

# 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)<sup>1</sup>. 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 effects<sup>2</sup>. Three classes of stimulatory CpG ODNs have been identified, classes A, B and C, which differ in their immunostimulatory activities<sup>3,4</sup>. 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](http://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 x10<sup>4</sup>) to each ODN-containing well.
- Incubate for 6-24 h at 37 °C, 5% CO<sub>2</sub>.
- 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-htlr9a
pUNO1-mTLR9 (murine TLR9 gene)	puno1-mtlr9
HEK-Blue™ hTLR9 Cells	hkb-htlr9
HEK-Blue™ mTLR9 Cells	hkb-mtlr9
QUANTI-Blue™	rep-qb1

### TECHNICAL SUPPORT

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