

ODN 2395 FITC

FITC labeled CpG oligonucleotide; Human TLR9 ligand

Catalog # tlrl-2395f (formerly tlrl-fodnc)

For research use only

Version # 16J31-MM

PRODUCT INFORMATION

Content

- 50 µg (6.5 nmol) lyophilized ODN 2395 labeled with FITC at the 3' terminus
- 1.5 ml endotoxin-free water

ODN2395 sequence

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

Note: Bases are phosphorothioate, palindrome is underlined.

Molecular weight: 7631 g/mol

Storage and stability

- ODN 2395 FITC is shipped at room temperature and should be stored at -20 °C for up to 1 year. Protect from light.
- Upon resuspension, prepare aliquots of ODN 2395 FITC and store at -20 °C protected from light. Resuspended product is stable for 6 months 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 FITC.
- Resuspend lyophilized ODN 2395 FITC with 13 µl of endotoxin-free water (provided).
 - Vortex until completely dissolved.
 - Prepare aliquots of ODN 2395 FITC and store at -20 °C.

TLR9 stimulation using ODN 2395 FITC

ODN 2395 FITC 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.
- Evaluate CpG ODN cellular uptake and localization by confocal laser-scanning microscopy (excitation 495 nm, emission 520 nm) or flow cytometry.

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	Catalog Code
ODN 2395	tlrl-2395
HEK-Blue™ hTLR9 cells	hkb-htlr9
HEK-Blue™ mTLR9 cells	hkb-mtlr9
QUANTI-Blue™	rep-qb1

TECHNICAL SUPPORT

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