

ODN D-SL03

Type C CpG oligonucleotide; Multispecies TLR9 ligand

Catalog # tlrl-dsl03

For research use only

Version # 16E31-MM

PRODUCT INFORMATION

Content

- 200 µg (21.4 nmol) of ODN D-SL03 provided lyophilized

Note: ODN D-SL03 is sterile filtered prior to lyophilization.

- 1.5 ml endotoxin-free water

D-SL03 sequence

5'-tcg cga acg ttc gcc gcg ttc gaa cgc gg-3' (29 mer)

Note: Bases are phosphorothioate (nuclease resistant).

Molecular weight: 9345 g/mol

Storage and stability

- ODN D-SL03 is shipped at room temperature. Upon receipt, store at -20 °C.

- Upon resuspension, prepare aliquots of ODN D-SL03 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 D-SL03 (double-stem loop ODN) 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 D-SL03 is a type C CpG ODN and a TLR9 agonist in a diverse vertebrate species, namely humans, mice, rats, rabbits, pigs and dogs. ODN D-SL03 is composed of double stem loops, a phosphorothioate backbone and two palindromes with AACGTT motif and TTCGAA motif in each loop. ODN D-SL03 is a robust inducer of IFN-α apparently due to the presence of the palindrome sequence. D-SL03 has been shown to potently activate human B cells, NK cells and mononuclear cells as well as PBMC/splenocytes obtained from diverse vertebrate species, namely mice, rats, rabbits, dogs and pigs. D-SL03 activates B cells and NK cells. ODN D-SL03 demonstrates anti-tumor activity in mice with established breast cancer¹.

1. Yang L. *et al.*, 2013. CpG oligodeoxynucleotides with double stem-loops show strong immunostimulatory activity. *Int Immunopharmacol.* 15(1):89-96.

METHODS

Preparation of stock solution (500 µM)

TLR9 activation can be achieved with 1-5 µM ODN D-SL03.

- Resuspend ODN D-SL03 with 43 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 using D-SL03

ODN D-SL03 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).

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	hkb-htrl9
HEK-Blue™ mTLR9 Cells	hkb-mtrl9
QUANTI-Blue™	rep-qb1
pNiFty2-Luc (Zeo [®])	pnifty2-luc
pNiFty2-SEAP (Zeo [®])	pnifty2-seap
pUNO1-hTLR9a (human gene)	puno1-htrl9a
pUNO1-mTLR9 (mouse gene)	puno1-mtrl9
pUNO1-pTLR9 (pig gene)	puno1-ptlr9
ODN D-SL01 (type B CpG ODN)	tlrl-dsl01
A&C-Class TLR9 Agonist Kit	tlrl-kit9ac

TECHNICAL SUPPORT

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