

ODN 2395

Class C CpG oligonucleotide; a human/murine TLR9 ligand

Catalog # tlr1-2395, tlr1-2395-1, tlr1-2395-5

For research use only

Version # 16E24-MM

PRODUCT INFORMATION

Content

- ODN 2395 is provided lyophilized and is available in three quantities:
 - 200 µg (**28.37 nmol**): tlr1-2395 (formerly tlr1-odnc)
 - 1 mg (**141.85 nmol**): tlr1-2395-1 (formerly tlr1-odnc-1)
 - 5 x 1 mg (5 mg; **709.25 nmol**): tlr1-2395-5 (formerly tlr1-odnc-5)

Note: ODN 2395 is sterile filtered prior to lyophilization.

- endotoxin-free water; 1.5 ml with #tlr1-2395 and tlr1-2395-1, and 10 ml with #tlr1-2395-5.

ODN 2395 sequence

5'-tcgtcgttttcggcgc:gcgccc-3' (22 mer)

Note: Bases are phosphorothioate, palindrome is underlined.

Molecular weight: 7048 g/mol

Storage and stability

- ODN 2395 is shipped at room temperature. Upon receipt, store at -20 °C.
- Upon resuspension, prepare aliquots of ODN 2395 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

- TLR9 activity has been tested using HEK-Blue™ TLR9 cells.
- The absence of bacterial contamination (e.g. lipoproteins and endotoxins) has been confirmed using HEK-Blue™ TLR2 and HEK-Blue™ TLR4 cells.

DESCRIPTION

CpG ODNs are synthetic oligonucleotides that contain unmethylated CpG dinucleotides in particular sequence contexts (CpG motifs)¹. These CpG motifs are present at a 20-fold greater frequency in bacterial DNA compared to mammalian DNA. CpG ODNs are recognized by Toll-like receptor 9 (TLR9) leading to strong immunostimulatory effects². Three classes of stimulatory CpG ODNs have been identified, classes A, B and C, which differ in their immunostimulatory activities^{3,4}. Class A CpG ODNs are characterized by a phosphodiester central CpG-containing palindromic motif and a phosphorothioate 3' poly-G string. They induce high IFN-α production from plasmacytoid dendritic cells (pDC) but are weak stimulators of TLR9-dependent NF-κB signaling. Class B CpG ODNs contain a full phosphorothioate backbone with one or more CpG dinucleotides. They strongly activate B cells but stimulate weakly IFN-α secretion. Class C CpG ODNs combine features of both classes A and B. They contain a complete phosphorothioate backbone and a CpG-containing palindromic motif. Class C CpG ODNs induce strong IFN-α production from pDC and B cell stimulation.

ODN 2395 is a Class C CpG ODN with a preference for human and murine TLR9.

METHODS

Preparation of stock solution (500 µM)

TLR9 activation can be achieved with 1-5 µM ODN 2395.

- Resuspend ODN 2395 with endotoxin-free water (provided).
 - Add 57 µl to 200 µg vial of ODN 2395
 - Add 285 µl to 1 mg vial of ODN 2395
- 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.

TLR9 stimulation using ODN 2395

ODN 2395 can be used to stimulate TLR9 in 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 stimulation using HEK-Blue™ TLR9 cells in a 96-well plate.

- Dispense 20 µl of stimulatory or control ODN per well of a 96-well plate.
- Prepare cell suspension of HEK-Blue™ TLR9 cells according to the data sheet.
- Add HEK-Blue™ TLR9 cells (4-8 x10⁴) to each ODN-containing well.
- Incubate for 6-24 h at 37 °C, 5% CO₂.
- Determine TLR9 stimulation by assessing cytokine expression using ELISA, or SEAP expression using QUANTI-Blue™, a SEAP detection medium.

References

1. **Krieg, A. et al., 1995.** CpG motifs in bacterial DNA trigger direct B-cell activation. *Nature*, 374:546-9.
2. **Bauer, S. et al., 2001.** Human TLR9 confers responsiveness to bacterial DNA via species-specific CpG motif recognition. *PNAS*, 98:9237-42.
3. **Krug A. et al., 2001.** Identification of CpG oligonucleotide sequences with high induction of IFN-alpha/beta in plasmacytoid dendritic cells. *Eur J Immunol*, 31:2154-63.
4. **Marshall J. et al., 2005.** Superior activity of the type C class of ISS in vitro and in vivo across multiple species. *DNA Cell Biol.* 24(2):63-72.

RELATED PRODUCT

Product	Catalog Code
ODN 2395 Control	tlr1-2395c-1
pUNO1-hTLR9a (human TLR9 gene)	puno1-hltr9a
pUNO1-mTLR9 (murine TLR9 gene)	puno1-mtlr9
HEK-Blue™ hTLR9 Cells	hkb-hltr9
HEK-Blue™ mTLR9 Cells	hkb-mtlr9
QUANTI-Blue™	rep-qb1

TECHNICAL SUPPORT

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