Description

TLR9/NF-κB Luciferase Reporter HEK293 Cell Line is a HEK293 cell line expressing firefly luciferase under the control of NF-κB response elements with constitutive expression of human TLR9 (Toll-like receptor 9) (GenBank Accession No. NM_017442), a member of the toll-like receptor (TLR) family.

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 (toll-like receptor 9), also known as CD289 (cluster of differentiation 289), is expressed in cells in immune cells, such as dendritic cells, macrophages and NK cells. It can be found in endosomes, where it 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 its movement to the Golgi and lysosomes and interaction with MyD88. This interaction activates a signaling cascade that leads to the activation of the transcription factor NF-κB, which controls the expression of an array of inflammatory cytokines. TLR9 has been identified as a major player in systemic lupus erythematosus (SLE) and erythema nodosum leprosum (ENL). TLR9 has also been linked to cancer, with breast and renal cell carcinoma having lower expression levels, while prostate cancer and glioma showing higher levels. TLR9 agonists, such as SDS-101 and tilsotolimod, are currently being tested in the clinic as combination cancer therapies. Further studies into the molecular pathways involved TLR9 and development of agonists can open new avenues for the treatment of TRL9-linked diseases.

Application

Screen for activators or inhibitors of TLR9 signaling in a cellular model.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains >1 x 10^6 cells in 1 ml of Cell Freezing Medium (BPS Bioscience #79796)

Parental Cell Line

HEK293, Human Embryonic Kidney, epithelial-like cells, adherent.

Mycoplasma Testing

The cell line has been screened to confirm the absence of Mycoplasma species.

Materials Required but Not Supplied

These materials are not supplied with the cell line but are necessary for cell culture and cellular assays. BPS Bioscience's reagents are validated and optimized for use with this cell line and are highly recommended for best results. Media components are provided in the Media Formulations section below.

Media Required for Cell Culture

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187
Growth Medium 1A	BPS Bioscience #79528

Materials Required for Cellular Assay

Name	Ordering Information
ODN2006	Invivogen #tlrl-2006
E6446 dihydrochloride	Selleckchem #S6719
96-well tissue culture treated white clear-bottom assay plate	Corning #3610
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
Luminometer	

Storage Conditions

Cells are shipped in dry ice and should immediately be thawed or stored in liquid nitrogen upon receipt. Do not use a -80°C freezer for long term storage. Contact technical support at support@bpsbioscience.com if the cells are not frozen in dry ice upon arrival.

Media Formulations

For best results, the use of validated and optimized media from BPS Bioscience is *highly recommended*. Other preparations or formulations of media may result in suboptimal performance.

Note: Thaw Media do *not* contain selective antibiotics. However, Growth Media *do* contain selective antibiotics, which are used to maintain selective pressure on the cell population expressing the gene of interest.

Cells should be grown at 37°C with 5% CO_2. BPS Bioscience's cell lines are stable for at least 10 passages when grown under proper conditions.

Media Required for Cell Culture

Thaw Medium 1 (BPS Bioscience #60187):

MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, and 1% Penicillin/Streptomycin.

Growth Medium 1A (BPS Bioscience #79528):

MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin, 400 µg/ml of Geneticin®, and 100 µg/ml of Hygromycin B.

Media Required for Functional Cellular Assay

Assay Medium:

Thaw Medium 1 (BPS Bioscience #60187)

Cell Culture Protocol

Note: HEK293 cells are derived from human material and thus the use of adequate safety precautions is recommended.

Cell Thawing

1. Swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. As soon as the cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire content of the vial to a tube containing 10 ml of pre-warmed Thaw Medium 1.
   Note: Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.

2. Immediately spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 5 ml of pre-warmed Thaw Medium 1.

3. Transfer the resuspended cells to a T25 flask and incubate at 37°C in a 5% CO_2 incubator.

4. After 24 hours of culture, check for cell viability and attachment. For a T25 flask, add 3-4 ml of Thaw Medium 1, and continue growing in a 5% CO2 incubator at 37°C until the cells are ready to passage.

5. Cells should be passaged before they are fully confluent. Switch to Growth Medium 1A at first and subsequent passages.

Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS) without Ca^{2+}/Mg^{2+}, and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 1A and transfer to a tube.

3. Spin down cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in Growth Medium 1A.

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:5 twice a week.

Cell Freezing

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS) without Ca^{2+}/Mg^{2+} and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 1A and count the cells.

3. Spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 4°C Cell Freezing Medium (BPS Bioscience #79796) at ~2 x 10^6 cells/ml.

4. Dispense 1 ml of cell suspension into each cryogenic vial. Place the vials in an insulated container for slow cooling and store at -80°C overnight.

5. Transfer the vials to liquid nitrogen the next day for long term storage.

Note: It is recommended to expand the cells and freeze at least 10 vials at an early passage for future use.

