP NEW	NFAT (x5) AP-1 (x5) NF-κB (x5)	IFN-β	Zeocin™	SEAP	pnf3-sp8

Description

pNiFty plasmids are composed of three key elements: a proximal 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.

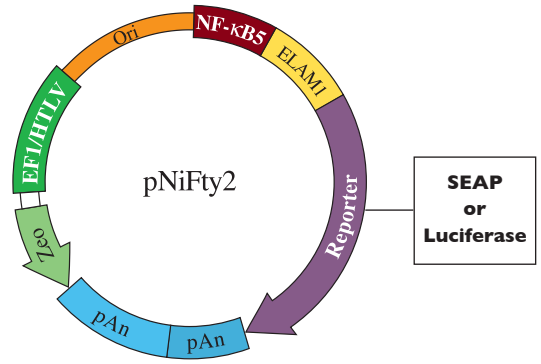


Minimal promoters

- **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.

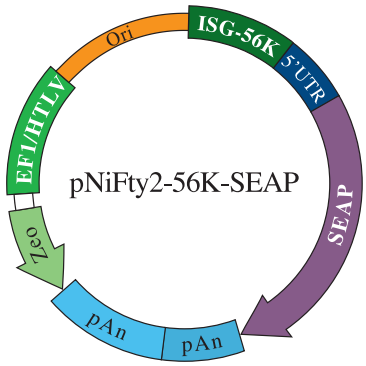
Reporter Genes

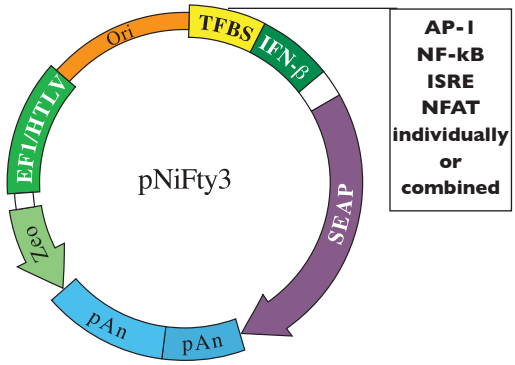
- SEAP reporter gene: Secreted alkaline phosphatase (SEAP) is a reporter widely used to study promoter activity or gene expression. SEAP expression can be rapidly and readily measured in supernatants of transfected cells. SEAP levels can be evaluated qualitatively with the naked eye and quantitatively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

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.

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

HEK-Blue™ Detection, page 15
QUANTI-Blue™, page 14
SEAP Reporter Assay Kit, page 13
Fast-Media®, page 45
Zeocin™, page 18

Pathogen-Associated Molecular Patterns

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 Agonist

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

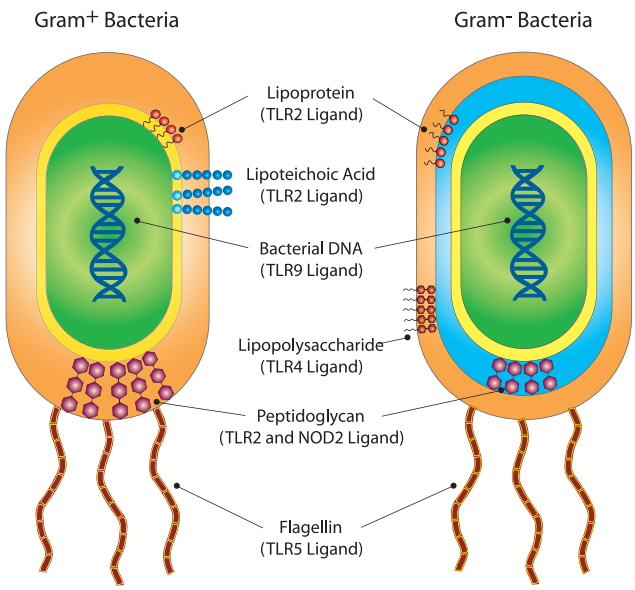
Dectin-1 is a specific receptor of β-glucans, which are glucose polymers found in the cell walls of fungi, including the yeasts *Saccharomyces cerevisiae* and *Candida albicans*.

NF-κB Activators

InvivoGen provides two TLR-independent activators of the transcription factor NF-κB: Tumor Necrosis Factor alpha (TNF-α) and Phorbol Myristate Acetate (PMA).

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 82
HKAL	Heat Killed *Acholeplasma laidlawii*	EndoFit™	10^6 - 10^8 cells/ml	10^9 cells	tlrl-hkal	p 82
HKEB **NEW**	Heat Killed *Escherichia coli* 0111:B4	>1 EU/10^9 cells	10^5 - 10^7 cells/ml	10^{10} cells	tlrl-hkeb	p 82
HKHP	Heat Killed *Helicobacter pylori*	EndoFit™	10^6 - 10^8 cells/ml	10^9 cells	tlrl-hkhp	p 82
HKLM	Heat Killed *Listeria monocytogenes*	EndoFit™	10^7 - 10^8 cells/ml	10^{10} cells	tlrl-hklm	p 82
HKLP	Heat Killed *Legionella pneumophila*	EndoFit™	10^7 - 10^8 cells/ml	10^9 cells	tlrl-hklp	p 82
HKLR	Heat Killed *Lactobacillus rhamnosus*	>1 EU/10^9 cells	10^8 - 10^9 cells/ml	10^{10} cells	tlrl-hklr	p 82
HKMF **NEW**	Heat Killed *Mycoplasma fermentans*	EndoFit™	10^6 - 10^8 cells/ml	10^9 cells	tlrl-hkmf	p 82
HKPA	Heat Killed *Pseudomonas aeruginosa*	>1 EU/10^8 cells	10^5 - 10^7 cells/ml	10^{10} cells	tlrl-hkpa	p 82
HKPG	Heat Killed *Porphyromonas gingivalis*	EndoFit™	10^6 - 10^8 cells/ml	10^{10} cells	tlrl-hkpg	p 82
HKSA	Heat Killed *Staphylococcus aureus*	>1 EU/10^9 cells	10^6 - 10^8 cells/ml	10^{10} cells	tlrl-hksa	p 82
HKSP	Heat Killed *Streptococcus pneumoniae*	EndoFit™	10^7 - 10^9 cells/ml	10^{10} cells	tlrl-hksp	p 82
LAM-MS	Lipoarabinomannan from *M. smegmatis*	EndoFit™	100 ng - 10 µg/ml	500 µg	tlrl-lams	p 82
LM-MS	Lipomannan from *Mycobacterium smegmatis*	>5 EU/mg	1 - 10 ng/ml	250 µg	tlrl-lmms2	p 82
LPS-PG Ultrapure	Ultrapure LPS from *P. gingivalis*	>10^4 EU/mg	10 ng - 10 µg/ml	1 mg	tlrl-pglps	p 83
LTA-BS	Lipoteichoic acid from *Bacillus subtilis*	10 EU/mg	100 ng - 1 µg/ml	5 mg	tlrl-lta	p 83
LTA-SA	Lipoteichoic acid from *S. aureus*	10 EU/mg	100 ng - 1 µg/ml	5 mg	tlrl-slta	p 83
LTA-SA Purified	Purified lipoteichoic acid from *S. aureus*	EndoFit™	1 ng - 1 µg/ml	5 mg	tlrl-pslta	p 83
Pam2CSK4	Synthetic diacylated lipoprotein - TLR2(6)	EndoFit™	1 - 100 ng/ml	100 µg 1 mg	tlrl-pam2 tlrl-pam2-1	p 83
Pam2CSK4 Biotin	Biotinylated Pam2CSK4	EndoFit™	1 - 100 ng/ml	50 µg	tlrl-bpam2	p 83
Pam2CSK4 Rhodamine	Rhodamine-labeled Pam2CSK4	EndoFit™	1 - 100 ng/ml	50 µg	tlrl-rpam2	p 83
Pam3CSK4	Synthetic triacylated lipoprotein - TLR1/2	EndoFit™	1 - 300 ng/ml	1 mg	tlrl-pms	p 83
Pam3CSK4 Biotin	Biotinylated Pam3CSK4	EndoFit™	1 - 100 ng/ml	50 µg	tlrl-bpms	p 83
Pam3CSK4 Rhodamine	Rhodamine-labeled Pam3CSK4	EndoFit™	1 - 300 ng/ml	50 µg	tlrl-rpms	p 83
PGN-BS	Peptidoglycan from *B. subtilis*	EndoFit™	1 - 10 µg/ml	5 mg	tlrl-pgnbs	p 83
PGN-EB	Peptidoglycan from *E. coli* 0111:B4	10^2 - 10^3 EU/mg	1 - 10 µg/ml	1 mg	tlrl-pgnec	p 83
PGN-EK	Peptidoglycan from *E. coli* K12	10^2 - 10^3 EU/mg	1 - 10 µg/ml	1 mg	tlrl-pgnek	p 83
PGN-SA	Peptidoglycan from *S. aureus*	1 EU/mg	1 - 10 µg/ml	5 mg	tlrl-pgnsa	p 83
Zymosan	Cell wall preparation of *S. cerevisiae*	EndoFit™	10 µg/ml	100 mg	tlrl-zyn	p 83
TLR3 Agonists						
Poly(A:U)	Polyadenylic–polyuridylic acid	<0.005 EU/µg	300 ng - 100 µg/ml	10 mg	tlrl-pau	p 84
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 tlr-pic-5	p 84
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 84
Poly(I:C) Fluorescein **NEW**	Fluorescein-labeled poly(I:C) (HMW)	EndoFit™	10 ng - 10 µg/ml	10 µg	tlrl-picf	p 84
Poly(I:C) Rhodamine **NEW**	Rhodamine-labeled poly(I:C) (HMW)	EndoFit™	10 ng - 10 µg/ml	10 µg	tlrl-picr	p 84
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 84
LPS-EB Ultrapure	Ultrapure lipopolysaccharide from *E. coli* 0111:B4	1 x 10^6 EU/mg	10 ng - 10 µg/ml	5 mg	tlrl-pelps	p 84
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 84
LPS-EK	Standard lipopolysaccharide from *E. coli* K12	1 x 10^6 EU/mg	1 ng - 10 µg/ml	5 mg	tlrl-eklps	p 84
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 84
LPS-SM Ultrapure	Ultrapure lipopolysaccharide from *S. minnesota*	1 x 10^5 EU/mg	10 ng - 10 µg/ml	5 mg	tlrl-smlps	p 84
MPLA	Monophosphoryl lipid A from *S. minnesota*	1 x 10^6 EU/mg	100 ng - 1 µg/ml	1 mg	tlrl-mpl	p 84
MPLAs	Synthetic monophosphoryl lipid A	1 x 10^6 EU/mg	10 ng - 10 µg/ml	500 µg	tlrl-mpls	p 84

