Description

The TLR7 (Toll-like receptor 7)/NF-κB Luciferase Reporter HEK293 Cell Line is a HEK293 cell line that expresses the Firefly luciferase reporter under the control of NF-κB (nuclear factor κB) response element, human TLR7 (Toll-like receptor 7) (NM_016562.4), and a mutated version of the chaperone protein UNC93B1 (inc-93 homolog B1) (NM_030930.4), to obtain a fully active NF-κB signaling pathway upon TLR7 stimulation.

This cell line has been validated with CL307, R848, KT474, Zimlovisertib and Edecesertib.

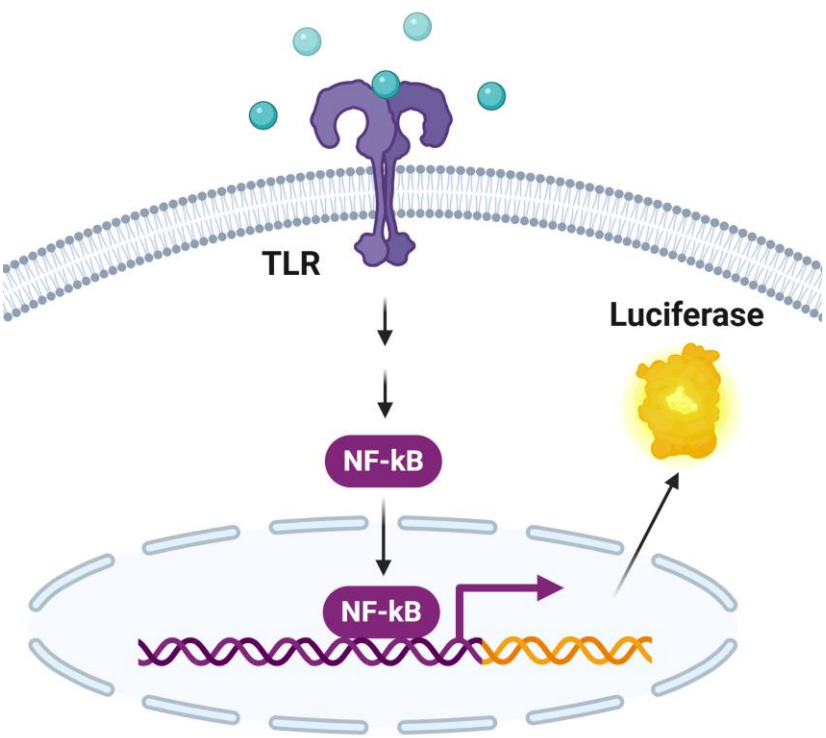

Figure 1: Schematic illustration of the mechanism of response of TLR7 (Toll-like receptor 7)/NF-κB Luciferase Reporter HEK293 Cell Line.

Background

The family of Toll-like receptors (TLRs) are cell surface receptors that detect a wide variety of microbial components and elicit innate immune responses. Toll-like receptor 7 (TLR7), however, is primarily located in endosomes. TLR7 signaling supports human systemic autoimmune diseases, such as systemic lupus erythematosus (SLE). Patients with SLE display phenotypes that are consistent with increased TLR7 signaling associated with elevated IgD−CD27 double-negative B cells. The TLR7 signaling pathway starts with the binding of ligands like single-stranded RNA from viruses, and the receptor is transported to lysosome-related organelles, a process that involves chaperone proteins like UNC93B1. Adapters such as MyD88 recruit proteins that eventually activate the transcription factors NK-κB (nuclear factor κB) and IRF7 (Interferon Regulatory Factor 7). Signaling through Toll-like receptor 7 (TLR7) drives the production of type I IFN (interferon) and promotes the activation of auto-reactive B cells. TLR7 inhibition has been proposed as a therapeutic approach for SLE and other autoimmune diseases.

Application

Screen for activators or inhibitors of the TLR7 signaling pathway.