Validation Data

- The following assays were designed for a 96-well format. To perform the assay in different tissue culture formats, the cell number and reagent volume should be scaled appropriately.
- All conditions should be performed in triplicate.
- Assay A should include “Stimulated”, “Cell-Free Control” and “Unstimulated Control” conditions.
- Assay B should include “No Inhibitor Control”, “No Inhibitor, No Agonist Control”, “Cell-Free Control” and “Test Inhibitor” conditions.

A. Dose Response of TLR9/NF-κB Luciferase Reporter HEK293 Cell Line to ODN2006

1. Harvest TLR9/NF-κB Luciferase Reporter HEK293 cells from culture in Growth Medium 1A and seed cells at a density of 35,000 cells per well in 90 µl of Assay Medium into a white clear-bottom 96-well microplate. Leave a few wells empty to use as the "Cell-Free Control" (Background Signal).
2. Incubate at 37°C with 5% CO_2 overnight (~16 hours).
3. Prepare a serial dilution of ODN 2006 in Assay Medium at 10x the final testing concentrations (10 µl/well).
4. Add 10 µl of diluted ODN 2006 to the "Stimulated Cells" wells.
5. Add 10 µl of Assay Medium to the "Unstimulated Control" wells.
6. Add 100 µl of Assay Medium to "Cell-Free Control" (for determining background luminescence signal).
7. Incubate at 37°C with 5% CO_2 for 5-6 hours.
8. Add 100 µl of ONE-Step™ Luciferase reagent per well.
9. Rock at Room Temperature (RT) for ~15 minutes.
10. Measure luminescence using a luminometer.
11. The background luminescence value should be subtracted from all readings.
12. Data Analysis: Subtract the average background luminescence from the luminescence reading of all other wells. The fold induction of luciferase reporter expression is the average background-subtracted luminescence of stimulated wells divided by the average background-subtracted luminescence of unstimulated control wells.

$$Fold\ induction = \frac{Luminescence\ of\ \ Stimulated\ Wells - avg.background}{Avg.Luminescence\ of\ Unstimulated\ Wells - avg.background}$$

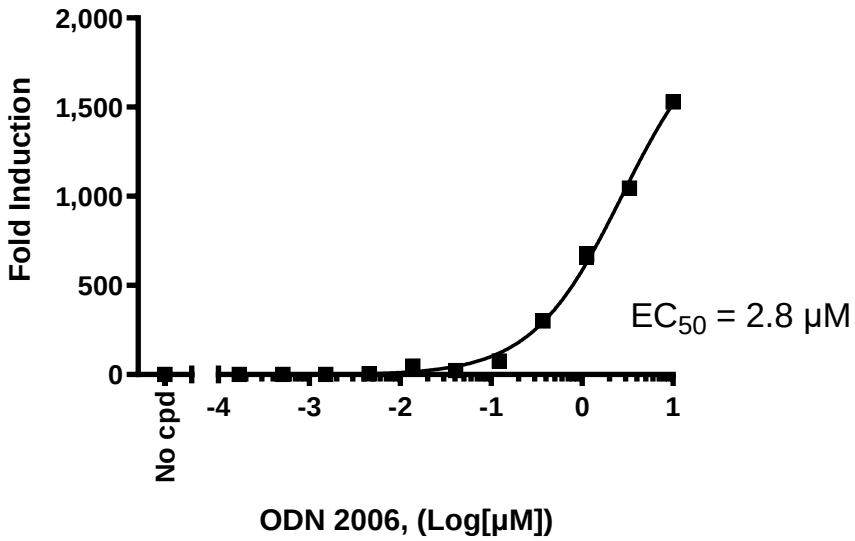

Figure 1. Dose response curve of TLR9/NF-κB Luciferase Reporter HEK293 Cell Line to ODN 2006. TLR9/NF-κB Luciferase Reporter HEK293 cells were treated with increasing concentrations of ODN 2006. The results are shown as fold induction of luciferase reporter expression. Luciferase activity was measured using the ONE-Step™ Luciferase Assay System. The results are shown as fold induction of luciferase reporter expression in relation to the activity of cells without treatment (unstimulated control).

B. Inhibition of ODN 2006 -induced reporter activity in the TLR9/NF-κB Luciferase Reporter HEK293 Cell Line

1. Harvest TLR9/NF-κB Luciferase Reporter HEK293 cells from culture in Growth Medium 1A and seed cells at a density of 35,000 cells per well in 50 µl of Assay Medium into a white clear-bottom 96-well microplate. Leave a few wells empty to use as the "Cell-Free Control" (Background Signal).
2. Incubate at 37°C with 5% CO_2 for 4-5 hours.
3. Prepare a serial dilution of inhibitor in Assay Medium at 2x the final testing concentrations (50 µl/well).
4. Add 50 µl of diluted inhibitor to the "Test Inhibitor" wells.
5. Add 50 µl of Assay Medium to the "No Inhibitor Control" and "No Inhibitor, No Agonist Control" wells.
6. Add 110 µl of Assay Medium to the "Cell-Free Control" (for determining background luminescence signal).
7. Incubate at 37°C with 5% CO_2 overnight (~16 hours).
8. The next day, prepare a solution of ODN 2006 in Assay Medium at 11 µM (10 µl/well).
9. Add 10 µl of diluted ODN 2006 to the "Test Inhibitor" and "No Inhibitor Control" wells.
10. Add 10 µl of Assay Medium to the "No Inhibitor and No Agonist Control" wells.
11. Incubate at 37°C with 5% CO_2 for 5-6 hours.