PRODUCT	ORIGIN/DESCRIPTION	ENDOTOXIN LEVELS*	WORKING CONCENTRATION	QTY	CATALOG CODE	INFO
TLR4 Antagonist						
LPS-RS	Lipopolysaccharide from *Rhodobacter sphaeroides*	1 x 10^6 EU/mg	10 ng - 10 µg/ml	5 mg	tlrl-rslps	p 84
TLR5 Agonists						
FLA-BS	Standard flagellin from *B. subtilis*	<10 EU/mg	10 ng - 10 µg/ml	100 µg	tlrl-bsfla	p 84
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 84
FLA-ST Ultrapure	Ultrapure flagellin from *S. typhimurium - >95% pure*	<50 EU/mg	10 - 100 ng/ml	10 µg	tlrl-pstfla	p 85
RecFLA-ST	Recombinant flagellin from *S. typhimurium*	EndoFit™	10 - 100 ng/ml	1 µg 10 µg	tlrl-flic tlrl-flic-10	p 85
TLR7 Agonists						
CL264	Adenine analog	EndoFit™	50 ng - 10 µg/ml	500 µg 5 mg	tlrl-c264s tlrl-c264-5	p 85
CL264 Biotin	Biotinylated CL264	EndoFit™	1 - 10 µg/ml	100 µg	tlrl-bc264	p 85
CL264 FITC	FITC-labeled CL264	EndoFit™	1 - 10 µg/ml	100 µg	tlrl-fc264	p 85
CL264 Rhodamine	Rhodamine-labeled CL264	EndoFit™	1 - 10 µg/ml	100 µg	tlrl-rc264	p 85
Gardiquimod	Imidazoquinoline compound	EndoFit™	0.1 - 3 µg/ml	500 µg 5 mg	tlrl-gdqs tlrl-gdq-5	p 85
Imiquimod	Imidazoquinoline compound	EndoFit™	1 - 5 µg/ml	500 µg 5 mg	tlrl-imqs tlrl-imq	p 85
Loxoribine	Guanosine analog	EndoFit™	1 mM (300 µg/ml)	50 mg	tlrl-lox	p 85
TLR8 Agonists						
ORN02/LyoVec	ssRNA with 6 UUAU repeats / LyoVec™	EndoFit™	0.25 - 5 µg/ml	4 x 25 µg	tlrl-orn2	p 85
ORN06/LyoVec	ssRNA with 6 UUGU repeats / LyoVec™	EndoFit™	0.25 - 5 µg/ml	4 x 25 µg	tlrl-orn6	p 85
ssPolyU Naked	RNA homopolymer	EndoFit™	1 - 10 µg/ml	10 mg	tlrl-sspu	p 85
ssPolyU/LyoVec	RNA homopolymer / LyoVec™	EndoFit™	1 - 10 µg/ml	4 x 25 µg	tlrl-lpu	p 85
ssRNA40/LyoVec	HIV-1 LTR-derived ssRNA / LyoVec™	EndoFit™	0.25 - 5 µg/ml	4 x 25 µg	tlrl-lrna40	p 85
ssRNA41/LyoVec	ssRNA40 control / LyoVec™	EndoFit™	0.25 - 5 µg/ml	4 x 25 µg	tlrl-lrna41	p 85
ssRNA-DR/LyoVec	ssRNA with 2 GUCCUUCAA repeats / LyoVec™	EndoFit™	1 - 10 µg/ml	4 x 25 µg	tlrl-ssdr	p 85
TLR7/8 Agonists						
CL075	Thiazoquinoline compound	EndoFit™	100 ng - 5 µg/ml	500 µg 5 mg	tlrl-c75 tlrl-c75-5	p 85
CL097	Imidazoquinoline compound	EndoFit™	50 ng - 5 µg/ml	500 µg 5 mg	tlrl-c97 tlrl-c97-5	p 85
Poly(dT)	Thymidine homopolymer ODN (17 mer)	EndoFit™	10 µM	100 nmol	tlrl-pt17	p 85
R848	Imidazoquinoline compound	EndoFit™	10 ng - 10 µg/ml	500 µg 5 mg	tlrl-r848 tlrl-r848-5	p 85
TLR9 Agonists						
***E. coli* DNA ef**	Endotoxin-free DNA from *E. coli K12*	EndoFit™	0.25 - 10 µg/ml	1 mg	tlrl-ednaef	p 86
***E. coli* ssDNA/LyoVec**	*E. coli* single stranded DNA/LyoVec complexes	EndoFit™	1 - 10 µg/ml	200 µg	tlrl-ssec	p 86
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 86
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 86
ODN 1585 FITC	FITC-labeled CpG ODN - mouse specific, type A	EndoFit™	10 ng - 10 µg/ml	50 µg	tlrl-1585f	p 86
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 86
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 86

PRODUCT	ORIGIN/DESCRIPTION	ENDOTOXIN LEVELS	WORKING CONCENTRATION	QTY	CATALOG CODE	INFO
TLR9 Agonists						
ODN 1668 FITC	FITC-labeled CpG ODN - mouse specific, type B	EndoFit™	10 ng - 10 µg/ml	50 µg	tlrl-1668f	p 86
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 86
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 86
ODN 1826 Biotin	Biotinylated CpG ODN - mouse specific, type B	EndoFit™	10 ng - 10 µg/ml	50 µg	tlrl-1826b	p 86
ODN 1826 FITC	FITC-labeled CpG ODN - mouse specific, type B	EndoFit™	10 ng - 10 µg/ml	50 µg	tlrl-1826f	p 86
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 86
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 86
ODN 2006 Biotin	Biotinylated CpG ODN - human specific, type B	EndoFit™	10 ng - 10 µg/ml	50 µg	tlrl-2006b	p 86
ODN 2006 FITC	FITC-labeled CpG ODN - human specific, type B	EndoFit™	10 ng - 10 µg/ml	50 µg	tlrl-2006f	p 86
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 86
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 86
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 86
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 86
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 86
ODN 2216 Biotin	Biotinylated CpG ODN - human specific, type A	EndoFit™	10 ng - 10 µg/ml	50 µg	tlrl-2216b	p 86
ODN 2216 FITC	FITC-labeled CpG ODN - human specific, type A	EndoFit™	10 ng - 10 µg/ml	50 µg	tlrl-2216f	p 86
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 86
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 86
ODN 2336 FITC	FITC-labeled CpG ODN - human specific, type A	EndoFit™	10 ng - 10 µg/ml	50 µg	tlrl-2336f	p 86
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 86
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 86
ODN 2395 FITC	FITC-labeled CpG ODN - human specific, type C	EndoFit™	10 ng - 10 µg/ml	50 µg	tlrl-2395f	p 86
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 86
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 86
ODN M362 FITC	FITC-labeled CpG ODN - human specific, type C	EndoFit™	10 ng - 10 µg/ml	50 µg	tlrl-m362f	p 86

