

# B-Class TLR9 Agonist Kit

Set of known multispecies TLR9 agonists (B-Class CpG ODNs)

Catalog # tlr1-kit9b

For research use only

Version # 14B07-MM

## PRODUCT INFORMATION

### Content:

ODNs are provided lyophilized:

- 100 µg (15.71 nmol) ODN 1668
- 100 µg (15.71 nmol) ODN 1826
- 100 µg (12.98 nmol) ODN 2006 (ODN 7909 or PF-3512676)
- 100 µg (14.16 nmol) ODN 2007
- 100 µg (13.62 nmol) ODN BW006 (ODN 684)
- 100 µg (11.99 nmol) ODN D-SL01
- 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<sup>1,2</sup>. CpG ODNs 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 immune-stimulatory activities<sup>3,4</sup>. 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.

CpG ODNs of this kit belong exclusively to the B class and are active in human and/or mouse and other species. The kit contains prototype CpG ODNs as well as less popular CpG ODNs.

- **ODN 1668** has a preference towards mouse TLR9.
- **ODN 1826** is a class B prototype ODN with a preference towards mouse TLR9.
- **ODN 2006** (also known as ODN 7909 or PF-3512676) is a class B prototype ODN with a preference towards human TLR9.
- **ODN BW006** (also known as ODN 684) is capable of activating human and mouse TLR9 as vigorously as ODN 2006.
- **ODN 2007** has a preference towards bovine and porcine TLR9.
- **ODN D-SL01** (double-stem loop ODN) is a TLR9 agonist in a diverse vertebrate species (humans, mice, rats, rabbits, pigs, swine and dogs).

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. 3. **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(7): 2154-63. 4. **Marshall JD. et al., 2005.** Superior activity of the type C class of ISS in vitro and in vivo across multiple species. *DNA Cell Biol.* 24(2):63-72.

## SEQUENCES (Class B CpG ODNs)

**ODN 1668 (mouse preferred):** 5'-tccatgacgttctgatct-3' (20 mer)

**ODN 1826 (mouse preferred):** 5'-tccatgacgttctgatct-3' (20 mer)

**ODN 2006 (human preferred):** 5'-tcgtcgtttgtcgtttgtcgtt-3' (24 mer)

**ODN BW006 (human/mouse):** 5'-tcgacgttcgtcgttcgttc-3' (23 mer)

**ODN 2007 (bovine/porcine):** 5'-tcgtcgttgcgtttgtcgtt-3' (22 mer)

**ODN D-SL01 (multispecies):** 5'-tcgcgacgttcgccgacgttcgta-3' (26 mer)

*Note: Bases shown in capital letters are phosphodiester and those in lower case are phosphorothioate (nuclease resistant).*

## METHODS

### Preparation of stock solution (500 µM)

- Resuspend ODN with endotoxin-free water provided.

### CpG ODN stimulation

Product	Working concentration	Stock solution concentration	Volume of solvent
ODN 1668	5 µM	500 µM	31 µl H <sub>2</sub> O
ODN 1826	5 µM	500 µM	31 µl H <sub>2</sub> O
ODN 2006	5 µM	500 µM	26 µl H <sub>2</sub> O
ODN 2007	5 µM	500 µM	28 µl H <sub>2</sub> O
ODN BW006	5 µM	500 µM	27 µl H <sub>2</sub> O
ODN D-SL01	5 µM	500 µM	24 µl H <sub>2</sub> O

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-tnfr](http://www.invivogen.com/hek-blue-tnfr)

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 10<sup>5</sup>) to each ODN-containing well.
- Incubate for 6 - 24 h at 37°C, 5% CO<sub>2</sub>.
- 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 (humanTLR9 gene)	hkb-htlr9
HEK-Blue™ mTLR9 cells (mouse TLR9 gene)	hkb-mtlr9
QUANTI-Blue™	rep-qb1
ODN 1668	tlr1-1668
ODN 1826	tlr1-1826
ODN 2006	tlr1-2006
ODN BW006	tlr1-bw006
ODN 2007	tlr1-2007
ODN D-SL01	tlr1-ds101

## TECHNICAL SUPPORT

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