

# ODN 2088

TLR9 antagonist; a suppressive oligonucleotide, mouse preferred

Catalog code: tlr1-2088, tlr1-2088-1, tlr1-2088-5

<https://www.invivogen.com/odn2088>

For research use only

Version 18J26-MM

## PRODUCT INFORMATION

### Contents

- ODN 2088 is provided lyophilized and is available in three quantities:
  - 200 µg (**41 nmol**): tlr1-2088 (formerly tlr1-minhodn)
  - 1 mg (**205 nmol**): tlr1-2088-1 (formerly tlr1-minhodn-1)
  - 5 x 1 mg (5 mg; **1.025 µmol**): tlr1-2088-5 (formerly tlr1-minhodn-5)

*Note: ODN 2088 is sterile filtered prior to lyophilization.*

- 1.5 ml endotoxin-free water

### ODN 2088 sequence

5'- tcc tgg cgg gga agt -3' (15 mer)

*Note: Bases are phosphorothioate (nuclease resistant).*

**Molecular weight:** 4874 g/mol

### Storage and stability

- ODN 2088 is shipped at room temperature. Upon receipt, store at -20°C.
- Upon resuspension, prepare aliquots of ODN 2088 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

- Biological 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

Studies suggest the existence of DNA sequences that can neutralize the stimulatory effect of CpG ODNs<sup>1</sup>. These sequences are characterized by three consecutive Gs downstream of a C or A, addition of a fourth G (G-tetrads) increases the inhibitory capability. The most potent inhibitory sequences are (TTAGGG)<sub>4</sub> found in mammalian telomeres<sup>2</sup> and ODN 2088 which derives from a stimulatory ODN by replacement of 3 bases<sup>1</sup>. Inhibitory ODNs act by disrupting the colocalization of CpG ODNs with TLR9 in endosomal vesicles without affecting cellular binding and uptake.

1. **Krieg A. et al., 1998.** Sequence motifs in adenoviral DNA block immune activation by stimulatory CpG motifs. PNAS 95(21):12631-6. 2. **Gursel I. et al., 2003.** Repetitive elements in mammalian telomeres suppress bacterial DNA-induced immune activation. J Immunol. 171(3):1393-400. 3. **Stunz LL. et al., 2002.** Inhibitory oligodinucleotides specifically block effects of stimulatory CpG oligonucleotides in B cells. Eur J Immunol. 32(5): 1212-22.

## METHODS

### Preparation of stock solution (500 µM)

Inhibition of CpG-mediated TLR9 activity can be obtained with 0.1-10 µM of ODN 2088.

- Resuspend ODN 2088 with endotoxin-free water (provided).
  - Add 82 µl to the 200 µg vial of ODN 2088
  - Add 410 µl to the 1 mg vial of ODN 2088
- 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 2088 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](http://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.

1. Dispense 20 µl of stimulatory ODN per well in a column, at concentrations ranging from 0 to 1 µM (see example below).
2. Add 20 µl of inhibitory or control ODN per well in a row, at concentrations ranging from 0 to 10 µM.
3. Prepare a cell suspension of HEK-Blue™ TLR9 cells according to the data sheet.
4. Add HEK-Blue™ TLR9 cells (4-8 x 10<sup>4</sup>) to each well.
5. Incubate for 6-24 h at 37°C, 5% CO<sub>2</sub>.
6. Determine inhibition of TLR9 stimulation by assessing cytokine expression using ELISA, or SEAP expression using QUANTI-Blue™, a SEAP detection medium.

Stimulatory ODN	ODN 2088 or control ODN						
	µM	10	3	1	0.3	0.1	0
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
ODN1826 (stimulatory CpG ODN)	tlr1-1826
ODN2088 Control	tlr1-2088c
QUANTI-Blue™ Solution	rep-qbs

### TECHNICAL SUPPORT

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