

# Mouse TLR1-9 Agonist Kit

Set of known agonists for mouse TLR1 to TLR7 and TLR9

Catalog code: tlr1-kit1mw

<https://www.invivogen.com/mouse-tlr1-9-agonist-kit>

For research use only

Version 24C14-MM

## PRODUCT INFORMATION

### Contents

- TLR1/TLR2 agonist - Pam3CSK4 (10 µg)
- TLR2 agonist - HKLM (10<sup>9</sup> cells)
- TLR2/TLR6 agonist - FSL-1 (10 µg)
- TLR3 agonist - Poly(I:C) HMW (500 µg)
- TLR3 agonist - Poly(I:C) LMW (500 µg)
- TLR4 agonist - LPS-EK standard (100 µg)
- TLR5 agonist - FLA-ST standard (10 µg)
- Murine TLR7 agonist - ssRNA40/LyoVec™ (25 µg)
- TLR9 agonist - ODN 1826 (100 µg - 15.71 nmol)
- 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 Agonists	Store lyophilized product at	Store resuspended product for
Pam3CSK4	4°C	1 month at 4°C, 6 months at -20°C
HKLM	4°C	1 month at 4°C, 6 months at -20°C
FSL-1	4°C	6 months at 4°C
Poly(I:C) HMW	4°C	1 month at 4°C, 1 year at -20°C
Poly(I:C) LMW	4°C	1 month at 4°C, 1 year at -20°C
LPS-EK	-20°C	1 month at 4°C, 6 months at -20°C
FLA-ST	-20°C	6 months at -20°C
ssRNA40/ LyoVec™	-20°C	1 week at 4°C
ODN 1826	-20°C	6 months at -20°C

### Quality control

The biological activity of each agonist has been confirmed using cellular assays.

## DESCRIPTION

### • Pam3CSK4 - TLR1/TLR2 agonist

Pam3CSK4 is a synthetic tripalmitoylated lipopeptide that mimics the acylated amino terminus of bacterial lipoproteins. Pam3CysSerLys4 (Pam3CSK4) is a potent activator of the pro-inflammatory transcription factor NF-κB<sup>1</sup>. 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<sup>2</sup>.

Molecular weight: 1852.33 g/mol

### • 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 non-specific response characterized by the secretion of pro-inflammatory cytokines. This response is mediated by TLR2<sup>3</sup>. Stimulation with HKLM induces immediate activation of NF-κB and the production of pro-inflammatory cytokines<sup>4</sup>.

### • FSL-1 - TLR2/TLR6 agonist

FSL-1 (Pam2CGDPKHPKSF) is a synthetic lipoprotein that represents the N-terminal part of the 44-kDa lipoprotein LP44 of *Mycoplasma salivarium*<sup>5</sup>. 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<sup>6</sup>. 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 pro-inflammatory cytokines.

Molecular weight: 1666.2 g/mol

### • Poly(I:C) HMW and Poly(I:C) LMW - TLR3 agonists

Poly(I:C) is a synthetic analog of double-stranded RNA (dsRNA), a molecular pattern associated with viral infection. Poly(I:C) is composed of a strand of poly(I) annealed to a strand of poly(C). The size of the strands varies. Poly(I:C) HMW has a high molecular weight (average size 1.5-8 kb), whereas Poly(I:C) LMW has a low molecular weight (average size 0.2-1 kb).

Poly(I:C) HMW and Poly(I:C) LMW may activate the immune system differently. dsRNA is known to induce interferons (IFNs) and other cytokines production. IFN induction is mediated by two different pathways. The first pathway leading to NF-κB activation depends on the dsRNA-responsive protein kinase (PKR)<sup>7</sup>, whereas the second pathway is PKR-independent and involves TLR3<sup>8</sup>.

### • LPS from *E. coli* K12 (LPS-EK) - TLR4 agonist

Lipopolysaccharide (LPS), the major structural component of the outer wall of Gram-negative bacteria, is a potent activator of the immune system. LPS recognition is mediated by TLR4 which forms a complex with MD2 and CD14<sup>9</sup> leading the production of pro-inflammatory cytokines through the MyD88 pathway. LPS signaling also involves a MyD88-independent cascade that mediates the expression of IFN-inducible genes via the adaptor protein TRIF<sup>10</sup>.

