

# ODN 2007

## Class B CpG oligonucleotide; a bovine/porcine TLR9 ligand

Catalog # tlr1-2007, tlr1-2007-1, tlr1-2007-5

For research use only

Version # 16E17-MM

### PRODUCT INFORMATION

#### Content

- ODN 2007 is provided lyophilized and is available in three quantities:
  - 200 µg (**28.33 nmol**): tlr1-2007 (formerly tlr1-podnb)
  - 1 mg (**141.7 nmol**): tlr1-2007-1 (formerly tlr1-podnb-1)
  - 5 x 1 mg (5 mg; **708 nmol**): tlr1-2007-5 (formerly tlr1-podnb-5)

*Note: ODN 2007 is sterile filtered prior to lyophilization.*

- endotoxin-free water; 1.5 ml with #tlr1-2007 and tlr1-2007-1, and 10 ml with #tlr1-2007-5.

#### ODN 2007 sequence

5'- tcg tcg ttg tcg ttt tgt cgt t -3' (22 mer)

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

**Molecular weight:** 7058 g/mol

#### Storage and stability

- ODN 2007 is shipped at room temperature. Upon receipt, store at -20 °C. Upon receipt, store at -20 °C.
- Upon resuspension, prepare aliquots of ODN 2007 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 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>.

ODN 2007 is a class B CpG ODN with a preference for bovine and porcine TLR9. 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.

**1. Krieg A. 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- $\alpha$ /beta in plasmacytoid dendritic cells. *Eur J Immunol*, 31(7): 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.

### METHODS

#### Preparation of stock solution (500 µM)

- Resuspend ODN 2007 with endotoxin-free water (provided).
  - Add 57 µl to 200 µg vial of ODN 2007
  - Add 285 µl to 1 mg vial of ODN 2007
- 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.*

#### ODN 2007 stimulation

- Transfect your TLR9-expressing cell line with an NF- $\kappa$ B reporter plasmid, such as a pNiFty plasmid, a plasmid carrying a SEAP or luciferase reporter gene under the control of the NF- $\kappa$ B-inducible ELAM-1 (E-selectin) promoter<sup>5</sup>.

*Note: If your cell line does not express TLR9, cotransfect with a TLR9 plasmid, such as pUNO1-pTLR9 plasmid expressing the porcine TLR9 gene.*

- Twenty-four to forty-eight hours after transfection, stimulate cells with 1-5 µM CpG ODN for 6 to 24 hours.
- Determine CpG ODN-induced TLR9 stimulation by assessing reporter gene expression using the appropriate detection system.

### RELATED PRODUCTS

Product	Catalog Code
ODN 2007 control	tlr1-2007c
pUNO1-pTLR9 (porcine TLR9 gene)	puno1-ptlr9
pNiFty-Luc (Amp <sup>R</sup> )	pnifty-luc
pNiFty-SEAP (Amp <sup>R</sup> )	pnifty-seap
pNiFty2-Luc (Zeo <sup>R</sup> )	pnifty2-luc
pNiFty2-SEAP (Zeo <sup>R</sup> )	pnifty2-seap

#### TECHNICAL SUPPORT

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