ODN 1668 FITC

FITC labeled CpG oligonucleotide - Mouse TLR9 ligand

Catalog # tlrl-1668f (formerly tlrl-fmodnb)

For research use only

Version # 16J28-MM

PRODUCT INFORMATION

Content:

- 50 µg (7.25 nmol) lyophilized ODN1668 labeled with FITC at the 3' terminus.
- 1.5 ml endotoxin-free water

ODN 1668 sequence

5'-tccatgacgttcctgatgct-3' (20 mer)

Note: Bases are phosphorothioate (nuclease resistant).

Molecular weight: 6947 g/mol

Storage :

- ODN 1668 FITC is shipped at room temperature. Store at -20˚C. Protect from light.
- Resuspended product should be stored at -20˚C and protected from light. Product is stable for 6 months. 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 CpG ODN solution (500 µM)

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

- Resuspend lyophilized ODN 1668 FITC with 14 µl of endotoxin-free water (provided).
- Vortex until complete solubilization.
- Store at -20 °C.

TLR9 stimulation using ODN 1668 FITC

ODN 1668 FITC 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% CO2.
- Determine TLR9 stimulation by assessing cytokine expression using ELISA, or SEAP expression using QUANTI-Blue™, a SEAP detection medium.
- Evaluate CpG ODN cellular uptake and localization by confocal laser-scanning microscopy (excitation 495 nm, emission 520 nm) or flow cytometry.

RELATED PRODUCTS

Product	Catalog Code
ODN 1668	tlrl-1668
pUNO-mTLR9 (mouse TLR9 gene)	puno-mtlr9
HEK-Blue™ mTLR9 Cells	hkb-mtlr9
QUANTI-Blue™	rep-qb1
293/mTLR9 Cells	293-mtlr9
pNiFty2-Luc (Zeo^R)	pnifty2-luc
pNiFty2-SEAP (Zeo^R)	pnifty2-seap

TECHNICAL SUPPORT
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