Mouse TLR9 Agonist Kit

Set of known agonists for mouse TLR9

Catalog # tlrl-kit9m

For research use only

Version # 14B07-MM

PRODUCT INFORMATION

Content:

ODNs are provided lyophilized:

- 100 µg (15.51 nmol) ODN 1585
- 100 µg (15.51 nmol) ODN 1585 Control
- 100 µg (15.71 nmol) ODN 1826
- 100 µg (15.71 nmol) ODN 1826 Control (ODN 2138)
- 100 µg (14.18 nmol) ODN 2395
- 100 µg (14.18 nmol) ODN 2395 Control
- 1.5 ml endotoxin-free water

Storage and stability:

- Products are shipped at room temperature and should be stored at -20°C.
- Upon resuspension, prepare aliquots of ODN and store at -20˚C. Product is stable 6 months at -20˚C. Avoid repeated freeze-thaw cycles.

Quality control

- TLR9 activity is tested using HEK-Blue™ TLR9 cells.
- The absence of bacterial contamination (endotoxins, peptidoglycans) is controlled using HEK-Blue™ TLR2 and HEK-Blue™ TLR4 cells.

DESCRIPTION

CpG ODNs are synthetic oligonucleotides that contain unmethylated CpG dinucleotides in particular sequence contexts (CpG motifs). These CpG motifs are present at a 20-fold greater frequency in bacterial DNA compared to mammalian DNA[1, 2]. CpG ODNs are recognized by Toll-like receptor 9 (TLR9) leading to strong immunostimulatory effects.

- **ODN 1585** is a class A CpG ODN with a preference towards mouse TLR9. Class 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.
- **ODN 1585 Control** contains GpC dinucleotides instead of CpGs and can be used as a negative control together with ODN 1585.
- **ODN 1826** is a class B CpG ODN with a preference towards mouse TLR9. Class 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.
- **ODN 1826 Control** (also known as ODN 2138) contains GpC dinucleotides instead of CpGs and can be used as a negative control together with ODN 1826.
- **ODN 2395** is a class C CpG ODN for human and mouse TLR9. Class C CpG ODNs combine features of both classes A and B. They contain a complete phosphorothioate backbone and a CpG-containing palindromic motif. Class C CpG ODNs induce strong IFN-α production from pDC and B cell stimulation.
- **ODN 2395 Control** contains GpC dinucleotides instead of CpGs and can be used as a negative control with ODN 2395.

1. Krieg AM. *et al.*, 1995. CpG motifs in bacterial DNA trigger direct B-cell activation. Nature, 374(6522):546-9. **2. Bauer, S. *et al.*, 2001.** Human TLR9 confers responsiveness to bacterial DNA via species-specific CpG motif recognition. PNAS 98(16):9237-42.

SEQUENCES

ODN 1585 (class A): 5'- ggGGTCAACGTTGAgggggg -3' (20 mer)
ODN 1585 Control: 5'- ggGGTCAAGCTTGAgggggg-3" (20 mer)

ODN 1826 (class B): 5'- tccatgacgttcctgacgtt-3' (20 mer)
ODN 1826 Control: 5'- tccatgagcttcctgagctt -3' (20 mer)

ODN2395 (class C): 5'- tcgtcgttttcggcgc:gcgccg-3'
ODN 2395 Control: 5'- tgctgcttttgggggccccc -3'

Note: Bases shown in capital letters are phosphodiester, those in lower case are phosphorothioate (nuclease resistant) and palindrome is underlined.

METHODS

Preparation of stock solution (500 µM)

- Resuspend ODN with endotoxin-free water provided.

Product	Working concentration	Stock solution concentration	Volume of solvent
ODN 1585	5 µM	500 µM	31 µl H_2O
ODN 1585 Control	5 µM	500 µM	31 µl H_2O
ODN 1826	5 µM	500 µM	31 µl H_2O
ODN 1826 Control	5 µM	500 µM	31 µl H_2O
ODN 2395	5 µM	500 µM	28 µl H_2O
ODN 2395 Control	5 µM	500 µM	28 µl H_2O

CpG ODN stimulation

ODNs can be used to stimulate TLR9 in HEK-Blue™ TLR9 cells. HEK-Blue™ TLR9 cells stably overexpress the TLR9 gene and an NF-κB-inducible secreted embryonic alkaline phosphatase (SEAP).
For more information, visit: www.invivogen.com/hek-blue-tlr9
Below is a protocol to study TLR9 stimulation using HEK-Blue™ TLR9 cells in a 96-well plate.

- Dispense 20 µl of stimulatory or control ODN per well of a 96-well plate.
- Prepare HEK-Blue™ TLR9 cell suspension according to the data sheet.
- Add HEK-Blue™ TLR9 cells (4-8 x10^4) to each ODN-containing well.
- Incubate for 6 - 24 h at 37°C, 5% CO_2.
- Determine TLR9 stimulation by assessing cytokine expression using ELISA, or SEAP expression using QUANTI-Blue™, a SEAP detection medium.

RELATED PRODUCTS

Product	Catalog Code
HEK-Blue™ mTLR9 cells	hkb-mtlr9
pUNO1-mTLR9 (mTLR9 gene)	puno1-mtlr9
QUANTI-Blue™	rep-qb1
ODN 1585	tlrl-1585
ODN 1826	tlrl-1826
ODN 2395	tlrl-2395

TECHNICAL SUPPORT
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