

ODN INH-18

TLR9 antagonist; Class B inhibitory ODN oligonucleotide

Catalog code: tlr1-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
- Note: 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)

Note: 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°C.
- Upon resuspension, prepare aliquots of ODN INH-18 and store at -20°C. Product is stable for 6 months at -20°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°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 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 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 10⁴) 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.

		ODN INH-18 or control ODN						
		µM	10	3	1	0.3	0.1	0
Stimulatory ODN	1							
	0.3							
	0.1							
	0.03							
	0.01							
	0							

RELATED PRODUCTS

Product	Catalog Code
HEK-Blue™ hTLR9 cells (human TLR9)	hkb-htlr9
HEK-Blue™ mTLR9 cells (mouse TLR9)	hkb-mtlr9
ODN 1826 (stimulatory CpG ODN)	tlr1-1826
ODN 2006 (stimulatory CpG ODN)	tlr1-2006
ODN 4084-F	tlr1-4084
QUANTI-Blue™ Solution	rep-qbs

TECHNICAL SUPPORT

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