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15-1021: CpG ODN (2216), TLR9 ligand (Class A)

Application : Functional Assay
Reactivity : Mouse,Human

Description

Sequence: 5'-gggggaCGatCGtCGggggg-3' (Class A ODN 2216: *Bases are phosphorothioate.*)

Synthetic oligodeoxynucleotides (ODN) containing unmethylated deoxycytosine-deoxyguanosine (CpG) motif are equivalent to bacterial DNA in the immunostimulatory activity, which can induce innate immunity via Toll-like receptor 9 (TLR9) in mammals. There are three major classes of CpG ODNs, and each ODN class exhibits different stimulatory effects on immune cell activation. Class A ODNs are potent inducers of IFN-alpha that leads to the plasmacytoid dendritc cell (pDC) maturation. Class B ODNs are relatively weak inducers of type I IFNs but strong stimulators of human B cells and monocyte maturation. Class C ODNs combine elements of both Classes A and B ODNs, which can induce IFN-alpha in pDC and activation of B cells.

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 2216-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 2216.

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.

B. CpG ODN 2216-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 2216.

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

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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.

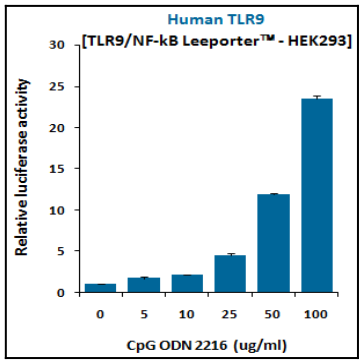

Fig-1: Induction of human TLR9 activity by CpG ODN 2216.

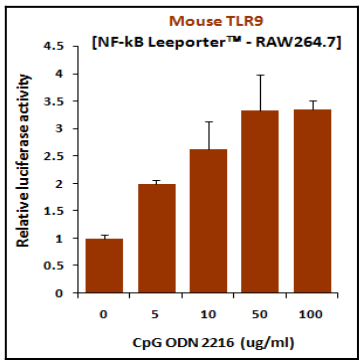

Fig-2. Induction of mouse TLR9 activity by CpG ODN 2216.

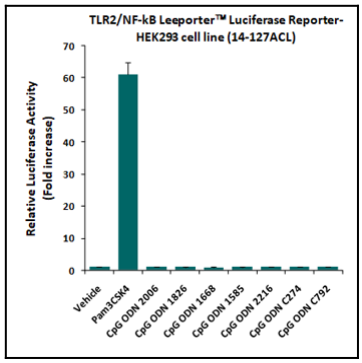

Fig-3: Abeomics' CpG ODNs did not show any TLR2 agonist activity. TLR2/NF-kB Leeporter™ HEK293 cells (14-127ACL) were treated with various CpG ODNs at 100 ug/ml as well as a positive TLR2 agonist, Pam3CSK4, at 10 ng/ml for 16 h, and luciferase activity was then analyzed.

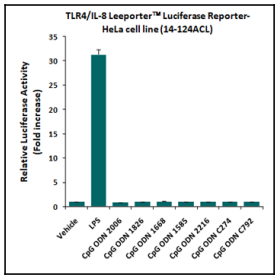

Fig-4: Abeomics' CpG ODNs did not show any TLR4 agonist activity. TLR4/IL-8 Leeporter™ HeLa cells (14-124ACL) were treated with various CpG ODNs at 100 ug/ml as well as a positive TLR4 agonist, LPS, at 10 ng/ml for 16 h, and luciferase activity was then analyzed.