### • Flagellin from *S. typhimurium* (FLA-ST) - TLR5 agonist

Flagellin is the major component of the bacterial flagellar filament, which confers motility on a wide range of bacterial species. Flagellin is a potent stimulator of innate immune responses in a number of eukaryotic cells and organisms, including both mammals and plants. In mammals, flagellin is recognized by TLR5<sup>11</sup> and triggers defense responses both systemically and at epithelial surfaces. Flagellin induces the activation of NF-κB and the production of cytokines and nitric oxide depending on the nature of the TLR5 signaling complex<sup>12</sup>.

## TECHNICAL SUPPORT

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● **ssRNA40/LyoVec™ - murine TLR7 agonist**

ssRNA40/LyoVec™ is a 20-mer phosphothioate protected single-stranded RNA (ssRNA) oligonucleotide containing a GU-rich sequence<sup>13</sup>. ssRNA40 is complexed with the cationic lipid LyoVec™ (ratio 1:2), to protect it from degradation and facilitate its uptake, and lyophilized to generate ssRNA40/LyoVec™. When complexed to cationic lipids, ssRNA can substitute for viral RNAs in inducing TNF-α and IFN-α production in peripheral blood mononuclear cells<sup>13, 14</sup>. ssRNA40 complexes are recognized by TLR8 in humans and TLR7 in mice.  
5'-GCCCGUCUGUUGUGACUC-3' (phosphorothioate bases)

● **ODN 1826 - TLR9 agonist**

CpG ODNs are synthetic oligonucleotides containing unmethylated CpG dinucleotides in particular sequence contexts that induce strong immunostimulatory effects through the activation of TLR9<sup>15, 16</sup>. ODN1826 is the optimal motif to induce the proliferation of B cells<sup>17</sup>.  
5'- tcc atg acg ttc ctg acg tt -3' (phosphorothioate bases)

1. Aliprantis A.O. *et al.*, 1999. Cell activation and apoptosis by bacterial lipoproteins through toll-like receptor-2. *Science*.285(5428):736-9. 2. 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. 3. Flo T.H. *et al.*, 2000. Human toll-like receptor 2 mediates monocyte activation by *Listeria monocytogenes*, but not by group B streptococci or lipopolysaccharide. *J Immunol*, 164(4):2064-9. 4. Hauf N. *et al.*, 1997. *Listeria monocytogenes* infection of P388D1 macrophages results in a biphasic NF-κB (RelA/p50) activation induced by lipoteichoic acid and phospholipases and mediated by IκBα and IκBβ degradation. *PNAS* 94(17):9394-9. 5. Shibata K.I. *et al.*, 2000. The N-terminal lipopeptide of a 44-kDa membrane-bound lipoprotein of *Mycoplasma salivarium* is responsible for the expression of intercellular adhesion molecule-1 on the cell surface of normal human gingival fibroblasts. *J. Immunol*. 165:6538-6544. 6. Okusawa T. *et al.*, 2004. Relationship between Structures and Biological Activities of Mycoplasma Diacylated Lipopeptides and Their Recognition by Toll-Like Receptors 2 and 6. *Infect Immun*. 72(3): 1657-1665. 7. Chu W.M. *et al.*, 1999. JNK2 and IKK beta are required for activating the innate response to viral infection. *Immunity*, 11(6):721-31. 8. Alexopoulou L. *et al.*, 2001. Recognition of double-stranded RNA and activation of NF-κappaB by Toll-like receptor 3. *Nature*, 413(6857):732-8. 9. Re F. & Strominger J.L., 2003. Separate Functional Domains of Human MD-2 Mediate Toll-Like Receptor 4-Binding and Lipopolysaccharide Responsiveness. 10. Yamamoto M. *et al.*, 2003. Role of adaptor TRIF in the MyD88-independent toll-like receptor signaling pathway. *Science*. 301(5633):640-3. 11. Hayashi F. *et al.*, 2001. The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. *Nature* 410(6832):1099-103. 12. Mizel S.B. *et al.*, 2003. Induction of macrophage nitric oxide production by Gram-negative flagellin involves signaling via heteromeric Toll-like receptor 5/Toll-like receptor 4 complexes. *J Immunol*. 170(12):6217-23. 13. Heil F. *et al.*, 2004. Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8. *Science*. 5:303(5663):1526-9. 14. Diebold S.S. *et al.*, 2004. Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. *Science*. 5:303(5663):1529-31. 15. Krieg A.M. *et al.*, 1995. CpG motifs in bacterial DNA trigger direct B-cell activation. *Nature*, 374(6522):546-9. 16. Bauer S. *et al.*, 2001. Human TLR9 confers responsiveness to bacterial DNA via species-specific CpG motif recognition. *PNAS* 98(16):9237-42. 17. Ballas Z.K. *et al.*, 2001. Divergent therapeutic and immunologic effects of oligodeoxynucleotides with distinct CpG motifs. *J Immunol*. 167(9):4878-86.

