ODN 4084-F

Class B inhibitory ODN

Catalog code: tlrl-4084

https://www.invivogen.com/odn4084-f

For research use only

Version 18J26-MM

PRODUCT INFORMATION

Contents

• 200 µg (51 nmol) lyophilized ODN 4084-F

Note: ODN 4084-F is sterile filtered prior to lyophilization.

• 1.5 ml endotoxin-free water

ODN 4084-F sequence

5'- cctggatgggaa -3'

Note: Bases are phosphorothioate (nuclease resistant).

Molecular weight: 3887 g/mol

Storage and stability

- ODN 4084-F is shipped at room temperature. Upon receipt, store at -20 °C.
- Upon resuspension, prepare aliquots of ODN 4084-F and store at -20 °C. 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 & endotoxins) has been confirmed using HEK-Blue™ TLR2 and HEK-Blue™ TLR4 cells.

DESCRIPTION

Toll-Like Receptor 9 (TLR9) detects unmethylated CpG dinucleotides in bacterial or viral DNA inducing strong immunostimulatory effects. TLR9 activation can be mimicked by synthetic phosphorothioate-stabilized oligodeoxynucleotides (ODN) containing immune stimulatory "CpG motifs". Studies suggest the existence of DNA sequences that can neutralize the stimulatory effect of CpG ODNs.

ODN 4084-F belongs to a new class of inhibitory ODNs[1-2]. It contains an inhibitory DNA motif consisting of two nucleotide triplets, a proximal CCT and a more distal GGG, spaced from each other by four nucleotides. ODN 4084-F is the shortest active inhibitory ODN. ODN 4084-F is linear and a class B ('broadly-active') inhibitory ODN. ODN 4084-F is potent inhibitor of TLR9-induced B cells and macrophages[3].

1. Lenert P. *et al*., 2003. Structural characterization of the inhibitory DNA motif for the type A (D)-CpG-induced cytokine secretion and NK-cell lytic activity in mouse spleen cells. DNA Cell Biol. 22(10):621-31. **2. Lenert PS., 2006.** Targeting Toll-like receptor signaling in plasmacytoid dendritic cells and autoreactive B cells as a therapy for lupus.Arthritis Res Ther. 8(1):203. **3. Lenert P. *et al*., 2009.** DNA-like class R inhibitory oligonucleotides (INH-ODNs) preferentially block autoantigen-induced B-cell and dendritic cell activation in vitro and autoantibody production in lupus-prone MRL-Fas(lpr/lpr) mice in vivo. Arthritis Res Ther. 11(3):R79.

METHODS

Preparation of stock solution (500 µM)

• Resuspend 200 µg of ODN 4084-F with 103 µl endotoxin-free water (provided).

• 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.

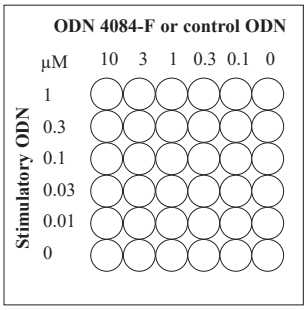
Inhibition of CpG ODN stimulation

Inhibition of CpG ODN stimulation is typically achieved with a 1-10:1 ratio of inhibitory ODN:stimulatory ODN. The inhibitory activity of ODN 4084-F on TLR9 can be assessed using 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 inhibition using HEK-Blue™ TLR9 cells in a 96-well plate.

We recommend to test several concentrations of the stimulatory ODN and inhibitory ODN, 3 or 10-fold apart.

- Dispense 20 µl of stimulatory ODN per well in a column, at concentrations ranging from 0 to 1 µM (see example below).
- Add 20 µl of inhibitory or control ODN per well in a row, at concentrations ranging from 0 to 10 µM.
- Prepare cell suspension of HEK-Blue™ TLR9 cells according to the data sheet.
- Add HEK-Blue™ TLR9 cells (4-8 x 10^4) to each well.
- Incubate for 6-24 h at 37 °C, 5% CO_2.
- Determine inhibition of TLR9 stimulation by assessing cytokine expression using ELISA, or SEAP expression using QUANTI-Blue™, a SEAP detection medium.



RELATED PRODUCTS

Product	Catalog Code
HEK-Blue™ hTLR9 cells (human TLR9)	hkb-htlr9
HEK-Blue™ mTLR9 cells (mouse TLR9)	hkb-mtlr9
ODN1826 (stimulatory CpG ODN)	tlrl-1826
ODN 2006 (stimulatory CpG ODN)	tlrl-2006
QUANTI-Blue™ Solution	rep-qbs

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