Description

The Notch1/CSL Luciferase Reporter HEK293 Cell Line is a HEK293 cell line that expresses the firefly luciferase reporter under the control of Notch-response elements (CSL responsive elements) and expression of Notch1ΔE (Notch1 that has a deletion of the entire extracellular domain). Inside the cells, the Notch1ΔE can be cleaved by γ-secretase. This active Notch1 NICD (Notch intracellular domain) translocated to the nucleus and induces the expression of the luciferase reporter. The cell line is validated for the inhibition of the expression of luciferase reporter using a known inhibitor of the Notch signaling pathway.

Background

The Notch signaling pathway controls cell fate decisions in vertebrates and invertebrates' tissues and is involved in embryonic development, tissue homeostasis, and regulation of the immune and angiogenic systems. Notch signaling is triggered through the binding of a transmembrane ligand, present in opposing cells, to one of the four existing Notch transmembrane receptors (Notch1/Notch2/Notch3/Notch4). This results in proteolytic cleavage of the Notch receptor, releasing the constitutively active intracellular domain of the Notch receptor (NICD). NICD translocate to the nucleus and associates with the transcription factor CSL (CBF1/RBPJκ/Suppressor of Hairless/Lag-1) and coactivator Mastermind to turn on the transcription of Notch-responsive genes. Dysfunction of Notch signaling has severe consequences, including developmental pathologies or cancer (such as T cell acute lymphoblastic leukemia, T-ALL, and urothelial bladder cancer). The use of Notch inhibitors, mainly gammasecretase inhibitors, as a cancer therapy option and in the regeneration of tissues is ongoing. Further studies will allow us to have a deeper understanding of Notch signaling and will benefit future therapeutic approaches.

Application(s)

- Monitor Notch signaling pathway activity.
- Screen for inhibitors of the Notch signaling pathway 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 Assays

Name	Ordering Information
DAPT	Selleckchem #S2215
CSL Reporter HEK293 Cell Line	BPS Bioscience #79754
Thaw Medium 1	BPS Bioscience #60187
96-well tissue culture-treated white clear-bottom assay plate	
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, 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 plus 100 µg/ml Hygromycin B and 400 µg/ml Geneticin®, G418 Sulfate.

Cell Culture Protocol

Cell Thawing

1. Retrieve a cell vial from liquid nitrogen storage. Keep on dry ice until ready to thaw.

2. When ready to thaw, swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. Once cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire content of the vial to an empty 50 ml conical tube.
Note: Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.

3. Using a 10 ml serological pipette, slowly add 10 ml of pre-warmed Thaw Medium 1 to the conical tube containing the cells. Thaw Medium 1 should be added dropwise while gently rocking the conical tube to permit gentle mixing and avoid osmotic shock.

4. 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.

5. Transfer the resuspended cells to a T25 flask or T75 flask and incubate at 37°C in a 5% CO_2 incubator.

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

7. Cells should be passaged before they are fully confluent. At first passage and subsequent passages, use Growth Medium 1A.

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 recommend sub-cultivation ratio of 1:10 to 1:20 weekly 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 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.

A. Validation Data

The following assay was designed for 96-well format. To perform the assay in a different format, the cell number and reagent volume should be scaled appropriately. This protocol is a general guideline.

- The assay should be performed in triplicate.
- This assay should include "Cell-Free Control" wells for determining background luminescence.
- CSL Reporter HEK293 Cell Line should be used as negative control.

Assay Medium: Thaw Medium 1.

A. Inhibition of Notch reporter activity by the inhibitor of Notch signaling pathway DAPT in the Notch1/CSL Luciferase Reporter HEK293 Cell Line.

1. Seed 3.5 x 10^4 cells in 90 µl of Assay Medim into each well of a white clear-bottom 96-well microplate. Leave a couple of wells empty for use as the cell-free control.

2. Prepare serial dilution of inhibitor in Assay Medium (10 µl/ well).

   3.1 For inhibitors soluble in Assay Medium, prepare a serial dilution in Assay Medium at 10x the desired concentrations.

   Assay Medium is the Diluent Solution.

   3.2 For inhibitors soluble in DMSO, such as DAPT, prepare a stock solution in 100% DMSO at a concentration 2,000x higher than the highest desired final concentration, then dilute it 200-fold with Assay Medium to prepare the highest concentration of the serial dilution. The concentration of DMSO is now 0.5% DMSO.

   Prepare a serial dilution at 10x the desired final concentrations using Assay Medium containing 0.5% DMSO.

   For controls use Assay Medium with 0.5% DMSO (Diluent Solution).

   Note: The concentration of DMSO should not exceed 0.5% in the final reaction.

