Human TLR3/7/8/9 Agonist Kit

Set of known agonists for human TLR3, TLR7, TLR8, and TLR9

Catalog code: tlrl-kit3hw3

https://www.invivogen.com/human-tlr3789-agonist-kit

For research use only

Version 19C07-MM

PRODUCT INFORMATION

Contents

- TLR3 agonist - **Poly(I:C) HMW** (500 µg)
- TLR3 agonist - **Poly(I:C) LMW** (500 µg)
- TLR3 agonist - **Poly(A:U)** (500 µg)
- TLR7 agonist - **Imiquimod** (25 µg)
- TLR7/8 agonist - **R848** (25 µg)
- TLR7/8 agonist - **CL075** (25 µg)
- TLR8 agonist - **ssRNA40/LyoVec™** (25 µg)
- ssRNA40 Control - **ssRNA41/LyoVec™** (25 µg)
- TLR9 agonist - **ODN2006** (100 µg - 12.98 nmol)
- ODN control - **ODN2006 Control** (100 µg - 12.98 nmol)
- TLR9 agonist - **ODN2216** (100 µg - 15.54 nmol)
- ODN control - **ODN2216 Control** (100 µg - 15.54 nmol)
- TLR9 agonist - **ODN2395** (100 µg - 14.18 nmol)
- ODN control - **ODN2395 Control** (100 µg - 14.18 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 Ligands	Lyophilized	Resuspended
Poly(I:C) HMW	1 year @ 4°C	1 month @ 4°C, 1 year @ -20°C
Poly(I:C) LMW	1 year @ 4°C	1 month @ 4°C, 1 year @ -20°C
Poly(A:U)	1 year @ 4°C	1 month @ 4°C, 1 year @ -20°C
Imiquimod	1 year @ -20°C	6 months @ -20°C
R848	1 year @ 4°C	6 months @ -20°C
CL075	1 year @ -20°C	6 months @ -20°C
ssRNA40/LyoVec	1 year @ -20°C	1 week @ 4°C
ssRNA41/LyoVec	1 year @ -20°C	1 week @ 4°C
ODN2006	1 year @ -20°C	6 months @ -20°C
ODN2006 Control	1 year @ -20°C	6 months @ -20°C
ODN2216	1 year @ -20°C	6 months @ -20°C
ODN2216 Control	1 year @ -20°C	6 months @ -20°C
ODN2395	1 year @ -20°C	6 months @ -20°C
ODN2395 Control	1 year @ -20°C	6 months @ -20°C

DESCRIPTION

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

Polyinosinic-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) - TLR3 agonist

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].

• Imiquimod - TLR7 agonist

Imiquimod (R837), an imidazoquinoline amine analogue to guanosine, is an immune response modifier with potent indirect antiviral activity. This low molecular synthetic molecule induces the production of cytokines such as IFN-α. Unlike R848, Imiquimod activates only TLR7 but not TLR8[5]. This activation is MyD88-dependent and leads to the induction of the transcription factor NF-κB[6].

Molecular weight: 240.3

• R848 - TLR7/8 agonist

R848 (Resiquimod) 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[7, 8]. Recently, R848 was shown to trigger NF-κB activation in cells expressing murine TLR8 when combined with poly(dT)[9]. Unlike other commercially available R848 preparations, InvivoGen's R848 is water soluble (1 mg/ml).

Molecular weight: 314.2

• 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[10]. CL075 seems also to induce the secretion of IFN-α through TLR7 but to a lesser extend. 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.

Molecular weight: 244.33

TECHNICAL SUPPORT
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Hong Kong: +852 3-622-34-80
E-mail: info@invivogen.com

• ssRNA40/LyoVec™ - TLR8 agonist

ssRNA40 is a 20-mer phosphothioate protected single-stranded RNA oligonucleotide containing a GU-rich sequence[11]. ssRNA40 is complexed with the cationic lipid LyoVec™, 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[11, 12]. ssRNA40 complexes are recognized by TLR8 in humans and TLR7 in mice.

5'-GCCCGUCUGUUGUGUGACUC-3' (phosphorothioate bases)

• ssRNA41/LyoVec™- ssRNA40 control

ssRNA41 is a 20-mer phosphothioate protected single-stranded RNA oligonucleotide. It derives from ssRNA40 by replacement of all U nucleotides with adenosine[13]. ssRNA41 is complexed with the cationic lipid LyoVec™, to protect it from degradation and facilitate its uptake, and lyophilized to generate ssRNA41/LyoVec™. Unlike ssRNA40, ssRNA41 is unable to induce the production of type I IFNs, and therefore can be used as a negative control for ssRNA40.

• ODN2006 (type B) / ODN2216 (type A) / ODN2395 (type C) - TLR9 agonists

CpG ODNs are synthetic oligonucleotides containing unmethylated CpG dinucleotides in particular sequence contexts that induce strong immunostimulatory effects through the activation of TLR9[14, 15]. 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-a production from plasmacytoid dendritic cells (pDC) but are weak stimulators of TLR9-dependent NF-kB 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-a 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-a production from pDC and B cell stimulation.

