Human & Mouse TLR2 Agonist Kit

Set of known agonists for human and mouse TLR2

Catalog code: tlrl-kit2hm

https://www.invivogen.com/tlr2-agonist-kit

For research use only

Version 18G26-MM

PRODUCT INFORMATION

Contents

- TLR6/2 agonist - **Pam2CSK4** (10 µg)
- TLR1/2 agonist - **Pam3CSK4** (10 µg)
- TLR6/2 agonist - **FSL-1** (10 µg)
- TLR2 agonist - **HKLM** (10^9 cells)
- TLR2 agonist - **LPS-PG** (100 µg)
- TLR2 agonist - **LTA-SA standard** (100 µg)
- TLR2 agonist - **PGN-SA** (100 µg)

- 2 x 1.5 ml endotoxin-free water

Storage and stability

- Products are shipped at room temperature and should be stored according to the table below.

TLR Ligands	Lyophilized	Resuspended
Pam2CSK4	1 year @ 4°C	1 month @ 4°C, 6 months @ -20°C
Pam3CSK4	1 year @ 4°C	1 month @ 4°C, 6 months @ -20°C
FSL-1	1 year @ 4°C	6 months @ 4°C
HKLM	1 year @ 4°C	1 month @ 4°C, 6 months @ -20°C
LPS-PG	1 year @ 4°C	1 month @ 4°C, 6 months @ -20°C
LTA-SA	1 year @ -20°C	1 month @ 4°C, 6 months @ -20°C
PGN-SA	2 years @ -20°C	1 year @ -20°C

DESCRIPTION

• Pam2CSK4 - TLR6/2 agonist

Pam2CSK4 is a synthetic diacylated lipopeptide (LP). Bacterial lipoproteins are strong immune modulators that activate early innate host responses after infection. LP analogues of these lipoproteins signal either through TLR1/2 or TLR6/2 heterodimers. According to the current model, diacylated LPs induce signaling through TLR6/2. However, it was reported that diacylated LP, such as Pam2CSK4, induce signaling in a TLR6-independent manner[1]. 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 tripalmitoylated lipopeptide that mimicks the acylated amino terminus of bacterial lipoproteins. Pam3CysSerLys4 (Pam3CSK4) is a potent activator of the proinflammatory transcription factor NF-κB[2]. Recognition of Pam3CSK4 is mediated by TLR2 which cooperates with TLR1 through their cytoplasmic domain to induce the signaling cascade leading to the activation of NF-κB[3].

• FSL1 - TLR6/2 agonist

FSL-1 (Pam2CGDPKHPKSF) is a synthetic lipoprotein that represents the N-terminal part of the 44-kDa lipoprotein LP44 of *Mycoplasma salivarium*[4]. The framework structure of FSL-1 is the same as that of MALP-2, a *Mycoplasma fermentans* derived lipopeptide (LP), but they differ in the amino acid sequence and length of the peptide portion[5]. FSL-1 is recognized by TLR2 and TLR6 inducing a MyD88-dependent signaling cascade that leads to the activation of NF-κB and the production of proinflammatory cytokines.

• HKLM - TLR2 agonist

HKLM is a freeze-dried heat-killed preparation of *Listeria monocytogenes* (LM), a facultative intracellular Gram-positive bacterium. Infection with LM induces a strong nonspecific response characterized by the secretion of proinflammatory cytokines. This response is mediated by TLR2[6]. Stimulation with HKLM induces immediate activation of NF-κB and the production of proinflammatory cytokines[7].

• LPS-PG - TLR2 agonist

Recognition of LPS from *P. gingivalis* (LPS-PG), a Gram-negative bacteria, is unusual as it appears mediated by either TLR2 and TLR4[8]. Indeed, bone marrow cells obtained from TLR2-/- or TLR4-/- mice respond to LPS-PG while bone marrow cells obtained from TLR2 and TLR4 double-knockout do not. LPS-PG has also been reported to act as a TLR4 antagonist in some cell types[9, 10]. This discrepancy may be explained by the ability of this bacterium to synthesize multiple, structurally different forms of lipid A. The TLR response to LPS-PG is dependent on the presence of key accessory molecules: CD14 is required for both TLR2 and TLR4 activation while MD-2 is only necessary for TLR4 activation[8].

TECHNICAL SUPPORT
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• LTA-SA - TLR2 agonist

LTA-SA is lipoteichoic acid (LTA) from *S.aureus* (SA). LTA is a major immunostimulatory component of Gram-positive bacteria. LTA is responsible for causing gram-positive sepsis. 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[11]. Recognition of LTA also involves LPS-binding protein (LBP) and CD14 but not TLR4 or MD2[12]. Activation of LTAs may require the involvement of TLR1[13].

• PGN-SA - TLR2 agonist

PGN-SA is peptidoglycan (PGN)from *S.aureus* (SA). 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. In Gram-negative bacteria, a thin layer of PGN is also found in the periplasmic space. PGN is known to be a potent activator of NF-κB and TNF-α through TLR2[14]. However, other pattern recognition proteins have been reported to mediate the immunostimulatory activity of PGN[15, 16, 17]. This discrepancy is correlated to the method of purification. PGN-SA which is purified by detergent lysis, enzymatic treatment, LiCl/EDTA and acetone cleaning is an activator of TLR2.

METHODS

Preparation of TLR agonist stock solutions

Product	Working concentration	Stock solution concentration	Volume of solvent
Pam2CSK4	1-100 ng/ml	100 µg/ml	100 µl H_2O
Pam3CSK4	1-300 ng/ml	100 µg/ml	100 µl H_2O
FSL-1	1-100 ng/ml	100 µg/ml	100 µl H_2O
HKLM	10^7-10^8 cells/ml	10^{10} cells/ml	100 µl H_2O
LPS-PG	100 ng-10 µg/ml	1 mg/ml	100 µl H_2O
LTA-SA	100 ng-1 µg/ml	200 µg/ml	500 µl H_2O
PGN-SA	1-10 µg/ml	200 µg/ml	500 µl H_2O

TLR stimulation

- Transfect your cell line with an NF-κB-inducible reporter plasmid, i.e. a plasmid carrying a reporter gene, such as SEAP or luciferase, under the control of an NF-κB-inducible ELAM-1 (E-selectin) promoter[18].

Note: InvivoGen provides pNiFty, a family of NF-κB-inducible reporter plasmids that can be transfected transiently (pNiFty) or stably (pNiFty2). pNiFty plasmids are available either with the SEAP or luciferase reporter genes.

If your cell line does not naturally express TLRs, cotransfect with a plasmid expressing a given TLR gene, such as the pUNO plasmid family.

Note: Alternatively, evaluate TLR2 activation using reporter cells, such as InvivoGen's HEK-Blue™ TLR2 cells which express the human and mouse TLR2 and SEAP reporter genes. NF-κB production in these cells can be easily quantified using a SEAP detection medium, such as QUANTI-Blue™ or HEK-Blue™ Detection.

- Twenty-four to forty-eight hours after transfection, stimulate cells with the corresponding agonist for 6 hours to 24 hours.
- Determine TLR stimulation by assessing reporter gene expression using the appropriate detection system.

References

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