

ODN 2216

Class A CpG oligonucleotide; a human TLR9 ligand

Catalog code: tlr1-2216, tlr1-2216-1, tlr1-2216-5

<https://www.invivogen.com/odn2216>

For research use only

Version 24E14-MM

PRODUCT INFORMATION

Contents

- ODN 2216 is provided lyophilized and is available in three quantities:
 - 200 µg (**31 nmol**): tlr1-2216
 - 1 mg (**155 nmol**): tlr1-2216-1
 - 5 x 1 mg (5 mg; **777 nmol**): tlr1-2216-5

Note: ODN 2216 is sterile filtered prior to lyophilization.

- endotoxin-free water; 1.5 ml with #tlr1-2216 and tlr1-2216-1, and 10 ml with #tlr1-2216-5.

ODN 2216 sequence

5'-ggGGGACGA:TCGTCgggggg-3' (20 mer)

Note: Bases shown in capital letters are phosphodiester, and those in lower case are phosphorothioate (nuclease resistant). Palindrome is underlined.

Molecular weight: 6432 g/mol

Storage and stability

- ODN 2216 is shipped at room temperature. Upon receipt, store at -20°C.
- Upon resuspension, prepare aliquots of ODN 2216 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 2216 is a class A CpG ODN with a preference for human TLR9. 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.

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/β 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.

TECHNICAL SUPPORT

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METHODS

Preparation of CpG ODN solution (500 µM)

TLR9 activation can be achieved with 1-5 µM ODN 2216.

- Resuspend ODN 2216 with endotoxin-free water (provided).
 - Add 62 µl to 200 µg vial of ODN 2216
 - Add 310 µl to 1 mg vial of ODN 2216
- 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 2216

ODN 2216 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: 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 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™ Solution, a SEAP detection medium.

RELATED PRODUCTS

Product	Description	Cat. Code
ODN 2216 Control (ODN 2243)	Negative control	tlr1-2243
pUNO1-hTLR9a	Human TLR9 gene	puno1-htlr9a
HEK-Blue™ hTLR9 cells	Reporter cells	hkb-htlr9
QUANTI-Blue™ Solution	SEAP detection medium	rep-qbs