PRODUCT	ORIGIN/DESCRIPTION	ENDOTOXIN LEVELS	WORKING CONCENTRATION	QTY	CATALOG CODE	INFO
TLR9 Agonists						
pCpG Giant	CpG-free *dcm*⁻ giant plasmid	EndoFit™	5 - 10 µg/ml	1 mg	tlrl-cpgg	p 86
Salmon sperm DNA	TLR9 negative control	EndoFit™	5 - 100 µg/ml	50 mg	tlrl-sdef	p 86
TLR9 Antagonists						
ODN 2088	Inhibitory ODN, mouse preferred	EndoFit™	50 nM - 1 µM	200 µg 1 mg	tlrl-2088 tlrl-2088-1	p 87
ODN 2088 control	Negative control for ODN 2088	EndoFit™	50 nM - 1 µM	200 µg 1 mg	tlrl-2088c tlrl-2088c-1	p 87
ODN 4084-F **NEW**	Class B inhibitory ODN	EndoFit™	50 nM - 1 µM	200 µg	tlrl-4084	p 87
ODN INH-1 **NEW**	Class R inhibitory ODN	EndoFit™	50 nM - 1 µM	200 µg	tlrl-inh1	p 87
ODN INH-47 **NEW**	Negative control for ODN INH-1	EndoFit™	50 nM - 1 µM	200 µg	tlrl-inh47	p 87
ODN TTAGGG	Inhibitory ODN, human preferred	EndoFit™	50 nM - 1 µM	200 µg 1 mg	tlrl-ttag tlrl-ttag-1	p 87
ODN TTAGGG control	Negative control for ODN TTAGGG	EndoFit™	50 nM - 1 µM	200 µg 1 mg	tlrl-ttagc tlrl-ttagc-1	p 87
G-ODN	Inhibitory guanosine-rich ODN	EndoFit™	50 nM - 1 µM	200 µg	tlrl-godn	p 87
NOD1 Agonists						
C12-iE-DAP	Acylated derivative of iE-DAP	EndoFit™	1 ng - 1 µg/ml	1 mg	tlrl-c12dap	p 87
iE-DAP	D-γ-Glu-mDAP	EndoFit™	1 - 100 µg/ml	5 mg	tlrl-dap	p 87
iE-Lys	iE-DAP negative control	EndoFit™	1 - 100 µg/ml	5 mg	tlrl-lys	p 87
Tri-DAP	L-Ala-γ-D-Glu-mDAP	EndoFit™	100 ng - 10 µg/ml	1 mg	tlrl-tdap	p 88
Tri-Lys	Tri-DAP negative control	EndoFit™	100 ng - 10 µg/ml	1 mg	tlrl-tlys	p 88
NOD2 Agonists						
MDP	Muramyldipeptide (L-D isoform, active)	EndoFit™	10 ng - 10 µg/ml	5 mg	tlrl-mdp	p 87
MDP control	Muramyldipeptide (D-D isoform, inactive)	EndoFit™	10 ng - 10 µg/ml	5 mg	tlrl-mdpc	p 88
MDP Biotin	Biotinylated Muramyldipeptide	EndoFit™	100 ng - 10 µg/ml	500 µg	tlrl-bmdp	p 88
MDP FITC	FITC-labeled Muramyldipeptide	EndoFit™	10 ng - 10 µg/ml	500 µg	tlrl-fmdp	p 88
MDP Rhodamine	Rhodamine-labeled Muramyldipeptide	EndoFit™	100 ng - 10 µg/ml	500 µg	tlrl-rmdp	p 88
L18-MDP	Muramyldipeptide with a C18 fatty acid chain	EndoFit™	1 - 100 ng/ml	1 mg	tlrl-lmdp	p 88
N-Glycolyl-MDP	N-glycolylated muramyldipeptide	EndoFit™	100 ng - 10 µg/ml	5 mg	tlrl-gmdp	p 88
Murabutide	Synthetic derivative of muramyldipeptide	EndoFit™	10 ng - 1 µg/ml	5 mg	tlrl-mbt	p 88
Murabutide control	Murabutide analog (D isoform, inactive)	EndoFit™	10 ng - 1 µg/ml	5 mg	tlrl-mbtc	p 88
NOD1/2 Agonists						
M-Tri$_{DAP}$	MurNAc-L-Ala-γ-D-Glu-mDAP	EndoFit™	1 - 100 µg/ml	1 mg	tlrl-mtd	p 88
PGN-ECndi ultrapure	Insoluble peptidoglycan from *E. coli K12*	EndoFit™	1 - 5 µg/ml	5 mg	tlrl-kipgn	p 88
PGN-ECndss ultrapure	Soluble sonicated peptidoglycan from *E. coli K12*	EndoFit™	1 - 5 µg/ml	1 mg	tlrl-ksspgn	p 88
PGN-SAndi ultrapure	Insoluble peptidoglycan from *S. aureus*	EndoFit™	1 - 5 µg/ml	5 mg	tlrl-sipgn	p 88
RIG-I/MDA-5 and CDS Agonists						
5'ppp-dsRNA **NEW**	5'triphosphate blunt-end double-stranded RNA	EndoFit™	300 ng - 1 µg/ml	25 µg 100 µg	tlrl-3prna tlrl-3prna-100	p 88
5'ppp-dsRNA Control **NEW**	5'triphosphate blunt-end double-stranded RNA	EndoFit™	300 ng - 1 µg/ml	25 µg 100 µg	tlrl-3prnac tlrl-3prnac-100	p 88
Poly(dA:dT) Naked **NEW**	Poly(dA-dT)•poly(dT-dA)	EndoFit™	1 - 5 µg/ml	200 µg 1 mg	tlrl-patn tlrl-patn-1	p 88
Poly(dA:dT)/LyoVec	Poly(dA-dT)•poly(dT-dA)/LyoVec complexes	EndoFit™	1 - 5 µg/ml	100 µg	tlrl-patc	p 88
Poly(dG:dC) Naked **NEW**	Poly(dG-dC)•poly(dG-dC)	EndoFit™	1 - 5 µg/ml	200 µg	tlrl-pgcn	p 88
Poly(dG:dC)/LyoVec **NEW**	Poly(dG-dC)•poly(dG-dC)/LyoVec complexes	EndoFit™	1 - 5 µg/ml	100 µg	tlrl-pgcc	p 88
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 88
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 88

PRODUCT	ORIGIN/DESCRIPTION	ENDOTOXIN LEVELS	WORKING CONCENTRATION	QTY	CATALOG CODE	INFO
Dectin-1 Agonists						
Curdlan NEW	Beta-1,3-glucan from *Alcaligenes faecalis*	<0.05 EU/µg	100 µg/ml	100 mg	tlrl-curd	p 89
HKCA	Heat-killed *Candida albicans*	EndoFit™	10^8 cells/ml	10^9 cells	tlrl-hkca	p 89
HKSC	Heat-killed *Saccharomyces cerevisiae*	EndoFit™	10^8 cells/ml	10^9 cells	tlrl-hksc	p 89
Zymosan	Cell wall preparation from S. cerevisiae	EndoFit™	1 - 100 µg/ml	100 mg	tlrl-zyn	p 89
Zymosan Depleted	Hot alkali treated zymosan	EndoFit™	100 µg/ml	10 mg	tlrl-dzn	p 89
NF-κB Activators						
PMA	Phorbol myristate acetate	NA	100 ng - 1 µg/ml	5 mg	tlrl-pma	p 89
Recombinant human TNF-α	Recombinant human tumor necrosis factor alpha	EndoFit™	0.1 - 10 ng/ml	20 µg	rhtnf-a	p 89

TLR & NOD Agonist Kits

The TLR and NOD agonist kits represent 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- **FSL1** (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- **ODN2006** (100 µg) - TLR9 Agonist	tlrl-kit1hw
TLR1-9 Agonist Kit Mouse (9 ligands)	1- **Pam3CSK4** (10 µg) - TLR1/2 Agonist 2- **FSL1** (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- **ODN1826** (100 µg) - TLR9 Agonist	tlrl-kit1mw
TLR2 Agonist Kit Human/Mouse (7 ligands)	1- **Pam3CSK4** (10 µg) - TLR1/2 Agonist 2- **FSL1** (10 µg) - TLR6/2 Agonist 3- **HKLM** (10^9 cells) - TLR2 Agonist 4- **LM-MS** (10 µg) - 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-kit2
TLR3/7/8/9 Agonist Kit Human (14 ligands)	1- **Poly(I:C) (HMW)** (500 µg) - TLR3 Agonist 2- **Poly(I:C) (LMW)** (500 µg) - TLR3 Agonist 3- **Imiquimod** (25 µg) - TLR7 Agonist 4- **R848** (25 µg) - TLR7/8 Agonist 5- **CL075** (25 µg) - TLR7/8 Agonist 6- **CL264** (25 µg) - TLR7 Agonist 7- **ssPolyU/LyoVec** (25 µg) - TLR8 Agonist	8- **ssRNA40/LyoVec** (25 µg) - hTLR8 Agonist 9- **ssRNA41/LyoVec** (25 µg) - Control 10- **ODN2006** (100 µg) - TLR9 Agonist 11- **ODN2006 control** (100 µg) - Control 12- **ODN2216** (100 µg) - TLR9 Agonist 13- **ODN2216 control** (100 µg) - Control 14- ***E.coli* ssDNA/LyoVec** (50 µg) - TLR9 Agonist	tlrl-kit3hw2
NOD1/2 Agonist Kit Human/Mouse (10 ligands)	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- **Murabutide** (100 µg) - NOD2 Agonist 7- **PGN-ECndi ultrapure** (100 µg) - NOD1/2 Agonist 8- **PGN-ECndss ultrapure** (25 µg) - NOD1/2 Agonist 9- **PGN-SAndi ultrapure** (100 µg) - NOD1/2 Agonist 10- **Tri-DAP** (25 µg) - NOD1 Agonist	tlrl-nodkit

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.

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*) **NEW**

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, 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 the 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*) **NEW**

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 wall. LM and LAM 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 involves also LPS-binding protein (LBP) and CD14[24].
InvivoGen provides LTA from *B. subtilis* (LTA-BS) and *S. aureus* (LTA-SA) and 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.

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].

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 yeast cell, 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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TLR3 Agonists

Poly(I:C) & 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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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*) - TLR4 (TLR2) Agonists

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]

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.

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 *E. coli* synthetic MPLA activates TLR4 but does not activate TLR2 even at high concentrations reflecting its high purity.

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TLR5 Agonists

FLA-BS, FLA-ST, FLA-ST Ultrapure & RecFLA-ST (*B. subtilis and S. typhimurium*)

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** 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** was purified by monoclonal anti-FliC affinity chromatography. The purity of FLA-ST ultrapure is >95%.
• **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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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 seems also 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]. It induces the activation of NF-κB at 0.4 μM (0.1 μg/ml) in TLR7-transfected HEK293 cells and at 4 μM (1 μg/ml) in TLR8-transfected HEK293 cells.

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[4]. 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].

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 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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TLR9 Agonists

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 induce differentially 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'-tgctgctttttgtgctttttgtgctt-3'
ODN 2007[7, 8]	(Type B, bovine/porcine)	5'-tcgtcgttgtcgttttgtcgtt-3'	**ODN 2007 control**	5'-tgctgcttgtgctttttgtgctt-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'-tgctgctttttgggggccccccc-3'
ODN D19[11, 12]	(Type A, human/porcine)	5'-ggTGCATC:GATGCAGggggg-3'	**ODN D19 control**	5'-ggTGCATG:CATGCAGggggg-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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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	**NEW**	5'-cctggatgggaa-3''
ODN INH-1	**NEW**	5'-cctggatgggaa:ttcccatccagg-3'
ODN INH-47 (control)	**NEW**	5'-tatggattttaa:ttaaaatccata-3'

Bases are phosphorothioate.

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NOD1/2 Agonists

iE-DAP & iE-Lys - 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 - NOD1 Agonist

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

MDP, MDP control, L18-MDP & N-glycolyl-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** - 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 safe 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

MurNAc-L-Ala-D-γ-Glu-mDAP (M-TriDAP), 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].

PGN-Ecndi & PGN-SAndi insoluble, ultrapure (*E. coli* and *S. aureus*) - 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 (*E. coli*) - 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.

1. Chamaillard M. *et al.*, 2003. An essential role for NOD1 in host recognition of bacterial peptidoglycan containing diaminopimelic acid. Nat. Immunol.4(7):702-7. **2. Girardin SE. *et al.*, 2003.** Nod2 is a general sensor of peptidoglycan through muramyl dipeptide (MDP) detection. J Biol Chem. 278(11):8869-72. **3. Inohara N. *et al.*, 2003.** Host recognition of bacterial muramyl dipeptide mediated through NOD2. Implications for Crohn's disease. J Biol Chem. 278(8):5509-12. **4. Traub S. *et al.*, 2004.** Structural requirements of synthetic muropeptides to synergize with lipopolysaccharide in cytokine induction. J Biol Chem. 279(10):8694-700. **5. Martinon F. *et al.*, 2004.** Identification of bacterial muramyl dipeptide as activator of the NALP3/cryopyrin inflammasome. Curr Biol. 14(21):1929-34. **6. Ishihara C. *et al.* 1985.** Effect of muramyl dipeptide and its stearoyl derivatives on resistance to Sendai virus infection in mice. Vaccine. 3(5):370-4. **7. Coulombe F,. *et al.*, 2009.** Increased NOD2-mediated recognition of N-glycolyl muramyl dipeptide. J Exp Med. 206(8):1709-16. **8. Chedid LA. *et al.*, 1982.** Biological activity of a new synthetic muramyl peptide adjuvant devoid of pyrogenicity. Infect Immun. 35(2):417-24. **9. Krueger JM. *et al.*, 1984.** Muramyl peptides. Variation of somnogenic activity with structure. J Exp Med. 159(1):68-76. **10. Girardin SE. *et al.*, 2003.** Peptidoglycan molecular requirements allowing detection by Nod1 and Nod2. J Biol Chem. 278(43):41702-8.

