ODN 2007

Class B CpG oligonucleotide; Bovine / porcine TLR9 ligand

Catalog code: tlrl-2007-1, tlrl-2007-5

https://www.invivogen.com/odn2007

For research use only

Version 23K22-MM

PRODUCT INFORMATION

Contents

- ODN 2007 is provided lyophilized and is available in two quantities:
  - 1 mg (**141.65 nmol**): tlrl-2007-1
  - 5 x 1 mg (5 mg; **708.25 nmol**): tlrl-2007-5

Note: ODN 2007 is sterile filtered prior to lyophilization.

- endotoxin-free water; 1.5 ml with #tlrl-2007-1, and 10 ml with #tlrl-2007-5.

ODN 2007 sequence

5'- tcg tcg ttg tcg ttt tgt cgt t -3' (22 mer)

Note: Bases are phosphorothioate (nuclease resistant).

Molecular weight: 7058 g/mol

Storage and stability

- ODN 2007 is shipped at room temperature. Upon receipt, store at -20 °C.
- Upon resuspension, prepare aliquots of ODN 2007 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 cellular assays.
- The absence of bacterial contamination (e.g. lipoproteins and endotoxins) has been confirmed using HEK-Blue™ TLR2 and HEK-Blue™ TLR4 cells.

DESCRIPTION

ODN 2007 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 2007 is a ligand of choice for bovine and porcine 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 2007, 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 2007.

- Add 285 µl of endotoxin-free water (provided) to 1 mg vial of ODN 2007.
- 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 2007

ODN 2007 can be used to stimulate TLR9 in cellular assays. If your cell line does not express TLR9, transfect with a TLR9 plasmid, such as pUNO1-bTLR9 or pUNO1-pTLR9 plasmids expressing the bovine and pig TLR9 genes, respectively. InvivoGen also provides a collection of inducible reporter plasmids for monitoring PRR (pattern recognition receptor) activation and cytokine signaling. Two families of pNiFty plasmids are available pNiFty2-N family (NF-κB-inducible reporter plasmids) and pNiFty3-I family (IRF-inducible reporter plasmids). For more information, visit: www.invivogen.com/innate-immunity-pnifty.

In addition, ODN 2007 can be used to stimulate TLR9 in HEK-Blue™ TLR9 cells. HEK-Blue™ TLR9 cells stably overexpress an NF-κB-inducible secreted embryonic alkaline phosphatase (SEAP) reporter gene and the human TLR9 (hTLR9) or murine TLR9 (mTLR9) gene. For more information, visit: https://www.invivogen.com/hek-blue-tlr9.

Below is a protocol to study TLR9 stimulation using TLR9-expressing 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
HEK-Blue™ hTLR9 cells	Reporter cells	hkb-htlr9
HEK-Blue™ mTLR9 cells	Reporter cells	hkb-mtlr9
pNiFty2-N-SEAP-Zeo	Reporter plasmid	pnf2-sp
pUNO1-bTLR9	Bovine TLR9 gene	puno1-btlr9
pUNO1-pTLR9	Pig TLR9 gene	puno1-ptlr9
QUANTI-Blue™ Solution	SEAP detection medium	rep-qbs

TECHNICAL SUPPORT
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