

# ODN 2006-G5

## Class B CpG oligonucleotide; a human TLR9 ligand

Catalog # tlr1-2006g5, tlr1-2006g5-1, tlr1-2006g5-5

For research use only

Version # 16E17-MM

### PRODUCT INFORMATION

#### Content

• ODN 2006-G5 is provided lyophilized and is available in three quantities:

- 200 µg (**21.22 nmol**): tlr1-2006g5 (formerly tlr1-hodnbg)
- 1 mg (**106.1 nmol**): tlr1-2006g5-1 (formerly tlr1-hodnbg-1)
- 5 x 1 mg (5 mg; **530.5 nmol**): tlr1-2006g5-5 (formerly tlr1-hodnbg-5)

*Note: ODN 2006-G5 is sterile filtered prior to lyophilization.*

• endotoxin-free water; 1.5 ml with #tlr1-2006g5 and tlr1-2006g5-1, and 10 ml with #tlr1-2006g5-5.

#### ODN 2006-G5 sequence

5'-TCGTCGTTTTGTGCGTTTTGTGCGTTGGGGG-3' (29 mer)

*Note: All bases are phosphodiester.*

**Molecular weight:** 8974 g/mol

#### Storage

- ODN 2006-G5 is shipped at room temperature. Upon receipt, store at -20 °C.

- Upon resuspension, prepare aliquots of ODN 2006-G5 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

Bacterial and viral DNA induce strong immunostimulatory effects through the activation of TLR9 due to the presence of unmethylated CpG dinucleotides in particular sequence contexts<sup>1,2</sup>. TLR9 activation can be mimicked by synthetic CpG oligodeoxynucleotides (ODNs). Three classes of stimulatory CpG ODNs have been identified, classes A, B and C, which differ in their immunostimulatory activities<sup>3,4</sup>.

Cellular uptake is a prerequisite for CpG-induced signal transduction as TLR9 is expressed in the endosome. Uptake of CpG-ODNs in mice is independent of CpG motifs while signaling is strictly dependent on such structures. Phosphorothiate (PTO) ODNs are taken up much more efficiently than their phosphodiester (PD) counterparts. However, PTO ODNs are associated with CpG-independent immunostimulatory effects and seem to induce a slightly different profile than PD ODNs. Class B prototype ODN 2006 in its PD form is poorly internalized. Addition of a 3' poly-G string (ODN 2006-G5) was reported to improve its internalization which was correlated with increased IL-6 secretion and PBMC proliferation<sup>5</sup>.

### METHODS

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

TLR9 activation can be achieved with 1-5 µM ODN 2006-G5.

- Resuspend ODN 2006 with endotoxin-free water (provided).
  - Add 45 µl to 200 µg of ODN 2006-G5
  - Add 225 µl to 1 mg of ODN 2006-G5
- 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 2006-G5

ODN 2006-G5 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.M. *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-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*. 24:63-72.
5. Bartz H. *et al.*, 2004. Poly-guanosine strings improve cellular uptake and stimulatory activity of phosphodiester CpG oligonucleotides in human leukocytes. *Vaccine*. 23:148-55.

### RELATED PRODUCT

Product	Catalog Code
ODN2006-G5 Control	tlr1-2006g5c
ODN2006	tlr1-2006
pUNO1-hTLR9a (human TLR9 gene)	puno1-htlr9a
HEK-Blue™ hTLR9 cells	hkb-htlr9
QUANTI-Blue™	rep-qb1

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