ODN2006 Sequence: 5'-tcgtcgttttgtcgttttgtcgtt-3'
ODN2216 Sequence: 5'-ggGGGACGA:TCGTCgggggg-3'
ODN2395 Sequence: 5'-tcgtcgtttt**cggcgc:gcgccg**-3'
Bases shown in capital letters are phosphodiester, those in lower case are phosphorothioate (nuclease resistant) and palindrome is underlined.

• ODN2006 Control / ODN2216 Control / ODN2395 Control - ODN controls

ODN2006 Control, ODN2216 Control and ODN 2395 have sequences with GpC dinucleotides instead of CpG dinucleotides.
ODN2006 Control Sequnce: 5'-tgctgcttttgtgcttttgtgctt-3'
ODN2216 Control Sequnce: 5'-ggGGGAGCA:TGCTGggggggc-3'
ODN2395 Control Sequnce: 5'-tgctgcttttgggggggcccccc-3'

1. Yamamoto M. *et al.*, 2003. Role of adaptor TRIF in the MyD88-independent toll-like receptor signaling pathway. Science 301(5633):640-3. **2. Alexopoulou L. *et al.*, 2001.** Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. Nature 413(6857):732-8. **3. Wang L. *et al.*, 2002.** Noncoding RNA danger motifs bridge innate and adaptive immunity and are potent adjuvants for vaccination. J Clin Invest. 110:1175–84. **4. Conforti R. *et al.*, 2010.** Opposing effects of toll-like receptor (TLR3) signaling in tumors can be therapeutically uncoupled to optimize the anticancer efficacy of TLR3 ligands. Cancer Res. 70(2):490-500. **5. Lee J. *et al.*, 2003.** Molecular basis for the immunostimulatory activity of guanine nucleoside analogs: Activation of Toll-like receptor 7. PNAS 100(11):6646-51. **6. Hemmi H. *et al.*, 2002.** Small anti-viral compounds activate immune cells via the TLR7 MyD88-dependent signaling pathway. Nat Immunol. 3(2):196-200. **7. Hemmi H. et al., 2002**. Small anti-viral compounds activate immune cells via the TLR7 MyD88-dependent signaling pathway. Nat Immunol. 3(2):196-200. **8. Jurk M. *et al.*, 2002.** Human TLR7 or TLR8 independently confer responsiveness to the antiviral compound R848. Nat Immunol. 3(6):499. **9. Gorden KKB. *et al.*, 2006.** Cutting Edge: Activation of Murine TLR8 by a Combination of Imidazoquinoline Immune Response Modifiers and PolyT Oligodeoxynucleotides. J Immunol. 177: 6584 - 6587. **10. Gorden KB. *et al.*, 2005.** Synthetic TLR agonists reveal functional differences between human TLR7 and TLR8. J Immunol. 174(3):1259-68. **11. 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. **12. Diebold SS. *et al.*, 2004.** Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. Science 5;303(5663):1529-31. **13. Salio M. *et al.*, 2007.** Modulation of human natural killer T cell ligands on TLR-mediated antigen-presenting cell activation. PNAS 104: 20490 - 20495. **14. Krieg, A.M. *et al.*, 1995.** CpG motifs in bacterial DNA trigger direct B-cell activation. Nature 374(6522):546-9. **15. Bauer S. *et al.*, 2001.** Human TLR9 confers responsiveness to bacterial DNA via species-specific CpG motif recognition. PNAS 98(16):9237-42.

METHODS

Preparation of TLR agonist stock solutions:

Product	Working concentration	Stock solution concentration	Volume of solvent
Poly(I:C) HMW	30 ng -10 µg/ml	1 mg/ml	500 µl H_2O*
Poly(I:C) LMW	30 ng -10 µg/ml	1 mg/ml	500 µl H_2O
Poly(A:U)	300 ng -100 µg/ml	1 mg/ml	500 µl H_2O
Imiquimod	1-5 µg/ml	100 µg/ml	250 µl H_2O
R848	10 ng -10 µg/ml	100 µg/ml	250 µl H_2O
CL075	100 ng -5 µg/ml	100 µg/ml	250 µl H_2O
ssRNA40/LyoVec™	0.25-10 µg/ml	100 µg/ml	250 µl H_2O
ssRNA41/LyoVec™	0.25-10 µg/ml	100 µg/ml	250 µl H_2O
ODN2006	5 µM	500 µM	26 µl H_2O
ODN2006 control	5 µM	500 µM	26 µl H_2O
ODN2216	5 µM	500 µM	31 µl H_2O
ODN2216 control	5 µM	500 µM	31 µl H_2O
ODN2395	5 µM	500 µM	28 µl H_2O
ODN2395 control	5 µM	500 µM	28 µl H_2O

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: https://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_2.
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	Catalog Code
HEK-Blue™ Detection	hb-det2
HEK-Blue™ hTLR3 cells	hkb-htlr3
HEK-Blue™ hTLR7 cells	hkb-htlr7
HEK-Blue™ hTLR8 cells	hkb-htlr8
HEK-Blue™ hTLR9 cells	hkb-htlr9

TECHNICAL SUPPORT
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Hong Kong: +852 3-622-34-80
E-mail: info@invivogen.com