

# ODN 2006 Biotin

Biotin labeled CpG oligonucleotide, type B - Human TLR9 ligand

Catalog # tlr1-2006b

<http://www.invivogen.com/odn2006-biotin>

For research use only

Version # 17A03-MM

## PRODUCT INFORMATION

### Content

- 2 x 50 µg (6.12 nmol) lyophilized ODN 2006 labeled with biotin at the 3' terminus.
- 1.5 ml endotoxin-free water

### ODN 2006 sequence

5'-tcgtcgtttgtcgtttgtcgtt-3' (24 mer)

*Note: Bases are phosphorothioate (nuclease resistant).*

**Molecular weight:** 8158 g/mol

### Storage

- ODN 2006 Biotin is shipped at room temperature and should be stored at -20°C. Lyophilized ODN 2006 Biotin is stable for at least 1 year.
- Upon resuspension, prepare aliquots of ODN 2006 Biotin and store at -20°C. Resuspended product is stable 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)<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 types of stimulatory CpG ODNs have been identified, types A, B and C, which differ in their immunostimulatory activities<sup>3,4</sup>.

ODN 2006 (also known as ODN 7909 or PF-3512676) is a CpG ODN class B with a preference for human TLR9. B-class CpG ODNs contain a full phosphorothioate backbone with one or more CpG dinucleotides. They strongly activate B cells but weakly stimulate IFN-α secretion.

1. **Krieg, A.M. et al., 1995.** CpG motifs in bacterial DNA trigger direct B-cell activation. *Nature*, 374(6522):546-9. 2. **Bauer, S. et al., 2001.** Human TLR9 confers responsiveness to bacterial DNA via species-specific CpG motif recognition. *PNAS*, 98(16):9237-42. 3. **Krug A. et al., 2001.** Identification of CpG oligonucleotide sequences with high induction of IFN-α/β in plasmacytoid dendritic cells. *Eur J Immunol*, 31(7): 2154-63. 4. **Marshall JD. 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.

## METHODS

### Preparation of stock solution (500 µM)

TLR9 activation can be achieved with 1-5 µM of ODN 2006 Biotin.

1. Resuspend 50 µg of lyophilized ODN 2006 Biotin with 12 µl of sterile endotoxin-free water provided.
2. Vortex gently until completely dissolved.
3. Prepare aliquots of ODN 2006 Biotin and store at -20°C.

### TLR9 stimulation using ODN 2006 Biotin

ODN 2006 Biotin 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/hek-blue-tlr9](http://www.invivogen.com/hek-blue-tlr9)

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

1. Dispense 20 µl 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 x 10<sup>4</sup>) 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™, a SEAP detection medium.
6. Alternatively, evaluate CpG ODN cellular uptake and localization using a biotin detection system and light microscopy.

## RELATED PRODUCTS

Product	Catalog Code
ODN 2006	tlr1-2006
ODN 2006 control	tlr1-2006c
ODN 2006 FITC	tlr1-2006f
pUNO1-hTLR9a (human TLR9a gene)	puno1-htlr9
HEK-Blue™ hTLR9 Cells	hkb-htlr9
QUANTI-Blue™	rep-qb1

## TECHNICAL SUPPORT

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