ODN 1668 Control

Negative control oligonucleotide for murine TLR9 ligand ODN 1668

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

For research use only

Version # 16E24-MM

PRODUCT INFORMATION

Content

• ODN 1668 Control is provided lyophilized and is available in 3 quantities:
- 200 µg (**31.42 nmol**): tlrl-1668c (formerly tlrl-modnbc)
- 1 mg (**157.10 nmol**): tlrl-1668c-1 (formerly tlrl-modnbc-1)
- 5 x 1 mg (5 mg; **785.50 nmol**): tlrl-1668c-5 (formerly tlrl-modnbc-5)

Note: ODN 1668 Control is sterile filtered prior to lyophilization.

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

ODN1668 Control sequence

5'-tccatgagcttcctgatgct-3' (20 mer)

Note: Bases are phosphorothioate (nuclease resistant).

Molecular weight: 6364 g/mol

Storage and stability

- ODN 1668 Control is shipped at room temperature. Upon receipt, store at -20 °C.
- Upon resuspension, prepare aliquots of ODN 1668 Control 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

- 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 Control contains GpC dinucleotides instead of CpGs and can be used as a negative control for ODN 1668 (class B CpG ODN).

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)

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

ODN 1668 Control can be used as a control ODN to study the stimulatory effect of ODN 1668 on 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.

Note: Use the ODN 1668 Control at the same concentration as the CpG-containing ODN 1668.

- 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	tlrl-1668
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