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 19A	BPS Bioscience #83825

Materials Used in Cellular Assay

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187
APC anti-human TLR7 Antibody	BioLegend#376905
CL307	InvivoGen # tlrl-c307
R848	Selleckchem# S8133
KT474	Selleckchem# E1655
Zimlovisertib	BPS Bioscience #83851
Edecesertib	BPS Bioscience #83852
Clear-bottom, white 96-well tissue culture-treated 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℃ 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 and Functional Cellular Assay

Thaw Medium 1 (BPS Bioscience #60187):

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

Growth Medium 19A (BPS Bioscience #83825):

MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin, 50 µg/ml Hygromycin, 0.5 µg/ml puromycin, 5 µg/ml blasticidin.

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 contents 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 or T75 flask and incubate at 37°C in a 5% CO_2 incubator.

4. After 24 hours of culture, check for cell attachment and viability. Change medium to fresh Thaw Medium 1 and continue growing in a 5% CO_2 incubator at 37°C until the cells are ready to passage.

5. Cells should be passaged before they are fully confluent. At first passage and subsequent passages, use Growth Medium 19A.

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 19A 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 19A.

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:5 to 1:10 once or twice per 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 19A 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

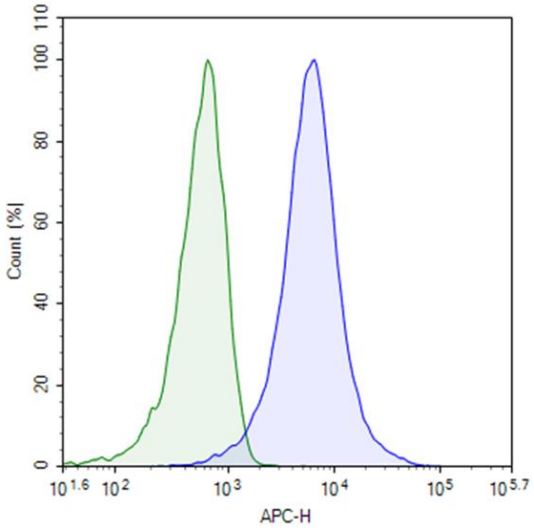

Figure 1: Expression of TLR7 in the TLR7 (Toll-like receptor 7)/NF-κB Luciferase Reporter HEK293 Cell Line.
TLR7 (Toll-like receptor 7)/NF-κB Luciferase Reporter HEK293 cells (blue) and HEK293 cells (green) were stained with APC anti-human TLR7 Antibody (BioLegend #376905) and analyzed by flow cytometry. The y axis represents the % of cells, while the x axis indicates APC intensity.

Functional Validation

- 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", "Background Control" and "Unstimulated Control" conditions.
- Assay B should include "Background Control", "No Inhibitor, Agonist Control", "No Inhibitor, No Agonist Control" and "Test Inhibitor, Agonist" conditions.

A. Dose-dependent Response of TLR7 (Toll-like receptor 7)/NF-κB Luciferase Reporter HEK293 Cell Line to a TLR7 agonist

1. Seed cells at a density of 20,000 cells per well in 100 µl of Assay Medium into a white clear-bottom 96-well cell culture plate. Leave a few wells empty to use as the "Background Control" (Cell-Free Control).

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

3. Prepare a serial dilution of CL307/R848 in Assay Medium at the testing concentrations (100 µl/well).

4. Carefully remove the medium from all wells.

5. Add 100 µl of Assay Medium containing the test compounds to the "Stimulated" wells.

6. Add 100 µl of Assay Medium to the "Unstimulated Control" wells (to determine the luminescence from the wells).

7. Add 100 µl of Assay Medium to the "Background Control" (cell-free control wells).

8. Incubate the plate at 37°C with 5% CO_2 for 4-5 hours.

9. Add 100 µl of ONE-Step™ Luciferase reagent to each well and rock at Room Temperature (RT) for ~15 to 30 minutes.

10. Measure luminescence using a luminometer.

11. 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.

