

ODN 2088 Control

Negative control for ODN 2088

Catalog code: tlr-2088c, tlr-2088c-1, tlr-2088c-5

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

For research use only

Version 18J26-MM

PRODUCT INFORMATION

Contents

- ODN 2088 Control is provided lyophilized and is available in 3 quantities:
 - 200 µg (**41 nmol**): tlr-2088c (formerly tlr-minhodnc)
 - 1 mg (**205 nmol**): tlr-2088c-1 (formerly tlr-minhodnc-1)
 - 5 x 1 mg (5 mg; **1.025 µmol**): tlr-2088c-5 (formerly tlr-minhodnc-5)

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

- 1.5 ml endotoxin-free water

ODN 2088 Control sequence

5'- tcc tga gct tga agt -3' (15 mer)

Note: Bases are phosphorothioate (nuclease resistant).

Molecular weight: 4808 g/mol

Storage and stability

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

- 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 ODNs¹. 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)₄ found in mammalian telomeres² and ODN 2088 which derives from a stimulatory ODN by replacement of 3 bases³. Inhibitory ODNs act by disrupting the colocalization of CpG ODNs with TLR9 in endosomal vesicles without affecting cellular binding and uptake.

ODN 2088 Control is a negative control for the inhibitory ODN 2088.

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 L. 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)

- Resuspend ODN 2088 Control with water (provided).
 - Add 83 µl to the 200 µg vial of ODN 2088 Control
 - Add 415 µl to the 1 mg vial of ODN 2088 Control
- 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

ODN 2088 Control can be used as a control ODN to study the inhibitory effect of ODN 2088. 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

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 a 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 2088 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
ODN2088 (inhibitory ODN)	tlr-2088
ODN1826 (stimulatory CpG ODN)	tlr-1826
QUANTI-Blue™ Solution	rep-qbs

TECHNICAL SUPPORT

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