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Data Sheet

TLR9/ NF- κ B Reporter – HEK293 Recombinant Cell Line

Catalog #: 60685

Product Description

Recombinant HEK293 cell expressing firefly luciferase gene under the control of NF- κ B response elements with constitutive expression of human TLR9 (Toll-like receptor 9, also been designated as CD289, a member of the toll-like receptor (TLR) family. GenBank Accession No. NM_017442)

Background

The family of Toll-like receptors (TLRs) acts as primary sensors that detect a wide variety of microbial components and elicit innate immune responses. Human TLR9 recognizes specific unmethylated CpG DNA motifs prevalent in microbial but not vertebrate genomic DNA, leading to innate and acquired immune responses. Stimulation of TLR9 triggers a signaling cascade leading to the activation of the transcription factor NF- κ B, which controls the expression of an array of inflammatory cytokine genes.

Application

- Screen for activators or inhibitors of TLR9 signaling in a cellular context
- Characterize the biological activity of TLR9 and its interactions with ligands

Format

Each vial contains $\sim 2 \times 10^6$ cells in 1 ml of 10% DMSO

Storage

Immediately upon receipt, store in liquid nitrogen.

Mycoplasma Testing

The cell line has been screened using the PCR-based Venor[®]GeM Mycoplasma Detection kit (Sigma-Aldrich #MP0025) to confirm the absence of *Mycoplasma* species.

General Culture Conditions

Thaw Medium 1 (BPS Cat. #60187): MEM medium (Hyclone #SH30024.01) supplemented with 10% FBS (Invitrogen #26140-079), 1% non-essential amino acids (Hyclone #SH30238.01), 1 mM Na pyruvate (Hyclone #SH30239.01), 1% Penicillin/Streptomycin (Hyclone SV30010.01)

Growth Medium 1A (BPS Cat. #79528): Thaw Medium 1 (BPS Cat. #60187) plus 400 μ g/ml of Geneticin (Life Technologies #11811031) and 100 μ g/ml of Hygromycin B (Life Technologies #10687-010).

Cells should be grown at 37°C with 5% CO₂ using **Growth Medium 1A**.

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It is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, then transfer the entire contents of the vial to a tube containing 10 ml of Thaw Medium 1 (**no Geneticin and Hygromycin B**). Spin down the cells, remove supernatant and resuspend cells in 5 ml of pre-warmed Thaw Medium 1 (**no Geneticin and Hygromycin B**). Transfer the resuspended cells to a T25 flask and incubate at 37°C in a 5% CO₂ incubator. After 24 hours of culture, add an additional ~3 ml Thaw Medium 1 (**no Geneticin and Hygromycin B**). At first passage, switch to Growth Medium 1A (**contains Geneticin and Hygromycin B**). Cells should be split before they reach 2.5 x 10⁶ cells/ml.

To passage the cells, dilute cell suspension into new culture vessels at no less than 0.2 x 10⁶ cells/ml. Subcultivation ratio: 1:5 to 1:10 twice a week.

Materials Required but Not Supplied

- Assay Medium: Thaw Medium 1 (BPS Cat. #60187)
- Growth Medium 1A (BPS Cat. #79528): Thaw Medium 1 (BPS Cat. #60187) plus 400 µg/ml of Geneticin (Life Technologies #11811031) and 100 µg/ml of Hygromycin B (Life Technologies #10687-010).
- ODN2006 (Invivogen tlr1-2006)
- 96-well tissue culture-treated white clear-bottom assay plate
- One-Step luciferase assay system (BPS Bioscience #60690) or other luciferase reagents for measuring firefly luciferase activity
- Luminometer

Assay Protocol

1. Harvest TLR9/NF-κB reporter-HEK293 cells from culture in **Growth Medium 1A** and seed cells at a density of ~35,000 cells per well into a white clear-bottom 96-well microplate in 90 µl of Thaw Medium 1. Incubate the plate at 37°C in a CO₂ incubator.
2. Twenty-four hours after seeding, Dilute the ODN2006 in assay medium. Add 10 µl of diluted ODN2006 to the treated wells.

Add 10 µl assay medium to control wells.

Add 100 µl of assay medium to cell-free control wells (for determining background luminescence)

Set up each treatment in at least triplicate

3. Incubate the plate at 37°C in a CO₂ incubator for ~ 6 hours.
4. Perform luciferase assay by using the ONE-Step luciferase assay system: Add 100 µl of One-Step Luciferase reagent per well and rock at room temperature for ~30 minutes.

