ODN 2006-G5 Control

Negative control for ODN 2006-G5

Catalog # tlrl-2006g5c (formerly tlrl-hodnbgc)

For research use only

Version # 16E17-MM

PRODUCT INFORMATION

Content

- 200 µg (**22.3 nmol**) lyophilized ODN2006-G5 Control

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

- 1.5 ml endotoxin-free water

ODN 2006-G5 Control sequence

5'- TGC TGC TTT TGT GCT TTT GTG CTT GGG GG -3' (29 mer)

Note: All bases are phosphodiester.

Storage

- ODN 2006-G5 Control is shipped at room temperature. Upon receipt, store at -20 °C.
- Upon resuspension, prepare aliquots of ODN 2006-G5 Control and store at -20 °C. Resuspended product is stable for 6 months at -20 °C when properly stored. Avoid repeated freeze-thaw cycles.

Molecular weight: 8974 g/mol

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

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[1,2]. 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[3-4].

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[5].

ODN 2006-G5 Control contains GpC dinucleotides instead of CpGs and can be used as a negative control for ODN 2006-G5 (class B CpG ODN).

METHODS

Preparation of CpG ODN solution (500 µM)

- Resuspend ODN 2006-G5 Control with 45 µl of endotoxin-free water (provided).
- 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

ODN 2006-G5 Control can be used as a control ODN to study the stimulatory effect of ODN 2006-G5 on 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.

Note: Use the ODN 2006-G5 Control at the same concentration as the CpG-containing ODN 2006-G5.

- 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 x10^4) to each ODN-containing well.
- Incubate for 6-24 h at 37 °C, 5% CO_2.
- 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-alpha/beta 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	tlrl-2006g5
pUNO1-hTLR9a (human TLR9 gene)	puno1-htlr9a
HEK-Blue™ hTLR9 cells	hkb-htlr9
QUANTI-Blue™	rep-qb1

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