Description

TLR8/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 TLR8 (Toll-like receptor 8) (GenBank Accession No. NM_138636), a member of the toll-like receptor (TLR) family.

This cell line has been validated with C1097, Motolimod, Afimetoran and Enpatoran.

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. TLR8 (toll-like receptor 8), also known as CD288 (cluster of differentiation 288), is expressed mainly in the lung and leukocytes. TLR8 is an endosomal receptor that recognizes single stranded RNA (ssRNA) that is GU-rich. TLR8 is involved in the recognition of ssRNA viruses such as Influenza, where TLR8 binding to the viral RNA recruits MyD88 and leads to activation of the transcription factor NF-κB and an antiviral response. TLR8 has also been linked to cancer, leading to production of inflammatory proteins in dendritic cells. TLR8 agonists such as motolimob have been used as adjuvant therapies in cancer therapy, with the aim of stimulating the immune system. Further studies into the molecular pathways involving TLR8 and the development of new agonists may open new avenues for the treatment of TLR8-related diseases.

Application

Screen for activators or inhibitors of TLR8 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 1W	BPS Bioscience #78854

Materials Required for Cellular Assay

Name	Ordering Information
CL097 (Imidazoquinoline compound)	Invivogen #tlrl-c97
Motolimod	MedChemExpress #HY-13773
Afimetoran	MedChemExpress #HY-139567
Enpatoran hydrochloride	MedChemExpress #HY-134581A
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 1W (BPS Bioscience #78854):

MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin, 200 µg/ml of Geneticin® and 50 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 reach a density of 2.5 x 10^6 cells/ml. Switch to Growth Medium 1W 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 1W 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 1W.

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:5-1:10 twice a week.

Cell Freezing

1. Aspirate the medium, wash the cells with 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 1W 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 assay was 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", "Background Control" and "Unstimulated Control" conditions.
- Assay B should include "Background Control". "No Inhibitor Control", "No Inhibitor, No Agonist Control" and "Test Inhibitor" conditions.

A. Dose Response of TLR8/NF-κB Luciferase Reporter HEK293 Cell Line to a TLR8 agonist

1. Harvest TLR8/NF-κB Luciferase Reporter HEK293 cells from culture in Growth Medium 1W 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 empty wells as cell-free control wells ("Background Control").

2. Incubate at 37°C with 5% CO_2 overnight (~16 hours).

3. Prepare a serial dilution of agonists of interest, such as CL097 and Motolimod, in Assay Medium at 10x the final testing concentrations (10 µl/well).

4. Add 10 µl of diluted agonist 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 the "Background Control" wells (cell-free wells).

7. Incubate at 37°C with 5% CO_2 for 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 Control" 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.

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

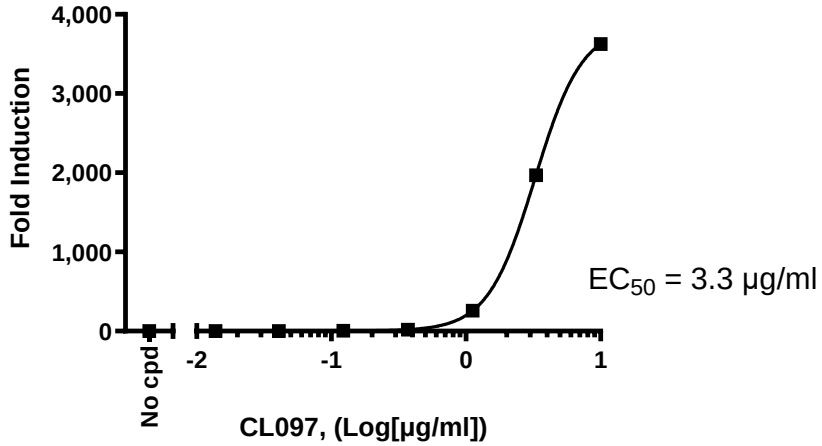

Figure 1: Dose response curve of TLR8/NF-κB Luciferase Reporter HEK293 Cell Line to CL097. TLR8/NF-κB Luciferase Reporter HEK293 cells were treated with increasing concentrations of CL097. Luciferase activity was measured with ONE-Step™ Luciferase Assay System. The results are shown as fold induction of luciferase reporter expression in relation to the activity of cells without treatment (unstimulated control).