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

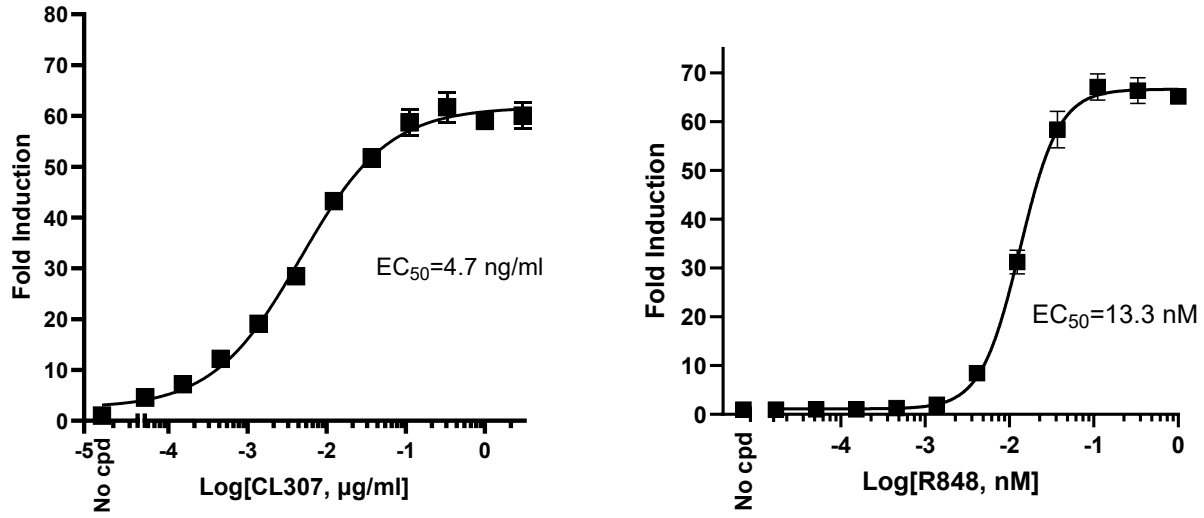

Figure 2: Dose response curve of TLR7 (Toll-like receptor 7)/NF-κB Luciferase Reporter HEK293 Cell Line to CL307 and R848.
TLR7/NF-κB Luciferase Reporter HEK293 Cells were incubated with increasing concentrations of human CL307 (left) and R848 (right) for 4 hours. Luciferase activity was measured using the ONE-Step™ Luciferase Assay System (#60690). The results are shown as fold induction of luciferase reporter expression in relation to the activity of unstimulated control.

B. Inhibition of CL307/R848-induced reporter activity in the TLR7 (Toll-like receptor 7)/NF-κB Luciferase Reporter HEK293 Cell Line

1. Seed cells at a density of 20,000 cells per well in 100 µl of Assay Medium into a white clear-bottom 96-well cell culture plate. Leave a few wells empty to use as the "Background Control ".
2. Incubate the cells at 37°C with 5% CO_2 overnight (~16 hours).
3. Prepare three-fold serial dilutions of inhibitor compounds in Assay Medium (90 µl/well).
4. Carefully remove the cell culture medium from the cells.
5. Add 90 µl of each dilution to the "Test Inhibitor, Agonist" wells.
6. Add 90 µl of Assay Medium to the "No Inhibitor, Agonist Control", "No Inhibitor, No Agonist Control" wells.
7. Add 100 µl of Assay Medium the the "Background Control" wells.
8. Incubate the cells at 37°C in 5% CO_2 for 24 hours if testing KT474, or for 3-4 hours if testing Zimlovisertib/Edecesertib.
9. Prepare a 10x solution of agonist CL307 (20 ng/ml) or R848 (100 nM) in Assay Medium (their final concentrations will be 2 ng/ml and 10 nM, respectively).

