ODN INH-18

TLR9 antagonist; Class B inhibitory ODN oligonucleotide

Catalog code: tlrl-inh18 https://www.invivogen.com/odn-inh18

> For research use only Version 18J26-MM

PRODUCT INFORMATION

Contents

 200 μg (25.9 nmol) of ODN INH-18 provided lyophilized <u>Note:</u> ODN INH-18 is sterile filtered prior to lyophilization.
 1.5 ml endotoxin-free water

ODN INH-18 sequence

5'-cct gga tgg gaa ctt acc gct gca-3' (24 mer) <u>Note:</u> Bases are phosphorothioate (nuclease resistant).

Molecular weight: 7723 g/mol

Storage and stability

- ODN INH-18 is shipped at room temperature. Upon receipt, store at -20 $^{\circ}\mathrm{C}.$

- Upon resuspension, prepare aliquots of ODN INH-18 and store at -20 $^{\circ}$ C. Product is stable for 6 months at -20 $^{\circ}$ C when properly stored. Avoid repeated freeze-thaw cycles.

Quality control

Inhibition of TLR9 activity has been tested using HEK-Blue™ TLR9 cells.
 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 have revealed the existence of DNA sequences that can neutralize the stimulatory effect of CpG ODNs¹.

ODN INH-18 is a linear and class R ('restricted') inhibitory ODN. 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 INH-18 is a potent inhibitor of TLR9-induced B cells and macrophages². ODN INH-18 strongly blocks TLR9 activation in both human and mouse TLR9-expressing cells.

1. Krieg A. *et al.*, 1998. Sequence motifs in adenoviral DNA block immune activation by stimulatory CpG motifs. PNAS 95(21):12631-6. 2. 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)

Inhibition of CpG-mediated TLR9 activity can be obtained with 0.1-10 μM ODN INH-18.

- Resuspend ODN INH-18 with 52 µl of endotoxin-free water (provided).
- + Vortex until completely dissolved. Prepare aliquots and store at -20 $^{\circ}\mathrm{C}.$
- · Prepare serial dilutions using endotoxin-free water.

<u>Note:</u> 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 INH-18 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.

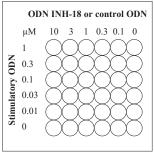
- Dispense 20 μ l of stimulatory ODN per well in a column, at concentrations ranging from 0 to 1 μ M (see example below).

- Add 20 μl of inhibitory or control ODN per well in a row, at concentrations ranging from 0 to 10 $\mu M.$

Prepare cell suspension of HEK-Blue[™] TLR9 cells according to the data sheet.
Add HEK-Blue[™] TLR9 cells

 $(4-8 \times 10^4)$ to each well.

Incubate for 6-24 h at 37 °C, 5% CO₂.
 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) HEK-Blue [™] mTLR9 cells (mouse TLR9) ODN 1826 (stimulatory CpG ODN) ODN 2006 (stimulatory CpG ODN) ODN 4084-F QUANTI-Blue [™] Solution	hkb-htlr9 hkb-mtlr9 tlrl-1826 tlrl-2006 tlrl-4084 rep-qbs



