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Data Sheet

TLR8/ NFκB Reporter – HEK293 Recombinant Cell Line

Catalog #: 60684

Product Description
Recombinant HEK293 cell expressing firefly luciferase gene under the control of NFκB response elements with constitutive expression of human TLR8 (Toll-like receptor 8, also known as CD288, a member of the toll-like receptor (TLR) family (GenBank Accession No. NM_138636).

Background
The TLR family plays a fundamental role in pathogen recognition and activation of innate immunity. TLR8 is an endosomal receptor that recognizes single stranded RNA (ssRNA), and can recognize ssRNA viruses such as Influenza, HIV and HCV. TLR8 binding to the viral RNA recruits MyD88 and leads to activation of the transcription factor NF-κB and an antiviral response. Human TLR8 is functional but murine TLR8 is non-functional because it lacks five amino acids.

Application
- Screen for activators or inhibitors of TLR8 signaling in a cellular context
- Characterize the biological activity of TLR8 and its interactions with ligands

Format
Each vial contains 2 x 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)

Complete Growth Medium: Thaw Medium 1 (BPS Cat. #60187) plus 200 µg/ml of Geneticin (Life Technologies, #11811031) and 50 µg/ml of Hygromycin B (Life Technologies, #10687-010).

Cells should be grown at 37°C with 5% CO_2 using complete growth medium.

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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% CO_2 incubator. After 24 hours of culture, add an additional ~3ml of Thaw Medium 1 without antibiotics. At first passage, switch to complete growth medium **(Thaw Medium 1 containing Geneticin and Hygromycin B)**. Cells should be split before they reach 2.5 x 10^6 cells/ml.

To passage the cells, dilute cell suspension into new culture vessels at no less than 0.2 x 10^6 cells/ml. Subcultivation ratio: 1:5 to 1:10 twice a week.

Materials Required but Not Supplied

- Thaw Medium 1 (BPS Bioscience, #60187)
- CL097 (Imidazoquinoline compound, InvivoGen, Cat # tlr1-c97), 1 mg/ml stock solution in water
- 96-well tissue culture-treated white clear-bottom assay plate
- One-Step luciferase assay system (BPS Bioscience, #60690)
- Luminometer

Assay Protocol

1. Harvest TLR8/NFkB reporter-HEK293 cells from culture in growth medium 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 CO_2 incubator.

2. 24 hours after seeding, Dilute the CL097 ligand in Thaw Medium 1.

3. Add 10 µl of diluted CL097 to the treated wells.
   Add 10 µl Thaw Medium 1 to control wells.
   Add 100 µl of Thaw Medium 1 to cell-free control wells (for determining background luminescence)
   Set up each treatment in at least triplicate

4. Incubate the plate at 37°C in a CO_2 incubator for ~ 6 hours.

5. Perform luciferase assay by using the ONE-Step luciferase assay system: Briefly, add 100 µl of One-Step Luciferase reagent per well and rock at room temperature for ~30 minutes. Measure luminescence using a luminometer. (*If using luciferase reagents from other vendors, follow the manufacturer's assay protocol.)*

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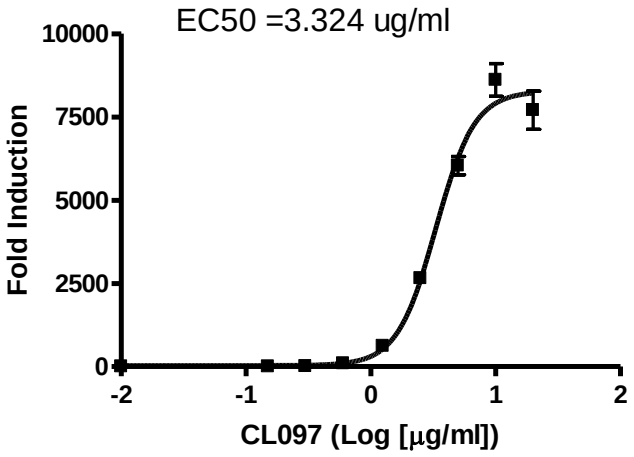
6. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells.
   The fold induction of NF-κB luciferase reporter expression = background-subtracted luminescence of stimulated well / average background-subtracted luminescence of unstimulated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