3. Add 10 µl of diluted inhibitor to the Notch1/CSL Luciferase Reporter HEK293 cells.

4. Add 100 µl of Assay Medium to the cell-free control wells (for determining background luminescence).

5. Incubate the plate at 37°C in a CO_2 incubator for 24 hours.

6. Add 100 µl of the ONE-Step™ Luciferase reagent per well.

7. Rock gently at Room Temperature for ~15 minutes.

8. Measure luminescence using a luminometer.

9. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells. The fold induction of CSL luciferase reporter expression is the average background-subtracted luminescence of treated well divided by the average background-subtracted luminescence of untreated control wells.

$$Fold\ induction = \frac{(luminescence\ of\ stimulated\ cells - average\ background)}{(average\ luminescence\ of\ unstimulated\ cells - average\ background)}$$

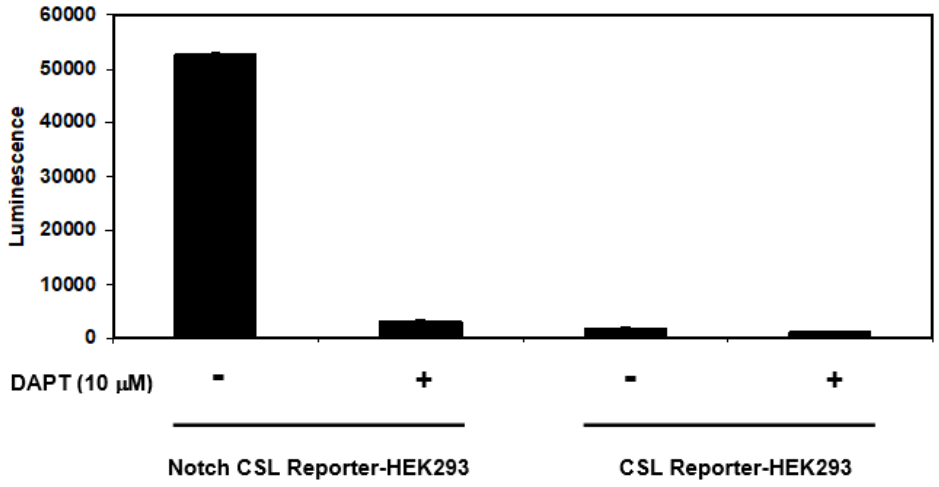

Figure 1. Response of Notch1/CSL Luciferase Reporter HEK293 Cell Line to DAPT, a Notch pathway inhibitor.
DAPT inhibits luciferase expression in Notch1/CSL Luciferase Reporter HEK293 cells. CSL Reporter HEK293 cells were used as control. CSL Reporter HEK293 Cell Line contains the firefly luciferase reporter under the control of Notch-response elements (CSL-responsive elements) but no expression of Notch1ΔE, so it is unable to express the Notch luciferase reporter. Activity was measured with ONE-Step™ Luciferase Assay System.

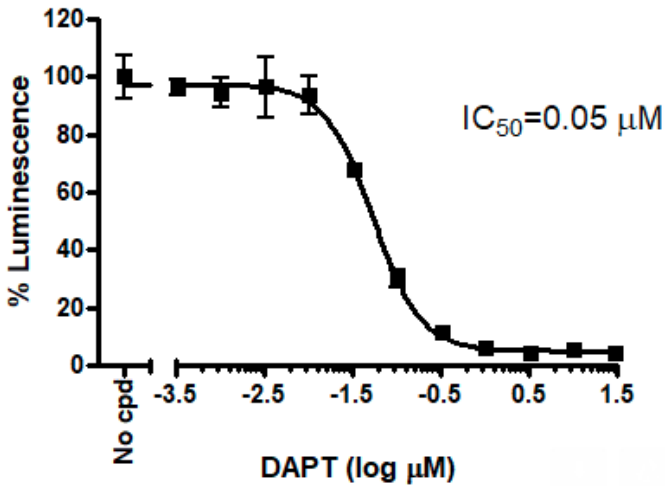

Figure 2. Dose response curve of Notch1/CSL Luciferase Reporter HEK293 Cell Line to DAPT, a Notch pathway inhibitor.
Notch1/CSL Luciferase Reporter HEK293 cells were incubated with increasing concentrations of DAPT. Activity was measured with ONE-Step™ Luciferase Assay System. The results were shown as percentage of luminescence. The background-subtracted luminescence of cells in the absence of DAPT was set at 100%.

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

References

1. Lu F.M., *et al.*, 1996 *Proc. Natl. Acad. Sci. USA* 93(11): 5663-5667.
2. Kanungo J., *et al.*, 2008 *J. Neurochem.* 106: 2236-48.
3. Cao L. *et al.*, 2023 *Blood Adv.* 10.1182/bloodadvances.2023010380

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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*
Notch1dE Lentivirus	78747	500 µl x 2
CSL (CBF1/RBP-Jk) Luciferase Reporter Lentivirus (Notch Signaling Pathway)	78746	500 µl x 2
Negative Control Luciferase Lentivirus	79578	500 µl x 2
Notch1 Pathway Reporter Kit (Human)	79503	500 reactions