ODN BW006 (ODN 684)

Type B CpG oligonucleotide; Human & mouse TLR9 ligand

Catalog # tlrl-bw006

For research use only

Version # 16F01-MM

PRODUCT INFORMATION

<u>Content</u>

 200 μg (27.24 nmol) of ODN BW006 provided lyophilized <u>Note:</u> ODN BW006 is sterile filtered prior to lyophilization.
1.5 ml endotoxin-free water

ODN BW006 sequence

5'-tcg acg ttc gtc gtt cgt cgt tc-3' (23 mer) <u>Note:</u> Bases are phosphorothioate (nuclease resistant).

Molecular weight: 7341 g/mol

Storage and stability

- ODN BW006 is shipped at room temperature. Upon receipt, store at -20 $^{\circ}\mathrm{C}.$

- Upon resuspension, prepare aliquots of ODN BW006 and store at -20 °C. 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 & endotoxins) has been confirmed using HEK-Blue[™] TLR2 and HEK-Blue[™] TLR4 cells.

DESCRIPTION

ODN BW006 (also known as ODN 684) is a synthetic oligonucleotide that contains unmethylated CpG dinucleotides in particular sequence contexts (CpG motifs), that are recognized by TLR9 leading to strong immunostimulatory effects. Three types of stimulatory CpG ODNs have been identified, types A, B and C, which differ in their immune-stimulatory activities.

ODN BW006 is a type B CpG ODN containing twice the optimal motif in human, GTCGTT¹. ODN BW006 is capable of inducing the proliferation of human peripheral blood mononucleated cell (PBMCs) peripheral blood mononucleated cell (PBMCs) and mouse splenocytes, as vigorously as ODN 2006, a class B prototype ODN. It was found to improve the rabies vaccine by inducing an earlier and more vigorous protective response¹. ODN BW006 promotes strong Th1 response^{2,3}.

 Wang X. et al., 2008. A CpG oligodeoxynucleotide acts as a potent adjuvant for inactivated rabies virus vaccine. Vaccine. 26(15):1893-901. 2. Yan Y. et al., 2012. A CpG oligodeoxynucleotide potentiates the anti-tumor effect of HSP65-Her2 fusion protein against Her2 positive B16 melanoma in mice. Int Immunopharmacol. 12(2):402-7.
Zhang X. et al., 2011. Enhanced specific immune responses by CpG DNA in mice immunized with recombinant hepatitis B surface antigen and HB vaccine. Virol J. 8:78.

METHODS <u>Preparation of stock solution (500 µM)</u>

TLR9 activation can be achieved with 1-5 μ M ODN BW006.

 \bullet Resuspend ODN BW006 with 54 μl of endotoxin-free water (provided).

• Vortex until completely dissolved. Prepare aliquots and store at -20 °C.

• Prepare serial dilutions using endotoxin-free water.

<u>Note:</u> 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 BW006

ODN BW006 can be used to stimulate TLR9 in HEK-Blue^T TLR9 cells. HEK-Blue^T TLR9 cells stably overexpress the TLR9 gene and an NF- κ B-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.

RELATED PRODUCTS

Product	Catalog Code	
HEK-Blue™ hTLR9 cells HEK-Blue™ mTLR9 cells pUNO1-hTLR9a pUNO1-mTLR9	hkb-htlr9 hkb-mtlr9 puno1-htlr9a puno1-mtlr9	