RIG-I/MDA5 and CDS Agonists

5'ppp-dsRNA - RIG-I Agonist **NEW**

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'- pppGCAUGCGACCUCUGUUUGA -3' 3'- CGUACGCUGGAGACAAACU -5'

Poly(dA:dT) - CDS Agonist **NEW**

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 **NEW**

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 & Poly(I:C)-LMW/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)/LyoVec and poly(I:C)-LMW/LyoVec are preformed complexes between poly(I:C) 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.

1. Hornung V. *et al.*, 2006. 5'-Triphosphate RNA is the ligand for RIG-I. Science. 314(5801):994-7. **2. Schlee m. *et al.*, 2009.** Recognition of 5' triphosphate by RIG-I helicase requires short blunt double-stranded RNA as contained in panhandle of negative-strand virus. Immunity. 17;31(1):25-34. **3. Takaoka A. *et al.*, 2007.** DAI (DLM-1/ZBP1) is a cytosolic DNA sensor and an activator of innate immune response. Nature. 448(7152):501-5. **4. Yang P. *et al.*, 2010.** The cytosolic nucleic acid sensor LRRFIP1 mediates the production of type I interferon via a beta-catenin-dependent pathway. Nat Immunol. 11(6):487-94. **5. Jones JW. *et al.*, 2010.** Absent in melanoma 2 is required for innate immune recognition of Francisella tularensis. PNAS, 107(21):9771-6. **6. Ablasser A. *et al.*, 2009.** RIG-I-dependent sensing of poly(dA:dT) through the induction of an RNA polymerase III-transcribed RNA intermediate. Nat Immunol. 10(10):1065-72. **7. Gitlin L. *et al.*, 2006.** Essential role of mda-5 in type I IFN responses to polyriboinosinic:polyribocytidylic acid and encephelamyocarditis picornavirus. PNAS 103(22):8459-8464. **8. Kato H. *et al.*, 2005.** Cell type-specific involvement of RIG-I in antiviral response. Immunity. 23(1):19-28.

Dectin-1 Agonists

Curdlan NEW

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 in robust activation of IL-1β–mediated inflammatory response[2].

HKCA (*Candida albicans*)

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*)

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].

Zymosan - TLR2 & Dectin-1 Agonist

Zymosan, an insoluble preparation of yeast cell, activates macrophages via TLR2. TLR2 cooperates with TLR6 and CD14 in response to zymosan[6]. 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[7].

Zymosan Depleted

Zymosan depleted is a *S. cerevisiae* cell wall preparation treated with hot alkali to remove all its TLR-stimulating properties[8]. Zymosan depleted activates Dectin-1 but not TLR2.

1. Goodridge HS, et al., 2009. Beta-glucan recognition by the innate immune system. Immunol Rev. 230(1):38-50. **2. Kankkunen P., 2010.** (1,3)-beta-glucans activate both dectin-1 and NLRP3 inflammasome in human macrophages. J Immunol. 184(11):6335-42. **3. Gow NA. et al., 2007.** Immune recognition of Candida albicans beta-glucan by dectin-1. J Infect Dis. 196(10):1565-71. **4. Giaimis J. *et al.*, 1993.** Both mannose and b-glucan receptors are involved in phagocytosis of unopsonized, heat-killed *Saccharomyces cerevisiae* by murine macrophages. J. Leukoc. Biol., 54: 564-571. **5. Brown GD. *et al.*, 2002.** Dectin-1 Is A Major ß-Glucan Receptor On Macrophages. J. Exp. Med., 196: 407-412. **6. Ozinsky A. *et al.*, 2000.** The repertoire for pattern recognition of pathogens by the innate immune system is defined by cooperation between toll-like receptors. PNAS. 97(25):13766-71. **7. Gantner BN. *et al.*, 2003.** Collaborative induction of inflammatory responses by dectin-1 and Toll-like receptor 2. J Exp Med. 197(9):1107-17. **8. Gantner BN. *et al.*, 2003.** Collaborative induction of inflammatory responses by dectin-1 and Toll-like receptor 2. J Exp Med. 197(9):1107-17.

NF-κB Activators

PMA

Phorbol 12-myristate 13-acetate (PMA), also known as 12-O-tetradecanoylphorbol 13-acetate (TPA) 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[1].

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[2]. It acts by activating transcription factors such as NF-κB and AP-1. InvivoGen provides a recombinant human TNF-α produced in CHO cells and purified by affinity chromatography.

1. Chang MS. *et al.*, 2005. Phorbol 12-myristate 13-acetate upregulates cyclooxygenase-2 expression in human pulmonary epithelial cells via Ras, Raf-1, ERK, and NF-kappaB, but not p38 MAPK, pathways. Cell Signal. 17(3):299-310. **2. Leong KG & Karsan A. 2000.** Signaling pathways mediated by tumor necrosis factor alpha. Histol Histopathol. 15(4):1303-25. Review.

HEK-Blue™ LPS Detection Kit

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

Description

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 11).

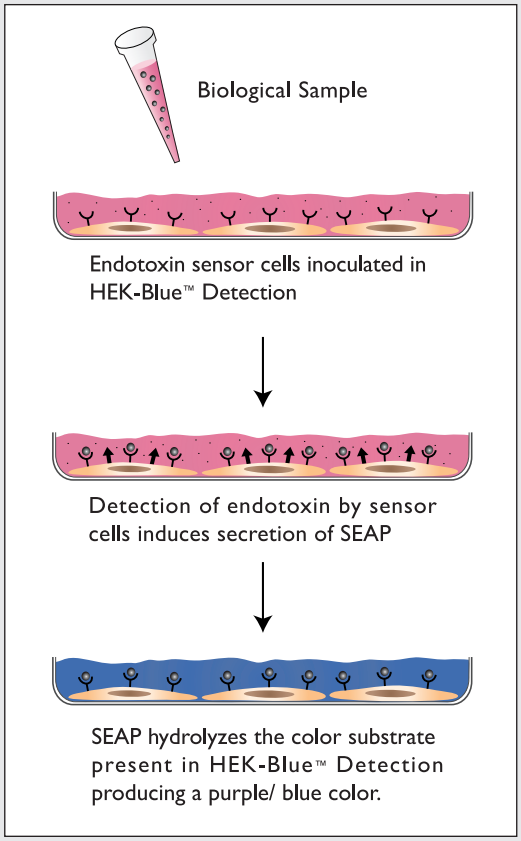
HEK-Blue™ Detection is a medium specifically designed for the detection of SEAP. It contains a color substrate that produces a purple/blue color following its hydrolysis by SEAP (see page 15).

Contents and Storage

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	2 pouches	hb-det1
LPS-EK (control)	5 mg	tlrl-eklps

TLR/NOD Ligand Screening

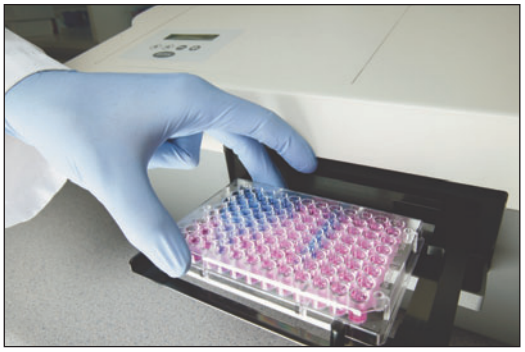
InvivoGen has developed a high-throughput method to determine whether a compound is recognized by Toll-Like Receptors or NOD1/NOD2 and acts either as an agonist or antagonist. This method is based on TLR- or NOD-induced activation of various transcription factors including NF-κB, AP1 and IRF7 in HEK293 clones.

Key Service Features

Short turnaround time - Screening turnaround: ONLY 2 weeks

Screening flexibility - Screening parameters can be selected and/or modified based on customer requirements. Level 1 and level 2 can be carried out on additional TLRs, including mouse TLRs. More compound concentrations can be tested.

Cost effective - A set-up charge applies for the first compound. Subsequent compounds are heavily discounted.



Description

TLR Ligand Screening

Two choices of services are offered, level 1 and level 2, that can be performed sequentially or individually.

Level 1: Single dose testing on a panel of human TLRs
Testing is carried out on seven TLRs.
- TLR2: wide array of microbial molecules
- TLR3: dsRNA
- TLR4: Gram-negative lipopolysaccharide
- TLR5: flagellin
- TLR7: single-stranded RNA and small antiviral molecules
- TLR8: single-stranded RNA and small antiviral molecules
- TLR9: specific unmethylated CpG oligonucleotide sequences

Screening is performed at a single concentration, typically a 1/10 dilution of the original compound solution provided, or customer specified. Duplicate tests (plus controls, see list).

Level 2: Dose response on one or two TLRs
Three concentrations of the compound(s), typically 1/10, 1/100 and 1/1000 dilutions of the original compound solution, are tested on the TLR(s) recognizing the compound(s) as determined in level 1 or specified by the customer. Triplicate tests (plus controls).

TLR ligands are typically recognized by a single TLR, potentially two (TLR7 and TLR8). Recognition by TLR4 usually reflects the presence of endotoxins in the sample.

NOD Ligand Screening

Two cytoplasmic proteins involved in pathogen recognition, NOD1 and NOD2, can be added to this screening service. Their activation by a given compound is detected using the same method as the one described above.

Compounds can be supplied as stock solutions or as solid materials for water-soluble compounds.
A detailed report will be prepared and provided to the customer electronically and in hard copy. All procedures are performed accordingly to strict guidelines. Confidentiality is guaranteed.