10. Add 10 µl of agonist to the "Test Inhibitor, Agonist" and "No Inhibitor, Agonist Control" wells.

11. Add 10 µl of Assay Medium to the "No Inhibitor, No Agonist Control" wells.

12. Incubate at 37°C in 5% CO_2 for 4-5 hours.

13. Add 100 µl/well of ONE-Step™ Luciferase Assay reagent.

14. Incubate with gentle agitation at RT for ~15 to 30 minutes.

15. Measure luminescence using a luminometer.

16. Data Analysis: Subtract the average background luminescence from the luminescence reading of all other wells. The percent luminescence of luciferase reporter activity is the background-subtracted luminescence of treated wells divided by the average background-subtracted luminescence of the "No Inhibitor, Agonist Control" (untreated control) wells x 100%.

$$Percent\ Luminescence = \left(\frac{luminescence\ of\ inhibitor\ \ treated\ cells - avg.background}{luminescence\ of\ \text{No Inhibitor Control}\ cells - avg.background}\right) \text{x}100$$

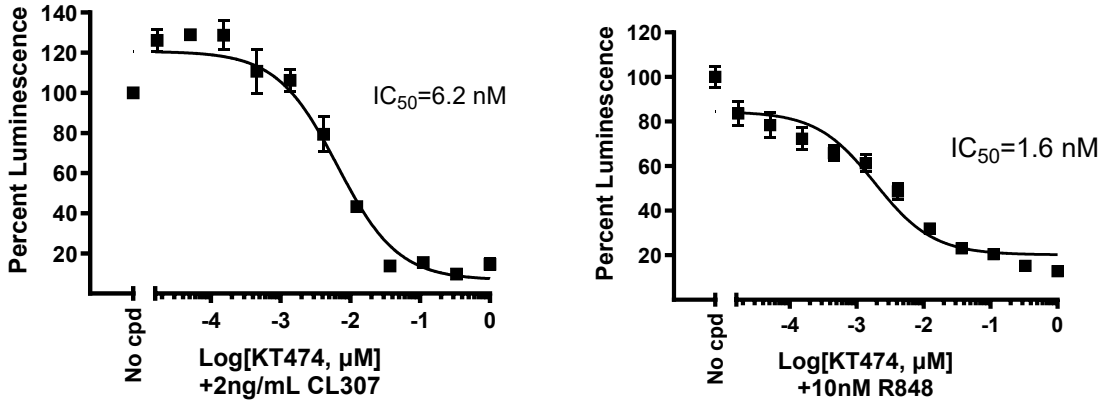

Figure 3. Dose response curve of TLR7 (Toll-like receptor 7)/NF-κB Luciferase Reporter HEK293 Cell Line to KT474.
Cells were incubated with increasing concentrations of KT474 for 24 hours, then with 2 ng/ml CL307 (Left) or 10 nM R848 (Right) for 4 hours. Luciferase activity was measured using One-Step™ Luciferase Assay System. Results are shown as percentage of luciferase reporter activity (in which agonist-stimulated cells in the absence of inhibitor were set at 100%).