**METHODS**

**Preparation of TLR agonist stock solutions**

Product	Working concentration	Stock solution concentration	Volume of solvent
Pam3CSK4	0.1-10 ng/ml	100 µg/ml	100 µl H <sub>2</sub> O
HKLM	10 <sup>7</sup> -10 <sup>8</sup> cells/ml	10 <sup>10</sup> cells/ml	100 µl H <sub>2</sub> O
FSL-1	0.01-100 ng	100 µg/ml	100 µl H <sub>2</sub> O
Poly(I:C) HMW	30 ng-10 µg/ml	1 mg/ml	500 µl H <sub>2</sub> O*
Poly(I:C) LMW	30 ng-10 µg/ml	1 mg/ml	500 µl H <sub>2</sub> O
LPS-EK	1 ng-10 µg/ml	100 µg/ml	1 ml H <sub>2</sub> O
FLA-ST	10 ng-10 µg/ml	100 µg/ml	100 µl H <sub>2</sub> O
ssRNA40/LyoVec™	0.25-5 µg/ml	100 µg/ml	250 µl H <sub>2</sub> O
ODN 1826	1-5 µM	500 µM	31 µl H <sub>2</sub> O

\*Note: Following resuspension of Poly(I:C) HMW, heat the solution for 10 minutes at 65-70°C, then allow the solution to cool at room temperature for 1 hour to ensure proper annealing.

**TLR activation**

Activation of TLRs can be monitored using InvivoGen's HEK-Blue™ TLR reporter cells. These cells stably express an NF-κB-inducible secreted embryonic alkaline phosphatase (SEAP) and overexpress a TLR gene.

For more information visit: [www.invivogen.com/hek-blue-tlr-cells](http://www.invivogen.com/hek-blue-tlr-cells).

1. Prepare HEK-Blue™ TLR cell suspension according to the data sheet.
2. Incubate cells with the corresponding agonist for 6-24 h at 37 °C, 5% CO<sub>2</sub>.
3. Determine TLR stimulation by assessing cytokine expression using an ELISA, or SEAP expression using a SEAP detection medium, such as HEK-Blue™ Detection.

**RELATED PRODUCTS**

Product	Description	Cat. Code
FLA-ST	TLR5 agonist	tlrl-stfla
FSL-1	TLR2/TLR6 agonist	tlrl-fsl
HEK-Blue™ Detection	SEAP detection medium	hb-det2
HEK-Blue™ mTLR2 cells	TLR2 reporter cells	hkb-mtlr2
HEK-Blue™ mTLR3 cells	TLR3 reporter cells	hkb-mtlr3
HEK-Blue™ mTLR4 cells	TLR4 reporter cells	hkb-mtlr4
HEK-Blue™ mTLR5 cells	TLR5 reporter cells	hkb-mtlr5
HEK-Blue™ mTLR7 cells	TLR7 reporter cells	hkb-mtlr7
HEK-Blue™ mTLR9 cells	TLR9 reporter cells	hkb-mtlr9
HKLM	TLR2 agonist	tlrl-hklm
LPS-EK	TLR4 agonist	tlrl-eklps
ODN 2006 (ODN 7909)	TLR9 agonist	tlrl-2006
Pam3CSK4	TLR1/TLR2 agonist	tlrl-pms
Poly(I:C) HMW	TLR3 agonist	tlrl-pic
Poly(I:C) LMW	TLR3 agonist	tlrl-picw
ssRNA40/LyoVec™	Murine TLR7 agonist	tlrl-irna40

**TECHNICAL SUPPORT**

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