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siehe unsere Liefer- und Versandbedingungen

Zuschläge

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- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Full Length Notch1/CSL Luciferase Reporter HEK293 Cell Line

#82201

Description

The Full Length 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 full-length Notch1 (accession number: NM_017617.5). Upon ligand-receptor interaction, the Notch1 receptor is cleaved by γ-secretase. This active Notch1 NICD (Notch intracellular domain) translocate to the nucleus and induces the expression of the luciferase reporter.

The cell line was functionally validated for both activation and inhibition of the expression of luciferase reporter with DAPT and DLL4.

Background

The Notch signaling pathway is highly evolutionarily conserved and plays an important role in cancer as a key driver in maintaining cancer stemness and inducing tumor angiogenesis. Mammals possess four different single transmembrane notch receptors, Notch1-4. Two families of Notch ligands of transmembrane proteins are Delta-like ligand (DLL1, DLL3, and DLL4) and Jagged (JAG1 and JAG2). The Notch signaling pathway is initiated by ligand-receptor binding, which undergoes a two-step proteolytic cleavage by the ADAM (A disintegrin and metalloproteinase with thrombospondin motifs) family proteases and gamma-secretases. The released Notch intracellular domain (NICD) translocates to the nucleus and then interacts with the transcription complex with DNA-binding transcription factors RBP-J (CSL), mastermind-like (MAML) protein, and other coactivator proteins to stimulate expression of Notch target genes. The Notch signaling pathway can be blocked by two major classes of Notch inhibitors: small-molecule gamma-secretase inhibitors and monoclonal antibodies that specifically bind Notch ligands or receptors. Notch signaling participates in the development and homeostasis of multiple tissues and organs. 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 gamma-secretase 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)

- Screen and validate antibodies against Notch1 for drug discovery and immunotherapy research.
- Screen for small molecule inhibitors of the Notch signaling pathway in a cellular model.
- Perform ligand/receptor binding assays to screen for potential Notch1 pathway inhibitors.

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
DLL4, Fc Fusion, Avi-Tag, Biotin-Labeled Recombinant	BPS Bioscience #101904
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 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 passed 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 recommended sub-cultivation ratio of 1:10 to 1:20 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 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

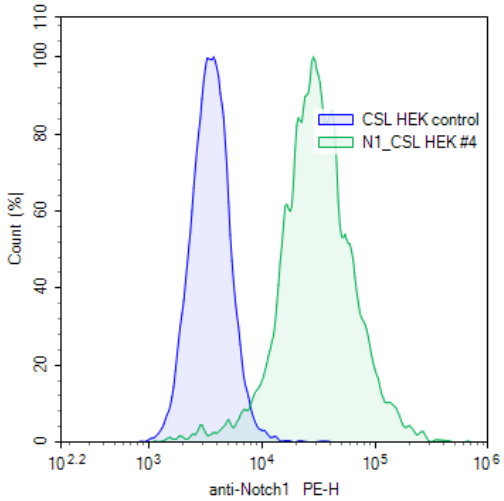

Figure 1: Flow cytometry analysis of the expression of Notch1 in Full Length Notch1/CSL Luciferase Reporter HEK293 Cell Line.
Full Length Notch1/CSL Luciferase Reporter HEK293 cells (green) and CSL Reporter HEK293 control cells (blue) were stained with PE-conjugated anti-human Notch 1 Antibody (Biolegend #352106) and analyzed by flow cytometry. Y-axis represents the % cell number. X-axis indicates the intensity of PE.

Functional Validation

- The following assays were 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", "Unstimulated Control" and "Stimulated" conditions.
- We recommend using the CSL Reporter HEK293 Cell Line as a negative control.

Assay Medium: Thaw Medium 1.

A. Activation of Notch reporter activity with DLL4 peptide, a Notch signaling ligand, in Full Length Notch1/CSL-Luciferase Reporter HEK293 Cell Line

1. Prepare a serial dilution of DLL4 ligand in PBS buffer (50 µl/well).
2. Add 50 µl of diluted DLL4 into each "Stimulated" well of a white clear-bottom 96-well microplate.
3. Add 50 µl of PBS to the "Unstimulated Control" wells.
4. Incubate the plate at 4°C overnight in the dark.
5. Wash the plate three times with PBS buffer.
6. Add 3.5 x 10^4 Full Length Notch1/CSL Luciferase Reporter HEK293 cells in 100 µl of Assay Medium into the "Stimulated" and "Unstimulated Control" wells.
7. Add 100 µl of Assay Medium to the "Cell-Free Control" wells (for determining background luminescence).
8. Incubate the plate at 37°C in a CO_2 incubator for 24 hours.
9. Add 100 µl of the ONE-Step™ Luciferase reagent per well.
10. Rock gently at Room Temperature for ~15 minutes.
11. Measure luminescence using a luminometer.
12. 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 unstimulated control wells.

