ODN 2216 Biotin

Biotin labeled CpG oligonucleotide, type B; Human TLR9 ligand

Catalog # tlrl-2216b

http://www.invivogen.com/odn2216-biotin

For research use only

Version # 17A31-MM

PRODUCT INFORMATION

Content

• 2 x 50 µg (7 nmol) lyophilized ODN 2216 labeled with biotin at the 3' terminus.

• 1.5 ml endotoxin-free water

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: 6893 g/mol

Storage

• ODN 2216 Biotin is shipped at room temperature and should be store at -20 °C. Lyophilized ODN 2216 Biotin is stable for at least 1 year.

• Upon resuspension, prepare aliquots of ODN 2216 Biotin and store at -20 °C. Resuspended product is stable 6 months 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 effects[2]. Three types of stimulatory CpG ODNs have been identified, types A, B and C, which differ in their immune-stimulatory 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.M. *et al.*, 1995. CpG motifs in bacterial DNA trigger direct B-cell activation. Nature, 374(6522):546-9. **2. Bauer, S. *et al.*, 2001.** Human TLR9 confers responsiveness to bacterial DNA via species-specific CpG motif recognition. PNAS, 98(16):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(7): 2154-63. **4. Marshall JD. *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 of ODN 2216 Biotin.

1. Resuspend 50 µg of lyophilized ODN 2216 Biotin with 15 µl of endotoxin-free water provided.
2. Mix gently until complete solubilization.
3. Prepare aliquots of ODN 2216 Biotin and store at -20 °C.

TLR9 stimulation using ODN 2216 Biotin

ODN 2216 Biotin 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.

1. Dispense 20 µl of stimulatory or control ODN per well of a 96-well plate.
2. Prepare cell suspension of HEK-Blue™ TLR9 cells according to the data sheet.
3. Add HEK-Blue™ TLR9 cells (4-8 x10^4) to each ODN-containing well.
4. Incubate for 6-24 h at 37 °C, 5% CO_2.
5. Determine TLR9 stimulation by assessing cytokine expression using ELISA, or SEAP expression using QUANTI-Blue™, a SEAP detection medium.
6. Alternatively, evaluate CpG ODN cellular uptake and localization using a biotin detection system and light microscopy.

RELATED PRODUCTS

Product	Catalog Code
ODN 2216	tlrl-2216
ODN 2216 control	tlrl-2216c
ODN 2216 FITC	tlrl-2216f
pUNO1-hTLR9a (human TLR9a gene)	puno1-htlr9
HEK-Blue™ hTLR9 Cells	hkb-htlr9
QUANTI-Blue™	rep-qb1

TECHNICAL SUPPORT
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Hong Kong: +852 3-622-34-80
E-mail: info@invivogen.com