12. Add 110 µl of ONE-Step™ Luciferase reagent per well.

13. Rock at Room Temperature (RT) for ~15 minutes.

14. Measure luminescence using a luminometer.

15. The background luminescence value should be subtracted from all readings.

16. Data Analysis: Subtract the average background luminescence from the luminescence reading of all other wells. The percent luminescence of luciferase reporter expression is the background-subtracted luminescence of treated wells divided by the average background-subtracted luminescence of the untreated control wells x 100%.

$$Percent\ Luminescence = \left(\frac{Luminescence\ of\ \ Treated\ Wells - avg.background}{Avg.Luminescence\ of\ Untreated\ Wells - avg.background}\right) x100$$

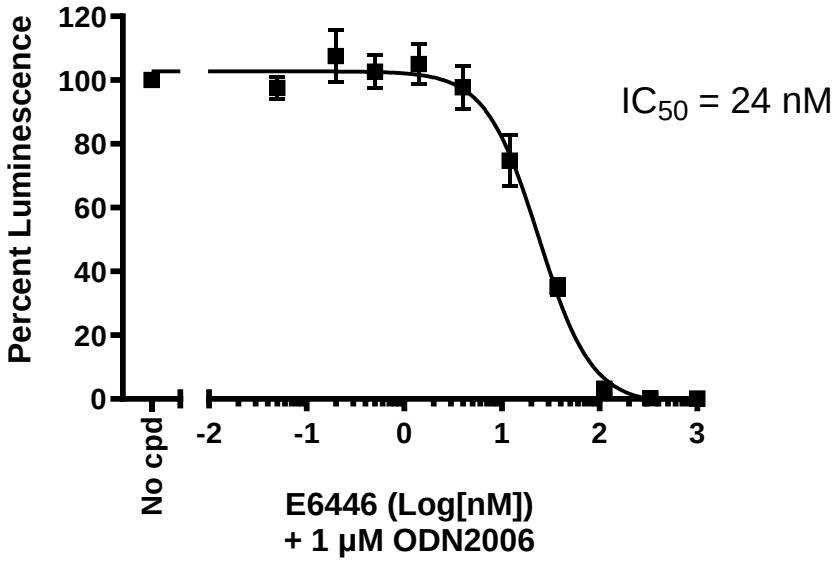

Figure 2: Dose response curve of TLR9/NF-κB Luciferase Reporter HEK293 Cell Line to E6446. TLR9/NF-κB Luciferase Reporter HEK293 cells were treated with increasing concentrations of E6446 overnight before addition of ODN 2006 to a final value of 1 µM. Luciferase activity was measured using the ONE-Step™ Luciferase Assay System. The results are shown a percent luminescence of luciferase reporter activity (in which ODN 2006-stimulated cells in the absence of inhibitor is set at 100%).

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

Sequence
hTLR9 sequence (accession number NM_017442)

MGFCRSALHPLSLLVQAIMLAMTLALGTLPAFLPCELQPHGLVNCNWLFLKSVPHFSMAAPRGNVTSLSLSSNRIHHLHDSDFAH
LPSLRHLNLKWNCPPVGLSPMHFPCHMTIEPSTFLAVPTLEELNLSYNNIMTVPALPKSLISLSLSHTNILMLDSASLAGLHALRFLF
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LTATMGEADGGEKVWLQPGDLAPAPVDTPSSEDFRPNCSTLNFTLDLSRNNLVTVQPEMFAQLSHLQCLRLSHNCISQAVNGS
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WRGRQSGRDEDALPYDAFVVFDKTQSAVADWVYNELRGQLEECRGRWALRLCLEERDWLPGKTLFENLWASVYGSRKTLFVL
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NRNFCQGPTAE

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Troubleshooting Guide
Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

Related Products

Products	*Catalog #*	*Size*
TLR8/ NF-κB Luciferase Reporter HEK293 Cell Line	60684	2 vials
Transfection Collection™: NF-κB Transient Pack (NF-κB Signaling Pathway)	79268	100 reactions
NF-κB Luciferase Reporter HEK293 Cell Line	60650	2 vials
NF-κB GFP Reporter HEK293 Cell Line	79402	2 vials
NF-κB Luciferase Reporter Lentivirus	79564	500 µl x 2
NF-κB Luciferase Reporter Jurkat Cell Line	60651	2 vials

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