TLR	Ligand	Concentration
TLR2	HKLM	10^8 cells/ml
TLR3	Poly(I:C)	1 µg/ml
TLR4	*E. coli* K12 LPS	100 ng/ml
TLR5	*S. typhimurium* flagellin	100 ng/ml
TLR7	Imiquimod	3 µg/ml
TLR8	ssPolyU/LyoVec	10 µg/ml
TLR9	CpG ODN 2006	10 µg/ml
NOD1	Tri-DAP	1 µg/ml
NOD2	Muramyl dipeptide	100 ng/ml

PRODUCT	CAT. CODE
TLR / NOD Ligand Screening	tlrl-test

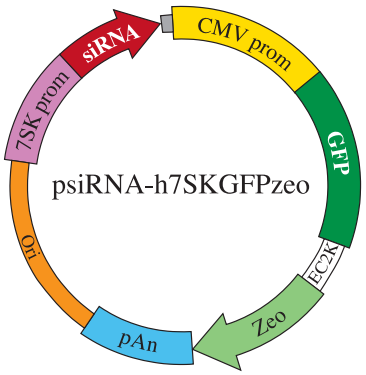
Contact us for more information.
info@invivogen.com

Ready-Made psiRNA - 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 the 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 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 in both *E. coli* and mammalian cells.



Contents and Storage

Ready-made psiRNA plasmids are available alone or in a kit.
Each ready-made psiRNA plasmids is 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
- 4 pouches of Fast-Media® Zeo

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

GENE NAME	SPECIES	CAT. CODE
Toll-Like Receptors (TLRs)		
TLR1	h, m	p 149
TLR2	h, m	p 149
TLR3	h, m	p 149
TLR4	h, m	p 149
TLR5	h, m	p 149
TLR6	h, m	p 149
TLR7	h, m	p 149
TLR8	h, m	p 149
TLR9	h, m	p 149
TLR10	h	p 149
NOD-Like Receptors (NLRs)		
IPAF / CARD12	h	p 149
NAIP5 / BIRC1E	m	p 149
NALP1 / CARD7	h	p 149
NALP2	h	p 149
NALP3 / NLRP3	h, m	p 149
NALP12 / Monarch1	h	p 149
NOD1 / CARD4	h, m	p 149
NOD2 / CARD15	h, m	p 149
NOD9 / NLRX1	h, m	p 149
RIG-I-Like Receptors (RLRs)		
LGP2 / DHX58	h, m	p 149
MDA5 / IFIH1	h, m	p 149
RIG-I / DDX58	h, m	p 149
Other Pathogen Sensors		
AIM2 / IFI210	h, m	p 148
CLEC9A	h, m	p 148
DAI / ZBP1	h	p 148
DC-SIGN / CD209	h, m	p 148
Dectin-1	h, m	p 148
IFI16 NEW	h, m	p 148

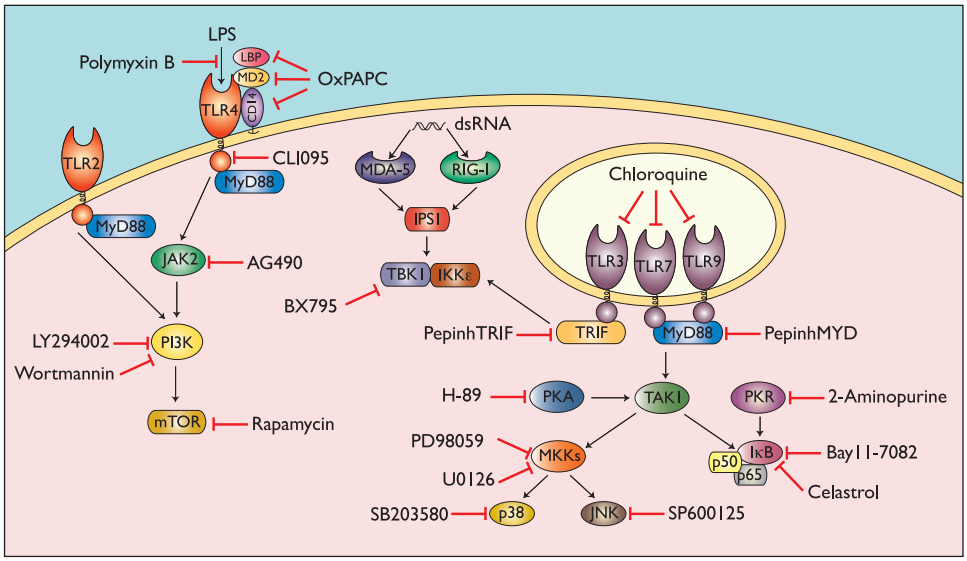
GENE NAME	SPECIES	CAT. CODE
Other Pathogen Sensors		
MBL2	h	p 149
SIGNR1	m	p 149
SIGNR2	m	p 149
SIGNR3	m	p 149
Adaptors		
ASC / CARD5	h, m	p 148
Cardinal / CARD8	h	p 148
IPS-1 / MAVS / VISA	h, m	p 149
MyD88	h, m	p 149
RAC1	h, m	p 149
SARM1	h	p 149
TICAM1 / TRIF	h, m	p 149
TICAM2 / TRAM	h, m	p 149
TIRAP / Mal	h, m	p 149
Co-receptors		
CD14	h, m	p 148
CD36	h, m	p 148
MD2 NEW	h	p 149
Signaling Effectors		
BCL10 / CLAP	h, m	p 148
CARD9	h, m	p 148
CD44	h, m	p 148
DDX3 / DDX3X	h	p 148
FADD / MORT1	h, m	p 148
IKKε / IKBKE / IKK-i	h, m	p 149
IRAK-1	h, m	p 149
IRAK-4	h, m	p 149
LRRFIP2	h	p 149
NAP1 / AZI2	h, m	p 149
Pellino1 / PELI1	h	p 149
Pellino2 / PELI2	h	p 149

GENE NAME	SPECIES	CAT. CODE
Signaling Effectors		
Pellino3 / PELI3	h,	p 149
PKD1 / PKRD1	h	p 149
PKR / EIF2AK2	h, m	p 149
PRKRA / PACT	h, m	p 149
RIP1 / RIPK1	h, m	p 149
RIP2 / RIPK2	h, m	p 149
STING NEW	h, m	p 149
SUGT1 NEW	h	p 149
TAK1 / MAP3K7	h, m	p 149
TANK	h, m	p 149
TBK1 / NAK	h, m	p 149
TRADD	h, m	p 149
TRAF3 / CAP-1	h, m	p 149
TRAF6 / RNF85	h, m	p 149
Signaling Inhibitors		
A20 / TNFAIP3	h, m	p 148
ATF3	h	p 148
Bcl-3	h, m	p 148
DAK	h, m	p 148
DUBA	m	p 148
FLII / Fliih	h	p 148
IRAK-M	h	p 149
MULAN / Dublin	h, m	p 149
PIN1 / DOB	h, m	p 149
RNF125 / TRAC-1	h, m	p 149
RP105 / CD180	h	p 149
SIGIRR / TIR8	m	p 149
SIKE	h, m	p 149
ST2 / IL1RL1 / T1	h	p 149
Tollip / IL-1RAcPIP	h	p 149
TRAFD1 / FLN29	h	p 149
Triad3A	h	p 149

Signal Transduction Inhibitors

InvivoGen offers a selection of known inhibitors of the signaling pathways initiated by PRRs. These inhibitory 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.

PRODUCT	DESCRIPTION	ENDOTOXIN LEVELS	WORKING CONCENTRATION	QUANTITY	CATALOG CODE	INFO
2-Aminopurine	PKR inhibitor	<0.125 EU/mg	1 - 10 mM	250 mg	tlrl-apr	p 94
AG490	JAK2 inhibitor	<0.125 EU/mg	1 -100 µM	10 mg	tlrl-ag4	p 94
Bay11-7082	IκB-α inhibitor	<0.125 EU/mg	1 - 10 µM	10 mg	tlrl-b82	p 94
BX795	TBK1/IKKε inhibitor	<0.125 EU/mg	10 nM - 1 µM	5 mg	tlrl-bx7	p 94
Celastrol	NF-κB inhibitor	<0.125 EU/mg	2.5 - 10 µM	1 mg	ant-cls	p 94
Chloroquine	Endosomal acidification inhibitor	<0.125 EU/mg	10 µM	250 mg	tlrl-chq	p 94
CLI-095	TLR4 signaling inhibitor	<0.125 EU/mg	50 nM - 1 µM	1 mg	tlrl-cli95	p 94
Cyclosporin A NEW	Calcineurin inhibitor	<0.125 EU/mg	50 nM - 1.5 µM	100 mg	tlrl-cyca	p 94
Gefitinib NEW	RIP2 tyrosine kinase inhibitor	<0.125 EU/mg	10 µM	10 mg	tlrl-gef	p 94
H-89	PKA inhibitor	<0.125 EU/mg	5 - 50 µM	5 mg	tlrl-h89	p 94
Leptomycin B NEW	Nuclear export inhibitor	<0.125 EU/mg	50 - 200 nM	5 µg	tlrl-lep	p 94
LY294002	PI3K inhibitor	<0.125 EU/mg	50 - 100 µM	5 mg	tlrl-ly29	p 94
OxPAPC	TLR2 and TLR4 inhibitor	<0.125 EU/mg	30 µg/ml	1 mg	tlrl-oxp1	p 94
PD98059	MAP kinase kinase inhibitor	<0.125 EU/mg	5 - 100 µM	10 mg	tlrl-pd98	p 94
Pepinh-Control	Control for Pepinh inhibitory peptides	<0.125 EU/mg	5 - 100 µM	2 mg	tlrl-pictrl	p 95
Pepinh-MYD	MyD88 inhibitory peptide	<0.125 EU/mg	5 - 100 µM	2 mg	tlrl-pimyd	p 95
Pepinh-TRIF	TRIF inhibitory peptide	<0.125 EU/mg	50 - 100 µM	2 mg	tlrl-pitrif	p 95
Polymyxin B	LPS-induced TLR4 activation inhibitor	<0.125 EU/mg	10 µg/ml	100 mg	tlrl-pmb	p 95
Rapamycin	mTOR inhibitor	<0.125 EU/mg	10-100 nM	5 mg	tlrl-rap	p 95
SB203580	p38 MAP kinase inhibitor	<0.125 EU/mg	1 - 20 µM	5 mg	tlrl-sb20	p 95
SP600125	JNK inhibitor	<0.125 EU/mg	10 - 500 µM	10 mg	tlrl-sp60	p 95
U0126	MEK1-MEK2 inhibitor	<0.125 EU/mg	10 - 50 µM	5 mg	tlrl-u0126	p 95
Wortmannin	PI3K inhibitor	<0.125 EU/mg	0.1 - 2.5 µM	5 mg	tlrl-wtm	p 95



Contents and Storage

Each product is provided as a solid and shipped at room temperature. Store at room temperature, 4°C or -20°C according to the product label.