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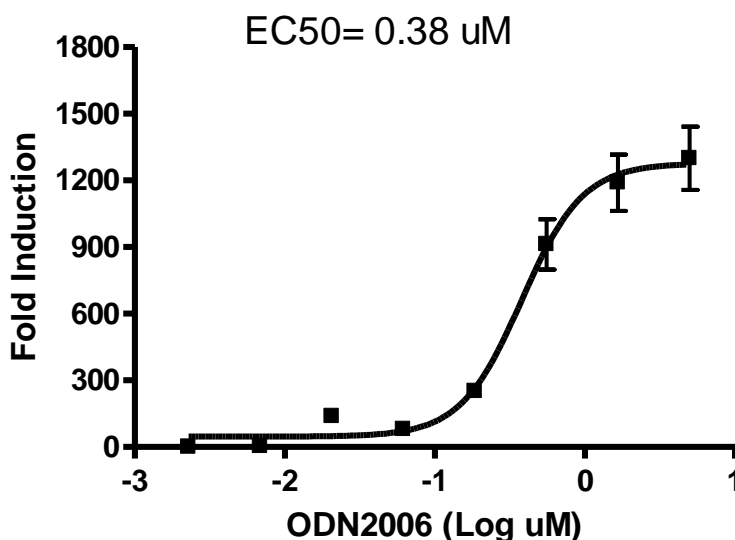
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Measure luminescence using a luminometer. (If using luciferase reagents from other vendors, follow the manufacturer's assay protocol.)

- Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells.
 The fold induction of NF- κ B luciferase reporter expression = background-subtracted luminescence of stimulated well / average background-subtracted luminescence of unstimulated control wells.

Figure 1. Dose Response of TLR9/NF- κ B Reporter HEK293 cells

The results are shown as fold induction of NF- κ B luciferase reporter expression. The EC₅₀ of ODN2006 is 0.38 μ M.



Sequence

hTLR9 sequence (accession number NM_017442)

```
MGFCRSALHPLSLLVQAIMLAMTLALGTLPAFLPCELQPHGLVNCNWFLKSVPHFSMAAPRGNVTLSLSLSSNRIHH
LHSDFAHLPSLRHLNLKWNCPVGLSPMHFPCHMTIEPSTFLAVPTLEELNLSYNNIMTVPALPKSLISLSLSHTN
ILMLDSASLAGLHALRFLFMDGNCYKNPCRQALEVAPGALLGLGNLTHLSLKYNNLTVVPRNLPSSLEYLLLSYNR
IVKLAPEDLANLTALRVLDVGGNCRRCDHAPNPCMECPRHFPQLHPDTFSHLSRLEGLVLKDSLSLWLNASWFRGLG
```

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NLRVLDLSENFYLYKCITKTKAFQGLTQLRKLNLNLSFNQKRVSF AHL SLAPSFGSLVALKELDMHGIFFRSLDETTLR
PLARLPMLQTLRLQMNFINQAQLGIFRAFPGLRYVDLSDNRISGASELTATMGEADGGKQVWLQPGDLAPAPVDTPS
SEDFRPN CSTLNFTL DLSRNNLVTVQPEMFAQLSHLQCLRLSHNCISQAVNGSQFLPLTGLQVLDL SHNKLDLYHEH
SFTELPRLEALDLSYNSQPFMGOVGHNF SFVAHLRTRLRHL SLAHNNIHSQVSQQLCSTSLRALDFSGNALGHMWAE
GDLYLHFFQGLSGLIWL DLSQNRLHTLLPQTLRNLPKSLQVLRRLRDNYLAFFKWWSLHFLPKLEVLDLAGNQLKALT
NGSLPAGTRLRRLDVSCNSISFVAPGFFSKAKELRELNLSANALKTV DHSWFGPLASALQILDVSANPLHCACGAAF
MDFLLEVQA AVPGLPSRVKCGSPGQLQGLSIFAQDLRLCLDEALSWDCFALSLLAVALGLGVPMLHHL CGWDLWYCF
HLCLAWLPWRGRQSGRDEDALPYDAFVVF DKTQSAVADWVYNELRGQLEECRGRWALRLCLEERDWLP GKTLFENLW
ASVYGSRKTLFVLAHTDRVSGLLRASFLLAQQRLLED R KDVVVLVILSPDGRRSRYVRLRQRLCRQSVLLWPHQPSG
QRSFWAQLGMALTRDNHFFYNRNFCQGPTAE

Related Products

| <u>Product</u> | <u>Cat. #</u> | <u>Size</u> |
|---|---------------|-------------|
| NF-kB Reporter (Luc)-HEK293 Recombinant Cell Line | 60650 | 2 vials |
| ONE-Step™ Luciferase Assay System | 60690-1 | 10 ml |
| ONE-Step™ Luciferase Assay System | 60690-2 | 100 ml |

Notes

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