

# 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 21L21-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)<sup>1</sup>. 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 effects<sup>2</sup>. Three classes of stimulatory CpG ODNs have been identified, classes A, B and C, which differ in their immunostimulatory activities<sup>3,4</sup>. 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-alpha/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.

### 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: <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<sup>4</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™ Solution, a SEAP detection medium.

## RELATED PRODUCTS

Product	Description	Cat. Code
ODN 2216 Control (ODN 2243)	Stimulatory ODN	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