AG490 - JAK2 Inhibitor

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

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[4]. PKR plays also a role in TLR-induced antiviral activities as an intermediary in TLR3, TLR4 and TLR9 signaling[5].

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[6]. TNF-α-dependent effects of NF-κB are important for TLR expression and cytokine production[7].

BX795 - TBK/IKKε Inhibitor

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

Chloroquine - Inhibitor of Endosomal Acidification

Chloroquine is a lysosomotropic agent that prevents endosomal acidification[11]. 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[12].

CLI095 - TLR4 Signaling Inhibitor

CLI095, also known as TAK-242, is a novel cyclohexene derivative that suppresses specifically TLR4 signaling, inhibiting the production of NO and pro-inflammatory cytokines[13]. It acts by blocking the signaling mediated by the intracellular domain of TLR4, but not the extracellular domain. It potently suppresses both ligand-dependent and -independent signaling of TLR4[14].

Cyclosporin A - Calcineurin inhibitor **NEW**

Cyclopsporin 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[15]. Conversely, it has been demonstrated that the inhibition of calcineurin in macrophages can trigger TLR signaling and enhance NF-κB activation[16].

Gefitinib - RIP2 Tyrosine Kinase inhibitor **NEW**

Geftinib (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[17].

H-89 - PKA Inhibitor

H-89 is a selective, potent cell permeable inhibitor of cAMP-dependent protein kinase (PKA)[18]. 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[19].

Leptomycin B - Nuclear export inhibitor **NEW**

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[20]. The cellular target of Leptomycin B has been identified as CRM1 (exportin 1), an evolutionarily conserved receptor for the nuclear export signal of proteins.

LY294002 - PI3K Inhibitor

LY294002 is a potent, cell permeable inhibitor of phosphatidylinositol 3-kinase (PI3K) that acts on the ATP binding site of the enzyme[21]. The PI3K pathway is extensively studied for its property in inhibiting apoptosis. PI3K is also known to regulate TLR-mediated inflammatory responses[22].

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[23].

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[24].

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)[25] preceeded by a protein transduction sequence (RQIKIWFQNRMKWKK) derived from antennapedia which enables the peptide to translocate through the cell membrane[26]. Pepinh-MYD is provided with a control peptide.

Pepinh-TRIF - TRIF inhibitory peptide

Pepinh-TRIF is a 30 aa peptide that blocks TRIF signaling by interfering with TLR-TRIF interaction. Pepinh-TRIF contains the 14 aa that correspond to the sequence of the BB loop of TRIF (FCEEFQVPGRGELH)[27] linked to the cell-penetrating segment of the antennapedia homoedomain (RQIKIWFQNRMKWKK)[26]. Pepinh-TRIF is provided with a control peptide.

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[28]. PMB is widely used to eliminate the effects of endotoxin contamination, both *in vitro* and *in vivo*.

Rapamycin - mTOR Inhibitor

Rapamycin is an inhibitor of the Ser/Thr protein kinase named "mammalian target of rapamycin" (mTOR) that regulates cell growth and metabolism in response to environmental cues. mTOR is a downstream target of PI3K, an important actor in TLR signaling. Rapamycin was shown to block TLR2- and TLR4-mediated expression of TNF-α and IL-6 in neutrophils stimulated with Pam3CSK4 or LPS[29].

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[30]. SB203580 inhibits also the phosphorylation and activation of protein kinase B (PKB, also known as Akt)[31]. 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)[32]. 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.

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

Wortmannin - PI3K Inhibitor

Wortmannin is a cell-permeable, fungal metabolite that acts as a potent, selective and irrevesible inhibitor of phosphatidylinositol 3-kinase (PI3K). There is increasing evidence of the involvement of PI3K in TLR signaling. Inhibition of PI3K by wortmannin was shown to greatly enhance TLR-mediated inducible nitric-oxide synthase (iNOS) expression and cytokine production in the mouse macrophage cell line RAW264.7. The effect of wortmannin was common to TLR2, -3, -4, and -9 and was accompanied by activation of NF-κB and up-regulation of cytokine mRNA production[34].

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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. Certain members of the NLR family sense pathogen-associated molecular patterns (PAMPs) in the cytosol and induce the assembly of large caspase-1-activating complexes called inflammasomes[1]. 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. Several inflammasomes have been identified and defined by the NLR protein that they contain. IPAF (ICE protease-activating factor) inflammasome is triggered by bacterial flagellin[2], whereas NALP1b (NACHT domain-, leucine-rich repeat-, and PYD-containing protein 1b) inflammasome is induced by anthrax lethal toxin[3]. NALP3/cryopyrin/NLRP3 inflammasome assembles in response to a variety of PAMPs and 'danger' signals, such as uric acid crystals. A new inflammasome has just been identified composed of AIM2 (absent in melanoma 2) which recognizes cytoplasmic double-stranded DNA.

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-1β signaling initiates signaling cascades leading to the activation of NF-κB and MAP kinases which trigger the secretion of inflammatory cytokines. IL-18 induces IFN-γ production and contributes to T-helper 1 (Th1) cell polarization. IL-18 signaling pathways are similar to those initiated by IL-1β. Both cytokines share a common maturation mechanism that requires caspase-1. 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 which assemble into a heterotetramer[4]. Caspase-1 is activated within the inflammasome through interaction with ASC (apoptosis-associated speck-like protein containing a carboxy-terminal CARD), a bipartite adapter protein that bridges NLRs and caspase-1[5].

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.

NALP3 Inflammasome

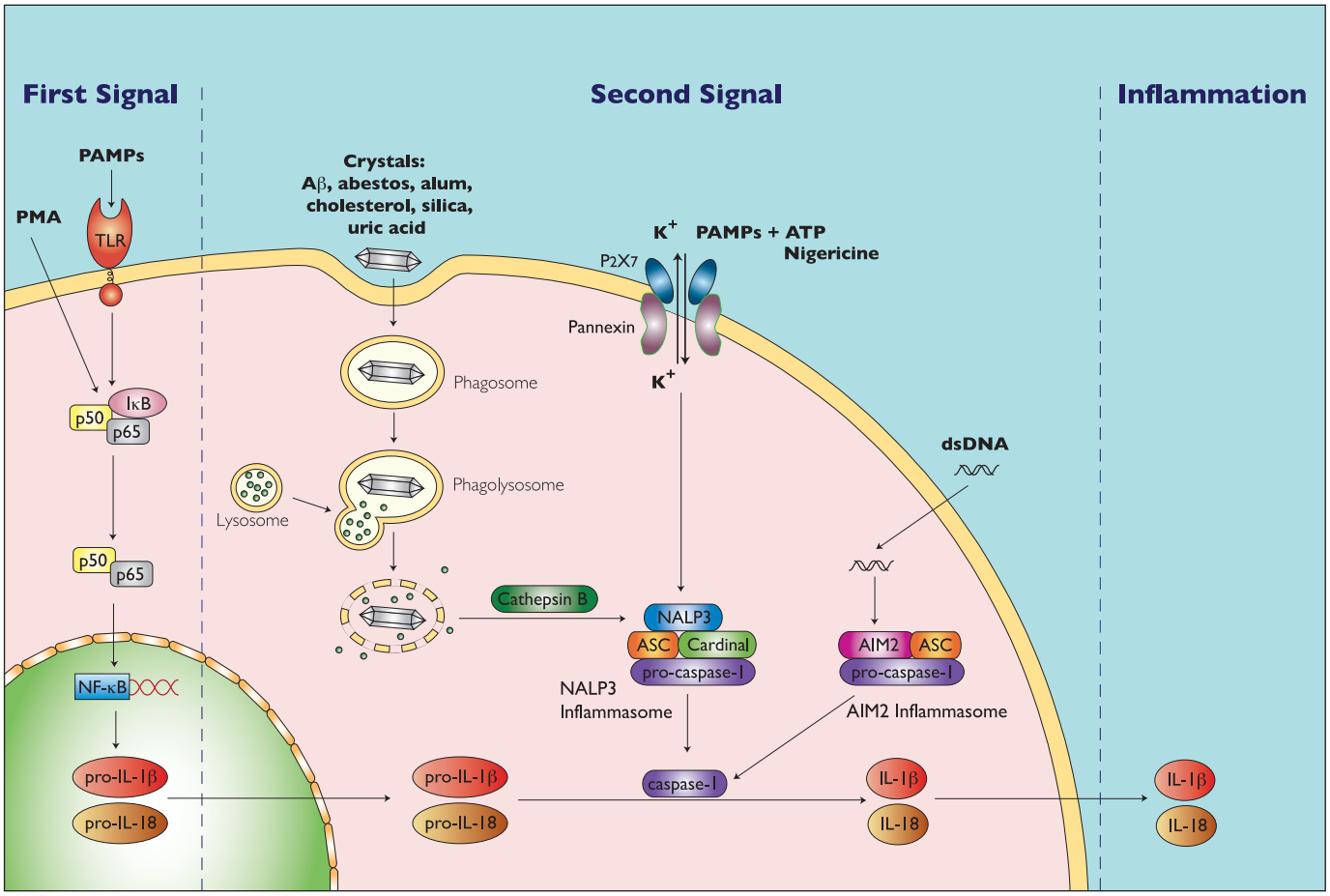
Among the inflammasomes, NALP3 inflammasome is the most studied. Its activation in macrophages can be achieved with PAMPs, such as LPS, peptidoglycan, and bacterial nucleic acids, provided the cells are exposed to ATP. Indeed, in the absence of ATP, macrophages stimulated with LPS produce large quantities of pro-IL-1β, but release little mature cytokine to 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 NALP3 inflammasome. The effect of ATP is mediated by the purinergic P2X7 receptor, which causes a rapid potassium efflux from the cytosol upon activation. ATP induces rapid opening of the non-selective cation channel of P2X7, followed by the gradual opening of a larger pore. The larger pore is mediated by the hemichannel pannexin-1 which is recruited upon activation of the P2X7 receptor. Recent studies have demonstrated that pannexin is required for caspase-1 activation in response to ATP, nigericin and maitotoxin[6]. However, the trigger between pannexin-1 and the NALP3 inflammasome remains unclear. Potassium (K+) efflux is thought to be an essential trigger of NALP3-induced caspase-1 activation. Macrophages derived from mice lacking the P2X7 receptor failed to activate caspase-1[7]. However, K+ efflux is not sufficient for activation of the NALP3 inflammasome, since activation of the P2X7 receptor does not induce caspase-1 maturation in macrophages that have not been exposed to LPS. It has been suggested that pannexin-1 may mediate the delivery of PAMPs into the cytosol which might explain the lack of requirement for TLR signaling in caspase-1 activation induced via the pannexin-1/NALP3 pathway[8].