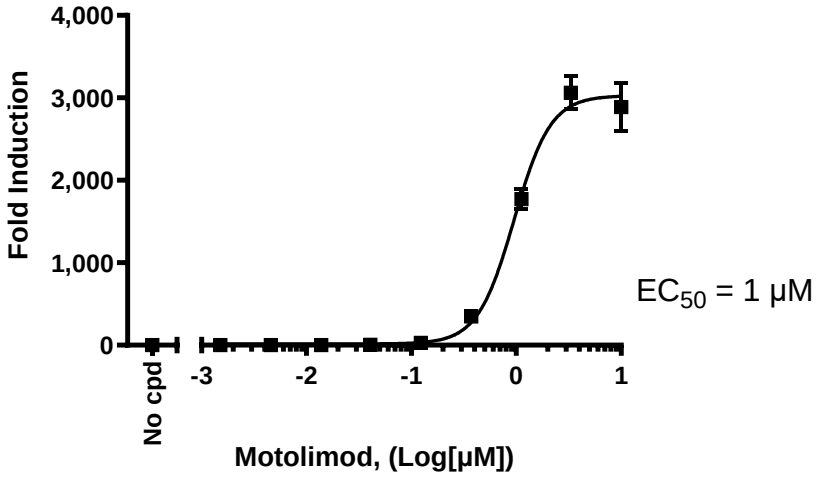

Figure 2: Dose response curve of TLR8/NF-κB Luciferase Reporter HEK293 Cell Line to Motolimod. TLR8/NF-κB Luciferase Reporter HEK293 cells were treated with increasing concentrations of Motolimod. Luciferase activity was measured with ONE-Step™ Luciferase Assay System. The results are shown as fold induction of luciferase reporter expression in relation to the activity of cells without treatment (unstimulated control).

B. Inhibition of CL097-induced reporter activity in the TLR8/NF-κB Luciferase Reporter HEK293 Cell Line

1. Harvest TLR8/NF-κB Luciferase Reporter HEK293 cells from culture in Growth Medium 1W 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 "Background Control" (cell-free control).

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 "Background Control" (for determining background luminescence signal).

7. Incubate at 37°C with 5% CO_2 overnight (~16 hours).

8. The next day, prepare solution of CL097 in Assay Medium at a concentration 11x higher than the final desired concentration: [CL097] = 3 µg/ml (10 µl/well).

9. Add 10 µl of diluted CL097 to the "Test Inhibitor" and "No Inhibitor Control" wells.

10. Add 10 µl of Assay Medium to the "No Inhibitor, 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 Control" 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%.

$$Percent\ Luminescence = \left(\frac{Luminescence\ of\ \ Test\ Inhibitor\ Wells - avg.background}{Avg.Luminescence\ of\ No\ Inhibitor\ Wells - avg.background}\right) x100$$

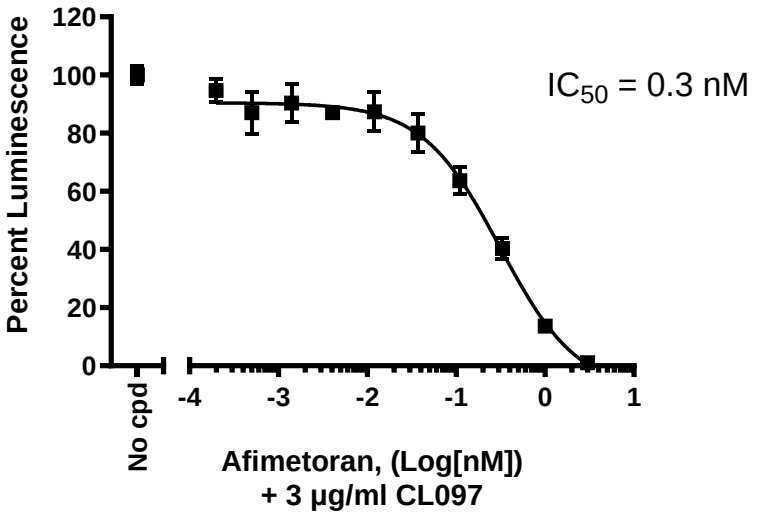

Figure 3: Dose response curve of TLR8/NF-κB Luciferase Reporter HEK293 Cell Line to Afimetoran.
TLR8/NF-κB Luciferase Reporter HEK293 cells were treated with increasing concentrations of Afimetoran overnight before addition of CL097, at a final concentration of 3 µg/ml. Luciferase activity was measured with ONE-Step™ Luciferase Assay System. The results are shown as percent luminescence of luciferase reporter activity (in which CL097-stimulated cells in the absence of inhibitor is set at 100%).

