ABGENEX Pvt. Ltd.,
E-5, Infocity, KIIT Post Office,
Tel : +91-674-2720712, +91-9437550560
Email : info@abgenex.com
Bhubaneswar, Odisha - 751024, INDIA

15-1022: CpG ODN (1826) with negative control oligo, TLR9 ligand (mouse)

Application : Functional Assay
Reactivity : Mouse,Human

Description

MW: 6,383g/mol
Sequence: 5'-tccatgagcttcctgagctt-3'

Unmethylated CG dinucleotides within particular sequence contexts are responsible for the immunostimulatory activity of bacterial DNA. Synthetic oligonucleotides (ODN) that contain such CpG motifs (CpG ODNs) mimic microbial DNA. The innate immune system of vertebrates has the ability to recognize CpG motifs in microbial DNA via the Toll-like receptor (TLR) 9 if the CpG ODN were free of additional immune stimulatory contaminants often present in synthetic commercial CpG ODN preparations designed for molecular biology applications (i.e. PCR). Given that high quality CpG ODNs were used [i.e. endotoxin-free], a close link has been established between the expression of TLR9 on certain immune cell subsets and the modulation of the immune system by CpG DNA. Different types of CpG ODNs were identified based on their differing biological effects on different cell types: ODN Type A is a potent inducer of IFN-α in human PDC, (i.e. ODN 1585 or 2216) leading to antigen presenting cell (APC) maturation, whereas ODN Type B (i.e. ODN 2006 or ODN 1668 / ODN 1826) is a weak inducer of IFN-α but rather stimulates IL-8 production and increasing costimulatory and Ag-presenting molecules and triggers proliferation of B-cells and IgM and IL-6 production. A third type of CpG ODN has been identified, termed ODN Type C, with both high induction of INF-α in PDC and activation of B-cells. The sequence of CpG Type C (also called K) (i.e. ODN 2395 or M362) combines elements of both Type A and Type B and contains a central palindromic sequence with CG dinucleotides, a characteristic feature of Type A, and a TCGTCG motif at the 5' end, present in Type B CpG ODNs. Although the CpG motifs are thought to differ between mice and humans, in both species the recognition of CpG ODNs is mediated by TLR9. The optimal CpG motif in humans is GTCGTT and GACGTT for the murine sequence. However, recent evidence suggests that this sequence specificity is restricted to phosphorothioate (PS)-modified ODN and is not observed when a natural phosphodiester backbone is used. In recent years sequence requirements, specificity, signalling pathways and kinetics of the TLR9 suppression by inhibitory ODNs (iODNs) have been investigated.

Product Info

Amount :	1 mg / 0.1 mg
Content :	1 mg/ml in entotoxin-free water
Storage condition :	Upon receipt, store at -20°C (Stable for at least 6 months). Avoid frequent freeze/thaw cycles.

Application Note

A. CpG ODN C274-mediated human TLR9 activation in TLR9/NF-kB Leeporter™ – HEK293 cells (Figure 2).

1. Harvest TLR9/NF-kB Leeporter™ – HEK293 cells and seed cells into a white solid-bottom 96-well microplate in 100 ul of growth medium at 5 x 10^4 cells/well.

2. Incubate cells at 37°C in a CO_2 incubator for overnight.

3. The next day, stimulate cells with various amounts of CpG ODN C274.

4. Incubate at 37°C in a CO_2 incubator for 6-16 hours.

5. Add 30-50 ul of luciferase assay reagent per well.

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6. Incubate at room temperature for 1-5 minutes and measure luminescence using a microplate luminometer.

B. CpG ODN C274-mediated mouse TLR9 activation in NF-kB Leeporter™ – RAW 264.7 cells (Figure 3).

1. Harvest NF-kB Leeporter™ – RAW 264.7 cells and seed cells into a white solid-bottom 96-well microplate in 100 ul of growth medium at 5 x 10^4 cells/well.

2. Incubate cells at 37°C in a CO_2 incubator for overnight.

3. The next day, stimulate cells with various amounts of CpG ODN C274.

4. Incubate at 37°C in a CO_2 incubator for 6-16 hours.

5. Add 30-50 ul of luciferase assay reagent per well.

6. Incubate at room temperature for 1-5 minutes and measure luminescence using a microplate luminometer.

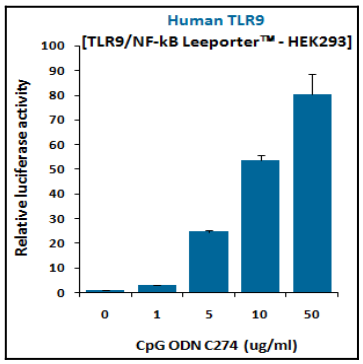

Fig-1: Induction of human TLR9 activity by CpG ODN C274.

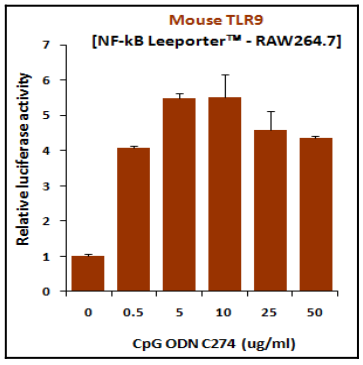

Fig-2: Induction of mouse TLR9 activity by CpG ODN C274.

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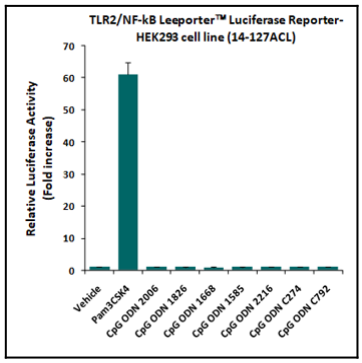

Fig-3: Abeomics' CpG ODNs did not show any TLR2 agonist activity. TLR2/NF-kB Leeporter™ HEK293 cells (14-127ACL) were treated with various CpG ODNs at 100 ug/ml as well as a positive TLR2 agonist, Pam3CSK4, at 10 ng/ml for 16 h, and luciferase activity was then analyzed.

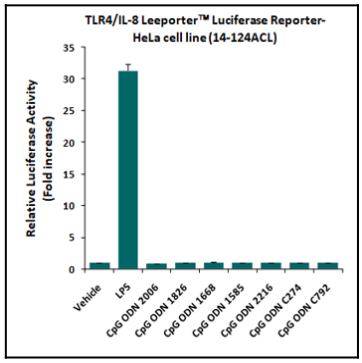

Fig-4: Abeomics' CpG ODNs did not show any TLR4 agonist activity. TLR4/IL-8 Leeporter™ HeLa cells (14-124ACL) were treated with various CpG ODNs at 100 ug/ml as well as a positive TLR4 agonist, LPS, at 10 ng/ml for 16 h, and luciferase activity was then analyzed.