A recent study demonstrated that monosodium urate (MSU) and calcium phosphate dihydrate (CPPD) crystals activate caspase-1 in a NALP3-dependent manner[9]. Deposition of MSU and CPPD crystals in joints is responsible for the inflammatory conditions gout and pseudogout, respectively. Thus, the NALP3 inflammasome was suggested to participate in the etiology of these auto-inflammatory diseases. In addition to its role in gout, uric acid is a major component released into the extracellular milieu by necrotic cells, suggesting an important role of NALP3 in the detection of endogenous 'danger' signal. Crystalline silica and asbestos were shown to induce the activation of the NALP3 inflammasome, suggesting that the NALP3 inflammasome participates in the pathogenesis of silicosis and asbestosis[10-12]. Aluminium salt (alum) crystals also activate the inflammasome formed by NALP3 and seem to require the presence of PAMPs. Indeed, several groups have reported that alum activates the NALP3 inflammasome only if the cells are pretreated with LPS[12-14]. NALP3 activation by crystals has been shown to require phagocytosis, causing lysosomal swelling and damage, and to involve cathepsin B, a lysosomal cysteine protease. As crystal-independent lysosomal damage was sufficient to induce NALP activation, it was suggested that rather than the crystal structure itself, the NALP3 inflammasome senses lysosomal pertubation as a 'danger' signal[12].

AIM2 Inflammasome

Recently, several groups have identified AIM2 (absent in melanoma 2) as a new receptor for cytoplasmic DNA, which forms an inflammasome with the ligand and ASC to activate caspase-1[15-17]. 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. Knockdown of AIM2 expression by RNA-mediated interference was shown to reduce DNA-induced maturation of IL-1β in macrophages whereas stable expression of AIM2 in the human embryonic kidney 293T cell line conferred responsiveness to cytoplasmic DNA. These data suggest that AIM2 is both required 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 Inducers & Inhibitors

The inflammasome is a multi-protein complex involved in the production of mature IL-1β. It is activated by a large variety of microbial molecules, danger signals and crystalline substances. InvivoGen provides a selection of these molecules known to induce the assembly of the NLRP3 or AIM2 inflammasomes. We also offer some inhibitors to confirm the ability of a given molecule to activate the inflammasome or to test the importance of phagocytosis or potassium efflux in this process. These inflammasome inducers and inhibitors have been validated using the **THP-1/HEK-Blue™-IL-1β Assay** (see page 100).

PRODUCT	DESCRIPTION	WORKING CONCENTRATION	QUANTITY	CATALOG CODE
Inflammasome Inducers				
Alum Crystals	Aluminium potassium sulfate	10 - 200 µg/ml	1 g	tlrl-alk
ATP	Adenosine 5′-triphosphate disodium salt	5 mM	1 g	tlrl-atp
CPPD Crystals	Calcium pyrophosphate dihydrate	50 - 200 µg/ml	5 mg	tlrl-cppd
Hemozoin NEW	Synthetic heme crystal	50 - 400 µg/ml	5 mg	tlrl-hz
MSU Crystals	Monosodium urate (uric acid)	50 - 200 µg/ml	5 mg	tlrl-msu
Nigericin	Nigericin, sodium salt	1 µM	10 mg	tlrl-nig
Poly(dA:dT), double-stranded B DNA	Poly(dA-dT)•poly(dT-dA)/LyoVec™ complex	1 - 5 µg/ml	100 µg	tlrl-pat
Inflammasome Inhibitors				
Glybenclamide	Proton pump inhibitor	25 µg/ml	1 g	tlrl-gly
Z-VAD-FMK	Pan-caspase Inhibitor	10 µg/ml (20 µM)	1 mg	tlrl-vad

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 triggers 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 NEW

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].

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[10].

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[11]. 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[12-14]. Poly(dA:dT) binds to AIM2 and induces its oligomerization, which is the first demonstration of an inflammasome bound to its ligand[12].

Inflammasome Inhibitors

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[15]. Glybenclamide was shown to potently block the activation of the NLRP3 inflammasome induced by PAMPs, DAMPs and crystalline substances[16, 17]. Recent data suggest that glybenclamide works downstream of the $P2X_7$ receptor but upstream of NLRP3[16].

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[18]. 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[17].

1. Li H. *et al.*, 2008. Cutting Edge: Inflammasome activation by Alum and Alum's adjuvant effect are mediated by NLRP3. J Immunol. 181:17-21. **2. Hornung V. *et al.*, 2008.** Silica crystals and aluminium salts activate the NALP3 inflammasome through phagosomal destabilization. Nature Immunol. 9:847-856. **3. Mariathasan S. *et al.*, 2006.** Cryopyrin activates the inflammasome and ATP. Nature 440;228-32. **4. Locovei S. *et al.*, 2007.** Pannexin1 is part of the pore forming unit of the P2X(7) receptor death complex. FEBS Lett. 581(3):483-8. **5. Perregaux D. & Gabel CA., 1994.** Interleukin-1b maturation and release in response to ATP and nigericin. J Biol. Chem. 269:15195-15203. **6. Shio MT. *et al.*, 2009.** Malarial hemozoin activates the NLRP3 inflammasome through Lyn and Syk kinases. PLoS Pathog. 5(8):e1000559. **7. Dostert C. *et al.*, 2009.** Malarial hemozoin is a Nalp3 inflammasome activating danger signal. PLoS One. 4(8):e6510. **8. Coban C. *et al.*, 2010.** The malarial metabolite hemozoin and its potential use as a vaccine adjuvant. Allergol Int. 59(2):115-24. **9. Martinon F. *et al.*, 2006.** Gout-associated uric acid crystals activate the NALP3 inflammasome. Nature.440(7081):237-41. **10. Pelegrin P. & Surprenant A., 2007.** Pannexin-1 couples to maitotoxin- and nigericin-induced interleukin-1beta release through a dye uptake-independent pathway. J Biol Chem. 282(4):2386-94. **11. Muruve DA. *et al.*, 2008.** The inflammasome recognizes cytosolic microbial and host DNA and triggers an innate immune response. Nature. 452(7183):103-7. **12. Hornung V. *et al.*, 2009.** AIM2 recognizes cytosolic dsDNA and forms a caspase-1-activating inflammasome with ASC. Nature. 458(7237):514-8. **13. Fernandes-Alnemri T. *et al.*, 2009.** AIM2 activates the inflammasome and cell death in response to cytoplasmic DNA. Nature. 458(7237):509-13. **14. Bürckstümmer T. *et al.*, 2008.** An orthogonal proteomic-genomic screen identifies AIM2 as a cytoplasmic DNA sensor for the inflammasome. Nat Immunol. 10(3):266-72. **15. Laliberte RE. *et al.*, 1999.** ATP treatment of human monocytes promotes caspase-1 maturation and externalization. J Biol Chem. 274(52):36944-51. **16. Lamkanfi M. *et al.*, 2009.** Glyburide inhibits the Cryopyrin/Nalp3 inflammasome. J. Cell Biol., 187: 61 - 70. **17. Dostert C. *et al.*, 2009.** Malarial hemozoin is a Nalp3 inflammasome activating danger signal. PLoS One. 4(8):e6510. **18. Slee EA. *et al.*, 1996.** Benzyloxycarbonyl-Val-Ala-Asp (OMe) fluoromethylketone (Z-VAD.FMK) inhibits apoptosis by blocking the processing of CPP32. Biochem J. 315 (Pt 1):21-4.

Contents and Storage

Each product is provided as a solid and shipped at room temperature. Store at room temperature, 4°C or -20°C according to the product label.

HEK-Blue™ IL-1β 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.

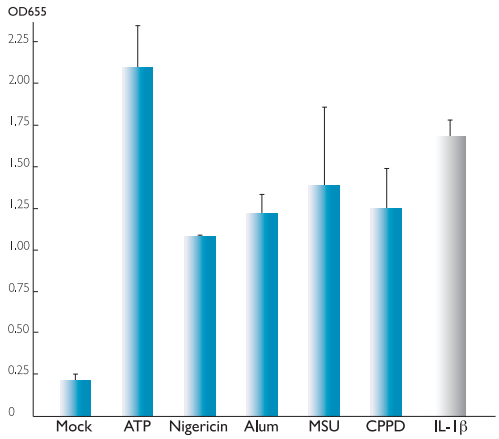
HEK-Blue™ IL-1β cells feature the SEAP (secreted embryonic alkaline phosphotase) reporter gene under the control of an NF-κB-inducible promoter. They naturally express the IL-1β receptor (IL-1R), and all the proteins involved in the MyD88-dependent IL-1R signaling pathway that leads to NF-κB activation. 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.

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.

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

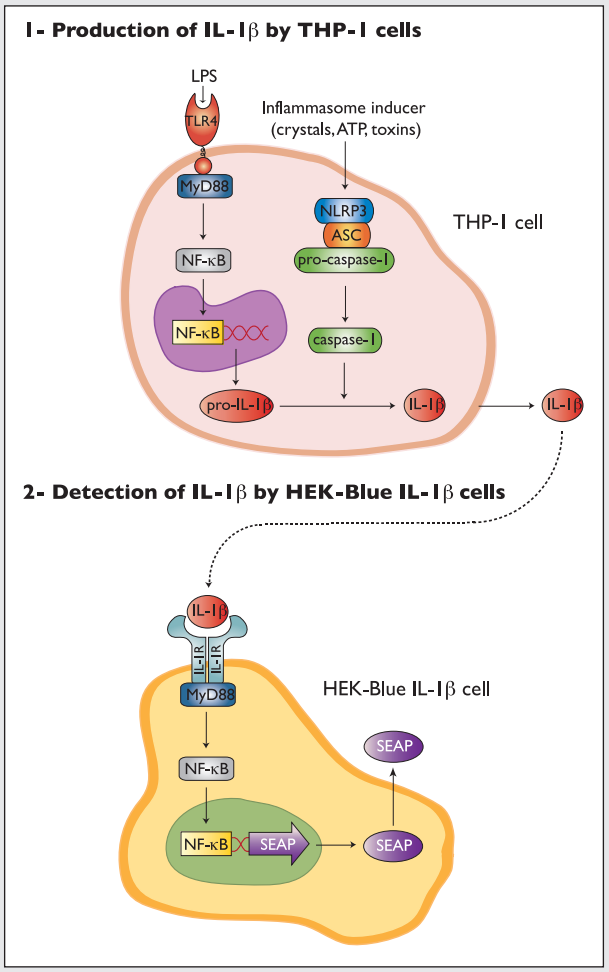
Contents and Storage

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.