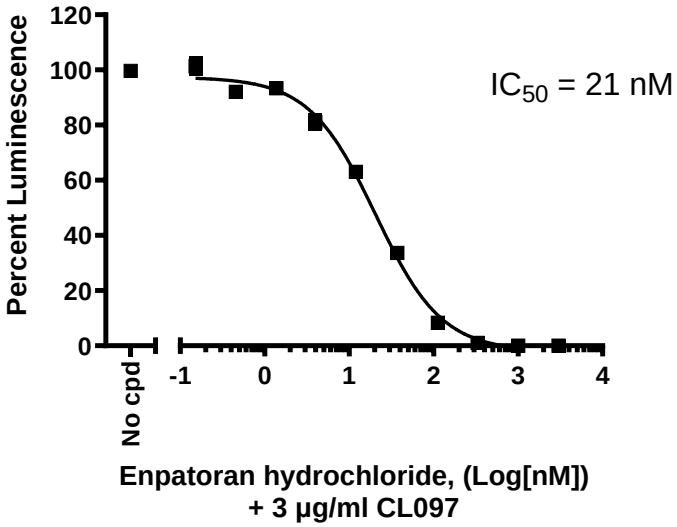

Figure 4: Dose response curve of TLR8/NF-κB Luciferase Reporter HEK293 Cell Line to Enpatoran hydrochloride.
TLR8/NF-κB Luciferase Reporter HEK293 cells were treated with increasing concentrations of Enpatoran hydrochloride overnight before addition of CL097, at a final concentration of 3 µg/ml. Luciferase activity was measured using the ONE-Step™ Luciferase Assay System. The results are shown as percent luminescence of luciferase reporter activity (in which CL097-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

hTLR8 sequence (accession number NM_138636)

MENMFLQSSMLTCIFLLISGSCELCAEENFSRSYPCDEKKQNDSVIAECSNRRLQEVPQTVGKYVTELDLSDNFITHITNESFQGLQ
NLTKINLNHNPNVQHQNGNPGIQSNGLNITDGAFLNLKNLRELLLEDNQLPQIPSGLPESLTELSLIQNNIYNITKEGISRLINLKNLY
LAWNCYFNKVCEKTNIEDGVFETLTNLELLSLSFNSLSHVPPKLPSSLRKLFLSNTQIKYISEEDFKGLINLTLLDLSGNCPRCFNAPFP
CVPCDGGASINIDRFAFQNLTQLRYLNLSSTSLRKINAAWFKNMPHLKVLDLEFNYLVGEIASGAFLTMLPRLEILDLSFNYIKGSYP
QHINISRNFSKLLSLRALHLRGYVFQELREDDFQPLMQLPNLSTINLGINFIKQIDFKLFQNFSNLEIIYLSENRISPLVKDTRQSYANS
SSFQRHIRKRRSTDFEFDPHSNFYHFTRPLIKPQCAAYGKALDLSLNSIFFIGPNQFENLPDIACLNLSANSNAQVLSGTEFSAIPHVK
YLDLTNNRLDFDNASALTELSDLEVLDLSYNSHYFRIAGVTHHLEFIQNFTNLKVLNLSHNNIYTLTDKYNLESKSLVELVFSGNRLDI
LWNDDDNRYISIFKGLKNLTRLDLSLNRLKHIPNEAFLNLPASLTELHINDNMLKFFNWTLLQQFPRLELLDLRGNKLLFLTDSLSDF
TSSLRTLLLSHNRISHLPSGFLSEVSSLKHLDLSSNLLKTINKSALETKTTTKLSMLELHGNPFECTCDIGDFRRWMDEHLNVKIPLVD
VICASPGDQRGKSIVSLELTTCVSDVTAVILFFFTFFITTMVMLAALAHHLFYWDVWFIYNVCLAKVKGYRSLSTSQTFYDAYISYDT
KDASVTDWVINELRYHLEESRDKNVLLCLEERDWDPGLAIIDNLMQSINQSKKTVFVLTKKYAKSWNFKTAFYLALQRLMDENM
DVIIFILLEPVLQHSQYLRLRQRICKSSILQWPDNPKAEGLFWQTLRNVVLTENDSRYNNMYVDSIKQY

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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*
TLR9/ NF-κB Luciferase Reporter HEK293 Cell Line	60685	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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