ODN 1668

Class B CpG oligonucleotide; a murine TLR9 ligand

Catalog # tlrl-1668, tlrl-1668-1, tlrl-1668-5

For research use only

Version # 16E17-MM

PRODUCT INFORMATION

Content:

• ODN 1668 is provided lyophilized and is available in three quantities:
- 200 µg (**31.42 nmol**): tlrl-1668 (formerly tlrl-modnb)
- 1 mg (**157.1 nmol**): tlrl-1668-1 (formerly tlrl-modnb-1)
- 5 x 1 mg (5 mg; **785.5 nmol**): tlrl-1668-5 (formerly tlrl-modnb-5)

Note: ODN 1668 is sterile filtered prior to lyophilization.

• endotoxin-free water; 1.5 ml with #tlrl-1668 and tlrl-1668-1, and 10 ml with #tlrl-1668-5.

ODN 1668 sequence

5'-tccatgacgttcctgatgct-3' (20 mer)

Note: Bases are phosphorothioate-linked (nuclease resistant).

Molecular weight: 6364 g/mol

Storage and stability

- ODN 1668 is shipped at room temperature. Upon receipt, store at -20 °C.
- Upon resuspension, prepare aliquots of ODN 1668 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 effects[2]. Three classes of stimulatory CpG ODNs have been identified, classes A, B and C, which differ in their immune-stimulatory activities[3-4].

ODN 1668 is a class B CpG ODN with a preference for mouse TLR9. Class B CpG ODNs contain a full phosphorothioate backbone with one or more CpG dinucleotides. They strongly activate B cells but stimulate weakly IFN-α secretion.

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.

METHODS

Preparation of stock solution (500 µM)

TLR9 activation can be achieved with 1-5 µM ODN 1668.

• Resuspend ODN 1668 with endotoxin-free water (provided).
- Add 63 µl to 200 µg of ODN 1668
- Add 315 µl to 1 mg of ODN 1668

• 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 1668

ODN 1668 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

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™, a SEAP detection medium.

RELATED PRODUCT

Product	Catalog Code
ODN 1668 Control	tlrl-1668c
pUNO1-mTLR9 (mouse TLR9 gene)	puno1-mtlr9
HEK-Blue™ mTLR9 Cells	hkb-mtlr9
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