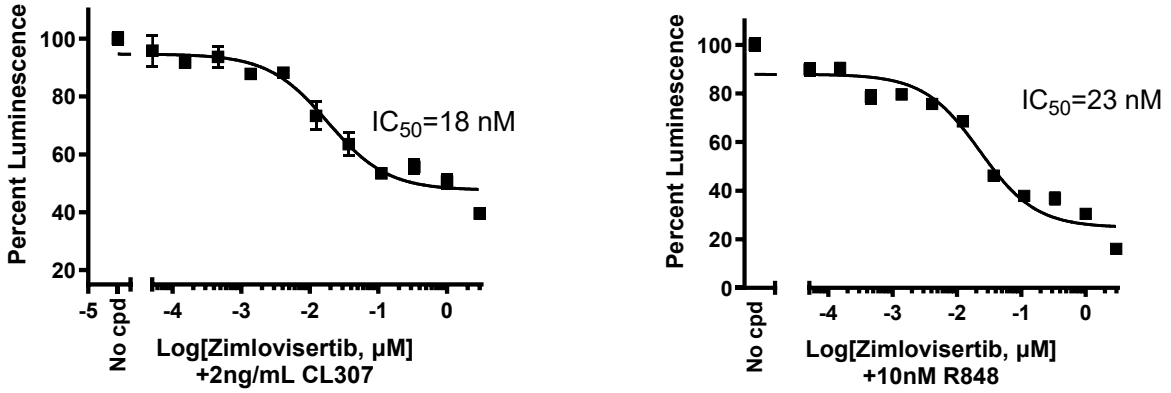

Figure 4. Dose response curve of TLR7 (Toll-like receptor 7)/NF-κB Luciferase Reporter HEK293 Cell Line to Zimlovisertib.

Cells were incubated with increasing concentrations of Zimlovisertib (#83851) for 4 hours, then with 2 ng/ml CL307 (Left) or 10 nM R848 (Right) for 4 hours. Luciferase activity was measured using One-Step™ Luciferase Assay System. Results are shown as percentage of luciferase reporter activity (in which agonist-stimulated cells in the absence of inhibitor is set at 100%).

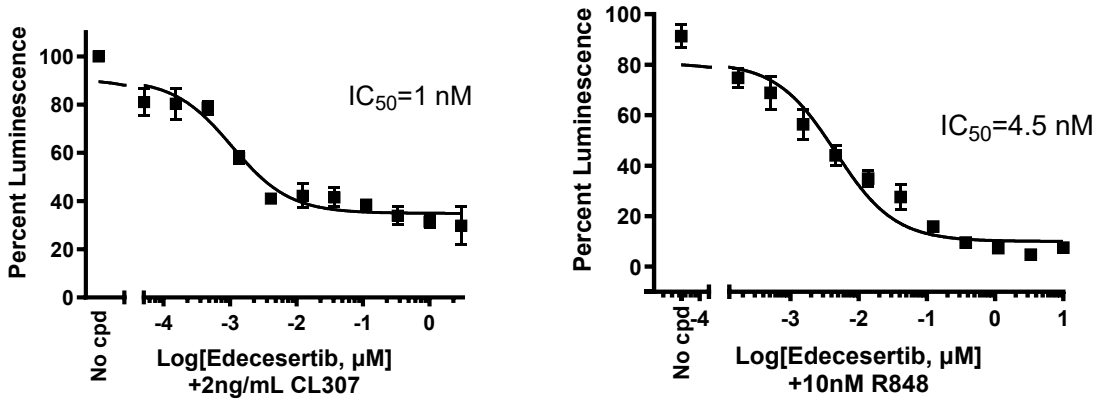

Figure 5. Dose response curve of TLR7 (Toll-like receptor 7)/NF-κB Luciferase Reporter HEK293 Cell Line to Edecesertib.

Cells were incubated with increasing concentrations of Edecesertib (#83752) for 4 hours, then with 2 ng/ml CL307 (Left) or 10 nM R848 (Right) for 4 hours. Luciferase activity was measured using One-Step™ Luciferase Assay System. Results are shown as percentage of luciferase reporter activity (in which agonist-stimulated cells in the absence of inhibitor are set at 100%).

Data shown is representative.

References

Brown G.J., *et al*., 2022 *Nature* 605: 349–356.
Jenks S.A., *et al*., 2018 *Immunity* 49(4):725-739.e6.
Majer. O., *et al.,* 2019 *Nature* 575: 366–370.

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Troubleshooting Guide
Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For all further questions, please visit https://bpsbioscience.com/contact.

Related Products

Products	*Catalog #*	*Size*
TLR8/NF-κB Reporter–HEK293 Recombinant Cell Line	60684	2 Vials
TLR9/NF-κB Reporter–HEK293 Recombinant Cell Line	60685	2 Vials

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