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) 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: 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.

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

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

Related Products

Zeocin™, page 18
QUANTI-Blue™, see page 14
Anti-hIL-1β-IgA, page 104

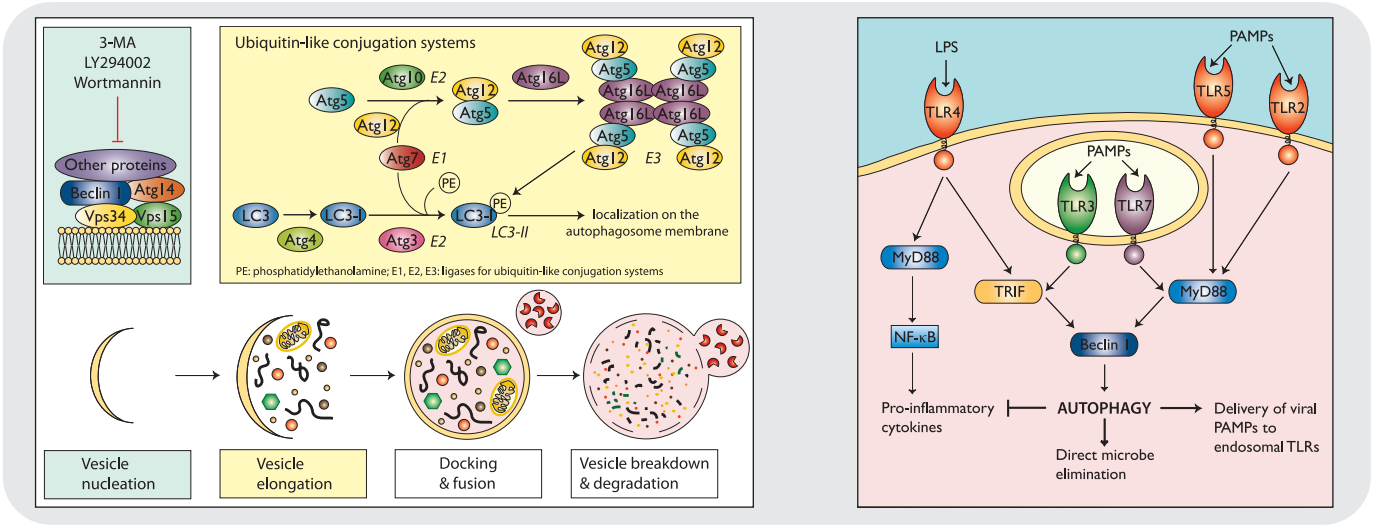
Autophagy and TLRs

Autophagy is a pathway by which cytoplasmic constituents, including organelles and intracellular pathogens, are sequestred in a double-membrane-bound autophagosome and delivered to the lysosome for degradation. 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 homeostatis during starvation and other stressful conditions. The 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 sequestred cytoplasmic material to lysosomal hydrolases[1, 2].

The 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 Atg16L1. Both conjugation systems are required for the generation of the autophagosome.

Autophagy plays a key role in the prevention of aging, cancer and neurodegeneration but also in the innate immune system against intracellular pathogens. Autophagy has been shown to interact with pattern recognition receptors (PRRs), such as the Toll-like receptors (TLRs). It was recently reported that the autophagic machinery 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 adapters trigger autophagy through a direct interaction with Beclin 1 (Shi). Autophagy seems also to participate in the regulation of inflammation. Macrophages from mice deficient for Atg16L1 produce more of the proinflammatory cytokines IL-1β and IL-18 after endotoxin stimulation of TLR4[7]. These data demonstrate that induction of autophagy and TLR signaling are connected.

1. Levine B. & Kroemer G., 2008. Autophagy in the pathogenesis of disease. Cell. 132(1):27-42. Review. **2. Mizushima N. *et al.*, 2008.** Autophagy fights disease through cellular self-digestion. Nature. 451(7182):1069-75. Review. **3. Lee HK. *et al.*, 2007.** Autophagy-dependent viral recognition by plasmacytoid dendritic cells. Science. 315(5817):1398-401. **4. Xu Y. *et al.*, 2007.** Toll-like receptor 4 is a sensor for autophagy associated with innate immunity. Immunity. 27(1):135-44. **5. Delgado MA,. *et al.*, 2008.** Toll-like receptors control autophagy. EMBO J. 27(7):1110-21. **6. Shi CS. &Kehrl JH., 2008.** MyD88 and Trif target Beclin 1 to trigger autophagy in macrophages. J Biol Chem. 283(48):33175-82. **7. Saitoh T. *et al.*, 2008.** Loss of the autophagy protein Atg16L1 enhances endotoxin-induced IL-1beta production. Nature. 2456(7219):264-8.



Autophagy Genes

Description

Autophagy genes are provided as open reading frames (coding sequences) from the Start codon to the Stop codon. They are cloned in the pUNO plasmid (see page 58) downstream of the EF-1α/HTLV promoter. pUNO plasmids are selectable in *E. coli* and mammalian cells with blasticidin.

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. They are provided with 4 pouches of *E. coli* Fast-Media® Blas (2 TB and 2 Agar). For more information about Fast-Media®, see pages 44-45.

PRODUCT	QTY	CAT. CODE (Human)	CAT. CODE (Mouse)
pUNO1-ATG3	*E. coli* disk	puno1-hatg3	puno1-matg3
pUNO1-ATG4A	*E. coli* disk	puno1-hatg4a	puno1-matg4a
pUNO1-ATG4B	*E. coli* disk	puno1-hatg4b	puno1-matg4b
pUNO1-ATG5	*E. coli* disk	puno1-hatg5	puno1-matg5
pUNO1-ATG7	*E. coli* disk	puno1-hatg7	puno1-matg7
pUNO1-ATG10	*E. coli* disk	puno1-hatg10	puno1-matg10
pUNO1-ATG16L1	*E. coli* disk	puno1-hatg16l1	puno1-matg16l1
pUNO1-BECLIN1	*E. coli* disk	puno1-hbecn1	puno1-mbecn1
pUNO1-LC3A	*E. coli* disk	puno1-hlc3a	puno1-mlc3a
pUNO1-LC3B	*E. coli* disk	puno1-hlc3b	puno1-mlc3b

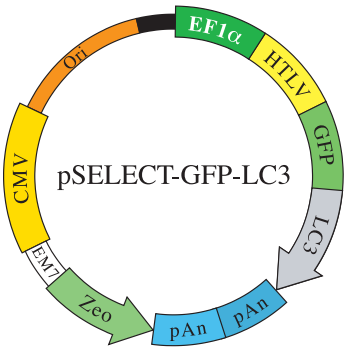
pSELECT-GFP-LC3 - GFP-LC3 Expression Plasmid

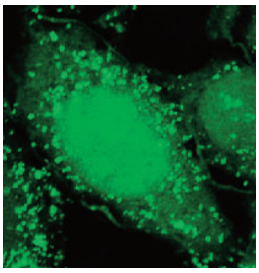
Description

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.

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 pages 44-45.

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

Autophagy Inducers & Inhibitors

Autophagy is regulated at different levels. Two connected signaling pathways encompassing class III phosphatidylinositol 3-kinase (PI3K) and mammalian target of rapamycin (mTOR) play a central role in this regulation. Autophagy requires PI3K autophagy but is negatively regulated by mTOR. Thus inhibitors of PI3K act as autophagy inhibitors while inhibitors of mTOR act as autophagy inducers.

PRODUCT	DESCRIPTION	WORKING CONCENTRATION	QTY	CATALOG CODE	REF.
Autophagy Inducers					
Rapamycin (mTOR inhibitor)	mimics cellular starvation by blocking signals required for cell growth and proliferation	10 - 500 nM	5 mg	tlrl-rap	1
Tamoxifen	stimulates autophagy by increasing the intracellular level of ceramide and abolishing the inhibitory effect of PI3K	10 - 100 µM	200 mg	tlrl-txf	2
Autophagy Inhibitors					
3-Methyladenine (3-MA, PI3K inhibitor)	inhibits the formation of autophagosome	5 mM	50 mg	tlrl-3ma	3
Bafilomycin A1 (V-ATPase inhibitor)	prevents maturation of autophagic vacuoles	0.1 - 1 µM	10 µg	tlrl-baf	4
LY294002 (PI3K inhibitor)	inhibits the formation of autophagosome	10 - 100 µM	5 mg	tlrl-ly29	3
Wortmannin (PI3K inhibitor)	inhibits the formation of autophagosome	0.1 - 10 µM	5 mg	tlrl-wtm	3

1. Jung CH. *et al.*, 2010. mTOR regulation of autophagy. FEBS Lett. 584(7):1287-95. **2. Scarlatti F. *et al.*, 2004.** Ceramide-mediated macroautophagy involves inhibition of protein kinase B and up-regulation of beclin 1. J Biol Chem. 279(18):18384-91. **3. Blommaart EF. *et al.*, 1997.** The phosphatidylinositol 3-kinase inhibitors wortmannin and LY294002 inhibit autophagy in isolated rat hepatocytes. Eur. J. Biochem. 243: 240–246. **4. Yamamoto A. *et al.*, 1998.** Bafilomycin A1 prevents maturation of autophagic vacuoles by inhibiting fusion between autophagosomes and lysosomes in rat hepatoma cell line, H-4-II-E cells. Cell Struct Funct. 23(1):33-42.

Contents and Storage

Each product is provided as a solid and shipped at room temperature. Store at room temperature, 4°C or -20°C according to the product label.