Figure 1. Dose Response of TLR8/NF-κB Reporter HEK293 cells

The results are shown as fold induction of NF-κB luciferase reporter expression. The EC50 of CL097 is 3.324 µg/ml



Sequence

hTLR8 sequence (accession number: NM_138636)

MENMFLQSSMLTCIFLLISGSCELCAEENFSRSYPCDEKKQNDSVIAECSNRRLQEVPQTVGKYVTELDL
SDNFITHITNESFQGLQNLTKINLNHNPNVQHQNGNPGIQSNGLNITDGAFLNLKNLRELLLEDNQLPQIPS
GLPESLTELSLIQNNIYNITKEGISRLINLKNLYLAWNCYFNKVCEKTNIEDGVFETLTNLELLSLSFNSLSHV
PPKLPSSLRKLFLSNTQIKYISEEDFKGLINLTLLDLSGNCPRCFNAPFPCVPCDGGASINIDRFAFQNLTQL
RYLNLSSTSLRKINAAWFKNMPHLKVLDLEFNYLVGEIASGAFLTMLPRLEILDLSFNYIKGSYPQHINISRN
FSKLLSLRALHLRGYVFQELREDDFQPLMQLPNLSTINLGINFIKQIDFKLFQNFSNLEIIYLSENRISPLVKD
TRQSYANSSSFQRHIRKRRSTDFEFDPHSNFYHFTRPLIKPQCAAYGKALDLSLNSIFFIGPNQFENLPDIA
CLNLSANSNAQVLSGTEFSAIPHVKYLDLTNNRLDFDNASALTELSDLEVLDLSYNSHYFRIAGVTHHLEFI
QNFTNLKVLNLSHNNIYTLTDKYNLESKSLVELVFSGNRLDILWNDDDNRYISIFKGLKNLTRLDLSLNRLK
HIPNEAFLNLPASLTELHINDNMLKFFNWTLLQQFPRLELLDLRGNKLLFLTDSLSDFTSSLRTLLLSHNRIS

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HLPSGFLSEVSSLKHLDLSSNLLKTINKSALETKTTTKLSMLELHGNPFECTCDIGDFRRWMDEHLNVKIPL
VDVICASPGDQRGKSIVSLELTTCVSDVTAVILFFFTFFITTMVMLAALAHHLFYWDVWFIYNVCLAKVKGY
RSLSTSQTFYDAYISYDTKDASVTDWVINELRYHLEESRDKNVLLCLEERDWDPGLAIIDNLMQSINQSKK
TVFVLTKKYAKSWNFKTAFYLALQRLMDENMDVIIFILLEPVLQHSQYLRLRQRICKSSILQWPDNPKAEGL
FWQTLRNVVLTENDSRYNNMYVDSIKQY

Related Products

Product	**Cat. #**	**Size**
NF-κB reporter (Luc)-HEK293 Recombinant cell line	60650	2 vials
TLR9/ NF-κB Reporter – HEK293 Recombinant Cell Line	60685	2 vials
ONE-Step™ Luciferase Assay System	60690-1	10 ml
ONE-Step™ Luciferase Assay System	60690-2	100 ml
Thaw Medium 1	60187	100 ml
NF-κB Reporter Kit (NF-κB Signaling Pathway)	60614	500 rxns.

Notes

License Disclosure: Purchase of this cell line grants you with a 10-year license to use this cell line in your immediate laboratory, for research use only. This license does not permit you to share, distribute, sell, sublicense, or otherwise make the cell line available for use to other laboratories, departments, research institutions, hospitals, universities, or biotech companies. The license does not permit the use of this cell line in humans or for therapeutic or drug use. The license does not permit modification of the cell line in any way. Inappropriate use or distribution of this cell line will result in revocation of the license and result in an immediate cease of sales and distribution of BPS products to your laboratory. BPS does not warrant the suitability of the cell line for any particular use, and does not accept any liability in connection with the handling or use of the cell line. Modifications of this cell line, transfer to another facility, or commercial use of the cells may require a separate license and additional fees; contact sales@bpsbioscience.com for details. Publications using this cell line should reference BPS Bioscience, Inc., San Diego.