

ODN 2006 (ODN 7909)

Class B CpG oligonucleotide; a human TLR9 ligand

Catalog # tlr1-2006, tlr1-2006-1, tlr1-2006-5

<http://www.invivogen.com/odn2006>

For research use only

Version # 16E05-MM

PRODUCT INFORMATION

Content

- ODN 2006 is provided lyophilized and is available in three quantities:
 - 200 µg (**25.96 nmol**): tlr1-2006 (formerly tlr1-hodnb)
 - 1 mg (**129.8 nmol**): tlr1-2006-1 (formerly tlr1-hodnb-1)
 - 5 x 1 mg (5 mg; **649 nmol**): tlr1-2006-5 (formerly tlr1-hodnb-5)

Note: ODN 2006 is sterile filtered prior to lyophilization.

- endotoxin-free water; 1.5 ml with #tlr1-2006 and tlr1-2006-1, and 10 ml with #tlr1-2006-5.

ODN 2006 sequence

5'-tcgtcgtttgcgtttgcgtt-3' (24 mer)

Note: Bases are phosphorothioate (nuclease resistant).

Molecular weight: 7698 g/mol

Storage and stability

- ODN 2006 is shipped at room temperature. Upon receipt, store at -20 °C.
- Upon resuspension, prepare aliquots of ODN 2006 and store at -20 °C. Resuspended product is stable for 6 months at -20 °C when properly stored. Avoid repeated freeze-thaw cycles.

Quality control

- TLR9 activity has been tested using HEK-Blue™ TLR9 cells.
- The absence of bacterial contamination (e.g. lipoproteins and endotoxins) has been confirmed 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)¹. These CpG motifs are present at a 20-fold greater frequency in bacterial DNA compared to mammalian DNA. CpG ODNs are recognized by Toll-like receptor 9 (TLR9) leading to strong immunostimulatory effects². Three classes of stimulatory CpG ODNs have been identified, classes A, B and C, which differ in their immunostimulatory activities^{3,4}. ODN 2006 (also known as ODN 7909 or PF-3512676) is a CpG ODN class B with a preference for human TLR9. B-class CpG ODNs contain a full phosphorothioate backbone with one or more CpG dinucleotides. They strongly activate B cells but weakly stimulate IFN- α secretion.

1. Krieg, A. et al., 1995. CpG motifs in bacterial DNA trigger direct B-cell activation. *Nature*, 374:546-9. **2. Bauer, S. et al., 2001.** Human TLR9 confers responsiveness to bacterial DNA via species-specific CpG motif recognition. *PNAS*, 98:9237-42. **3. Krug A. et al., 2001.** Identification of CpG oligonucleotide sequences with high induction of IFN- α /beta in plasmacytoid dendritic cells. *Eur J Immunol*, 31:2154-63. **4. 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(2):63-72.

METHODS

Preparation of stock solution (500 µM)

TLR9 activation can be achieved with 1-5 µM ODN 2006.

- Resuspend ODN 2006 with endotoxin-free water (provided).
 - Add 52 µl to 200 µg of ODN 2006
 - Add 260 µl to 1 mg of ODN 2006
- Vortex until completely dissolved. Prepare aliquots and store at -20 °C.
- Prepare serial dilutions using endotoxin-free water.

Note: The working concentration may vary depending on the levels of TLR9 gene expression and the species from which the gene was obtained.

TLR9 stimulation using ODN 2006

ODN 2006 can be used to stimulate TLR9 in HEK-Blue™ TLR9 cells. HEK-Blue™ TLR9 cells stably overexpress the TLR9 gene and an NF- κ B-inducible secreted embryonic alkaline phosphatase (SEAP) reporter gene. For more information, visit: www.invivogen.com

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 cell suspension of HEK-Blue™ TLR9 cells according to the data sheet.
- Add HEK-Blue™ TLR9 cells (4-8 x10⁴) 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
ODN 2006 control (ODN 2137)	tlr1-2006c-1
pUNO1-hTLR9a (human TLR9 gene)	puno1-htlr9a
HEK-Blue™ hTLR9 cells	hkb-htlr9
QUANTI-Blue™	rep-qb1

TECHNICAL SUPPORT

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