ODN 2006 (ODN 7909)

Class B CpG oligonucleotide; human TLR9 ligand

Catalog code: tlrl-2006, tlrl-2006-1, tlrl-2006-5

https://www.invivogen.com/odn2006

For research use only

Version 23K07-MM

PRODUCT INFORMATION

Contents

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

Note: ODN 2006 is sterile filtered prior to lyophilization.

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

ODN 2006 sequence

5'- tcgtcgttttgtcgttttgtcgtt -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

ODN 2006 (also known as ODN 7909 or PF-3512676) is a Class B CpG oligonucleotide (ODN). It is a short synthetic single-stranded DNA molecule containing unmethylated CpG dinucleotides (CpG motifs). These unmethylated CpG motifs mimic microbial DNA and act as immunostimulants. ODN 2006 is a ligand of choice for human Toll-like receptor 9 (TLR9). Activation of TLR9 triggers NF-κB- and interferon regulatory factor (IRF)-mediated pro-inflammatory responses upon the recognition of unmethylated cytosine-phosphorothioate-guanosine (CpG) forms of DNA[1, 2]. Unmethylated CpG dinucleotides are a hallmark of microbial (bacterial, viral, fungal, and parasite) DNA, as well as mitochondrial self-DNA[2, 3]. Class B (also called Type K) CpG ODNs, such as ODN 2006, contain a full phosphorothioate backbone with one or more CpG dinucleotides. They strongly activate B cells but weakly stimulate IFN-α secretion in plasmacytoid dendritic cells[4].

1. Kumagai Y. *et al.*, 2008. TLR9 as a key receptor of the recognition of DNA. Adv. Drug. Deliv. Rev. 60(7):795-804. 2. Kayraklioglu N. *et al.*, 2021. CpG oligonucleotides as vaccine adjuvants. DNA Vaccines: Methods and Protocols. Methods in Molecular Biology. Vol. 2197. p51-77. 3. Kumar V., 2021. The trinity of cGAS, TLR9, and ALRs: guardians of the cellular galaxy against host-derived self-DNA. Front. Immunol. 11:624597. 4. Krieg A.M. *et al.*, 1995. CpG motifs in bacterial DNA trigger direct B-cell activation. Nature. 374(6522):546-9.

METHODS

Preparation of CpG ODN 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 vial of ODN 2006
  - Add 260 µl to 1 mg vial 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-κB-inducible secreted embryonic alkaline phosphatase (SEAP) reporter gene.
For more information, visit: https://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 cell suspension of HEK-Blue™ TLR9 cells 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™ Solution, a SEAP detection medium.

RELATED PRODUCTS

Product	Description	Cat. Code
ODN 2006 Control (ODN 2137)	Negative control	tlrl-2006c-1
HEK-Blue™ hTLR9 cells	Reporter cells	hkb-htlr9
pUNO1-hTLR9a	Human TLR9 gene	puno1-htlr9a
QUANTI-Blue™ Solution	SEAP detection medium	rep-qbs

TECHNICAL SUPPORT
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