

# ODN 2395 Control

Negative control oligonucleotide for human/murine TLR9 ligand ODN 2395

Catalog code: tlr1-2395c-1

<https://www.invivogen.com/odn2395-control>

For research use only

Version 21L21-MM

## PRODUCT INFORMATION

### Contents

- 1 mg (141.85 nmol) of ODN 2395 Control provided lyophilized
- Note: ODN 2395 Control is sterile filtered prior to lyophilization.
- 1.5 ml endotoxin-free water

### ODN 2395 Control sequence

5'-tgctgcttttggggggccccc-3' (22 mer)

Note: Bases are phosphorothioate.

Molecular weight: 7048 g/mol

### Storage and stability

- ODN 2395 Control is shipped at room temperature. Upon receipt, store at -20°C.
- Upon resuspension, prepare aliquots of ODN 2395 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 stimulatory activity has been confirmed 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 interferon- $\alpha$  (IFN- $\alpha$ ) production from plasmacytoid dendritic cells (pDC) but are weak stimulators of TLR9-dependent NF- $\kappa$ B 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- $\alpha$  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- $\alpha$  production from pDC and B cell stimulation.

ODN 2395 Control contains GpC dinucleotides instead of CpGs and can be used as a negative control for ODN 2395 (Class C CpG ODN).

## METHODS

### Preparation of ODN solution (500 $\mu$ M)

- Add 285  $\mu$ l of endotoxin-free water (provided) to 1 mg vial of ODN 2395 Control.
- Vortex until completely dissolved. Prepare aliquots and store at -20°C.

### TLR9 stimulation

ODN 2395 Control can be used as a control ODN to study the stimulatory effect of ODN 2395 on TLR9 in HEK-Blue™ TLR9 cells. These cells stably overexpress the TLR9 gene and an NF- $\kappa$ B-inducible secreted embryonic alkaline phosphatase (SEAP) reporter gene. For more information, visit: <https://www.invivogen.com/hek-blue-tlr9>.

Below is a protocol to study TLR9 stimulation using HEK-Blue™ TLR9 cells in a 96-well plate.

Note: Use ODN 2395 Control at the same concentration as the CpG-containing ODN 2395.

1. Dispense 20  $\mu$ l of stimulatory or control ODN per well of a 96-well plate.
2. Prepare cell suspension of HEK-Blue™ TLR9 cells according to the data sheet.
3. Add HEK-Blue™ TLR9 cells ( $4-8 \times 10^4$ ) to each ODN-containing well.
4. Incubate for 6-24 h at 37°C, 5% CO<sub>2</sub>.
5. Determine TLR9 stimulation by assessing cytokine expression using ELISA, or SEAP expression using QUANTI-Blue™ Solution, 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 PRODUCTS

Product	Description	Cat. Code
HEK-Blue™ hTLR9 cells	Human TLR9 reporter cells	hkb-htlr9
ODN 2395	Stimulatory ODN	tlr1-2395
QUANTI-Blue™ Solution	SEAP detection medium	rep-qbs

## TECHNICAL SUPPORT

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