

A & C-Classes TLR9 Agonist Kit

Set of known multispecies TLR9 agonists (A & C-Classes CpG ODNs)

Catalog # tlr1-kit9ac

For research use only

Version # 14B07-MM

PRODUCT INFORMATION

Content:

ODNs are provided lyophilized:

- 100 µg (15.51 nmol) ODN 1585
- 100 µg (15.54 nmol) ODN 2216
- 100 µg (14.75 nmol) ODN 2336
- 100 µg (14.18 nmol) ODN 2395
- 100 µg (12.40 nmol) ODN M362
- 100 µg (10.7 nmol) ODN D-SL03
- 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) that are recognized by Toll-like receptor 9 (TLR9) leading to strong immunostimulatory effects.

Three classes of stimulatory CpG ODNs have been identified, classes A, B and C, which differ in their immunostimulatory activities^{1,2}. 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. Class C CpG ODNs contain a complete phosphorothioate backbone and a CpG-containing palindromic motif. Class C CpG ODNs are potent inducers of IFN-α from pDC and strong B cell activators^{3,4}. This kit contains CpG ODNs that belong to the A or C class. They are active in several species.

- **ODN 1585** is a A-class CpG ODN with a preference towards mouse TLR9.
- **ODN 2216 & ODN 2336** are A-class CpG ODN with a preference towards human and mouse TLR9.
- **ODN 2395 & ODN M362** are C-class CpG ODN with a preference towards human TLR9.
- **ODN D-SL03** (double-stem loop ODN) is a C-class CpG ODN and a TLR9 agonist in a diverse vertebrate species (humans, mice, rats, rabbits, pigs, swine and dogs).

1. Krug A. *et al.*, 2001. Identification of CpG oligonucleotide sequences with high induction of IFN-α/β in plasmacytoid dendritic cells. *Eur J Immunol*, 31:2154-63. 2. Marshall J. *et al.*, 2005. Superior activity of the type C class of ISS in vitro and in vivo across multiple species. *DNA Cell Biol*, 24:63-72. 3. Jurk M. *et al.*, 2004. C-Class CpG ODN: sequence requirements and characterization of immunostimulatory activities on mRNA level. *Immunobiology*, 209:141-54. 4. Abel K. *et al.*, 2005. Deoxycytidyl-deoxyguanosine oligonucleotide classes A, B, and C induce distinct cytokine gene expression patterns in rhesus monkey peripheral blood mononuclear cells and distinct alpha interferon responses in TLR9-expressing rhesus monkey plasmacytoid dendritic cells. *Clin Diagn Lab Immunol*, 12:606-21.

SEQUENCES

ODN 1585 (mouse preferred): 5'-ggGGTCAACGTTGAgggggg-3' (20 mer)

ODN 2216 (human/mouse): 5'-ggGGGACGA:TCGTCgggggg-3' (20 mer)

ODN 2336 (human/mouse): 5'-gggGACGAC:GTCGTGgggggg-3' (21 mer)

ODN 2395 (human preferred): 5'-tcgtcgtttcggcgc:gcgccg-3' (22 mer)

ODN M362 (human preferred): 5'-tcgtcgtcgttc:gaacgacgttgat-3' (25 mer)

ODN D-SL03 (multispecies): 5'-tcgcgaacgttcgccggttcgaacgcgg-3' (29 mer)

Note: Bases shown in capital letters are phosphodiester and those in lower case are phosphorothioate (nuclease resistant). 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 ₂ O
ODN 2216	5 µM	500 µM	31 µl H ₂ O
ODN 2336	5 µM	500 µM	28 µl H ₂ O
ODN 2395	5 µM	500 µM	28 µl H ₂ O
ODN M362	5 µM	500 µM	25 µl H ₂ O
ODN D-SL03	5 µM	500 µM	21 µl H ₂ O

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-tnfr9

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 x 10⁴) to each ODN-containing well.
- Incubate for 6 - 24 h at 37°C, 5% CO₂.
- 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™ hTLR9 cells (human TLR9 gene)	hkb-htlr9
HEK-Blue™ mTLR9 cells (mouse TLR9 gene)	hkb-mtlr9
QUANTI-Blue™	rep-qbl
ODN 1585	tlr1-1585
ODN 2216	tlr1-2216
ODN 2336	tlr1-2336
ODN 2395	tlr1-2395
ODN M362	tlr1-m362
ODN D-SL03	tlr1-dsl03

TECHNICAL SUPPORT

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