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

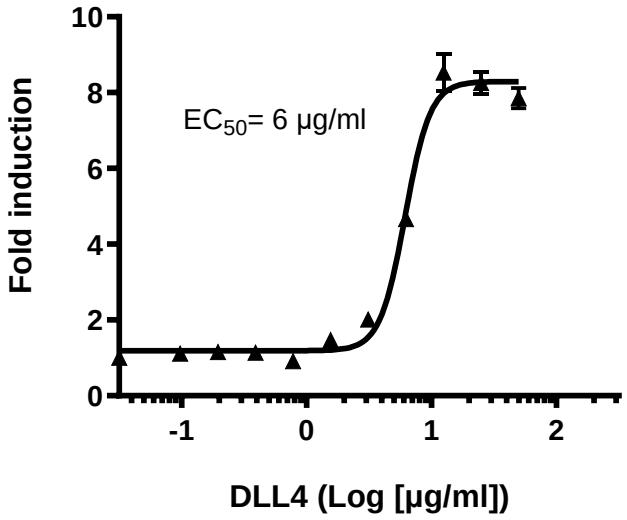

Figure 2. Response of Full Length Notch1/CSL Luciferase Reporter HEK293 Cell Line to DLL4, a Notch pathway activator.
Plate-coated DLL4 was incubated with Full Length Notch1/CSL Luciferase Reporter HEK293 cells. Luciferase activity was measured using ONE-Step™ Luciferase Assay System. The results are shown as fold induction.

B. Inhibition of Notch reporter activity with DAPT, an inhibitor of the Notch signaling pathway, in the Full Length Notch1/CSL Luciferase Reporter HEK293 Cell Line.

1. Prepare an 8 µg/ml DLL4 solution in PBS buffer (50 µl/well).
2. Add 50 µl of DLL4 solution into the "Stimulated" wells of a white clear-bottom 96-well microplate (DLL4 coated plate).
3. Add 50 µl of PBS in the "Unstimulated Control" wells.
4. Incubate the plate at 4°C in the dark overnight.
5. Prepare a 10x serial dilution of the inhibitor DAPT in Assay Medium (10 µl/well).
6. Seed 3.5 x 10^4 Full Length Notch1/CSL-Luciferase Reporter HEK293 cells in 90 µl of Assay Medium into each well of a non-tissue treated plate. Leave a couple of wells empty for use as the "Cell-Free Control".
7. Add 10 µl of diluted inhibitor DAPT to the wells containing Full Length Notch1/CSL Luciferase Reporter HEK293 cells.
8. Add 100 µl of Assay Medium to the "Cell-Free Control" wells.
9. Incubate the plate at 37°C in a 5% CO_2 incubator for 1 hour.
10. Wash the DLL4 coated plate three times with PBS buffer.

11. Transfer the preincubated cells to the corresponding wells on the DLL4 coated plate.
12. Incubate the plate at 37°C in a 5% CO_2 incubator for 28 hours.
13. Add 100 µl of the ONE-Step™ Luciferase reagent per well.
14. Rock gently at Room Temperature for ~15 minutes.
15. Measure luminescence using a luminometer.
16. Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells.

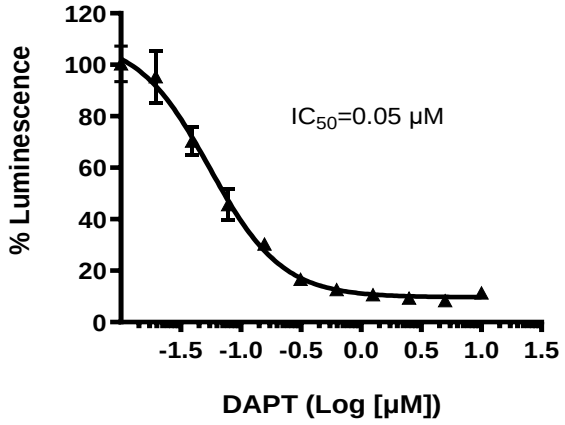

Figure 3. Dose response curve of Full Length Notch1/CSL Luciferase Reporter HEK293 Cell Line to DAPT, a Notch pathway inhibitor.
Increasing concentrations of DAPT were incubated with Full Length Notch1/CSL Luciferase Reporter HEK293 cells. Cells were then added to DLL4 coated plates. Luciferase activity was measured using 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*.*

Sequence

Human Notch1 sequence (accession number: NM_017617.5)

MPPLLAPLLCLALLPALAARGPRCSQPGETCLNGGKCEAANGTEACVCGGAFVGPRCQDPNPCLSTPCKNAGTCHVVDRRGVA
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References

Lu F.M., *et al.*, 1996 *Proc. Natl. Acad. Sci. USA* 93(11): 5663-5667.
Kanungo J., *et al.*, 2008 *J. Neurochem.* 106: 2236-48.
Cao L. *et al.*, 2023 *Blood Adv.* 10.1182/bloodadvances.2023010380
Zhou B., *et al.*, 2022 *Nature* 7:95
Benedito R., *et al*., 2009 *Cell*.12;137(6):1124-35.

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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
Notch1/CSL Luciferase Reporter HEK293 Cell Line	60652	2 vials
CSL (CBF1/RBP-Jk) Luciferase Reporter Lentivirus (Notch Signaling Pathway)	78746	500 µl x 2
DLL4, Fc Fusion, Avi-Tag, Biotin-Labeled Recombinant	101904	10 µg/50 µg
Negative Control Luciferase Lentivirus	79578	500 µl x 2
Notch1 Pathway Reporter Kit (Human)	79503	500 reactions

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