Notch Signaling Pathway
Notch CSL Reporter – HEK293 Cell line
Catalog #: 60652

Background

The Notch signaling pathway controls cell fate decisions in vertebrate and invertebrate tissues. Notch signaling is triggered through the binding of a transmembrane ligand to Notch transmembrane receptor (NOTCH1/ NOTCH2/NOTCH3/NOTCH4) on a neighboring cell. This results in proteolytic cleavage of the Notch receptor, releasing the constitutively active intracellular domain of NOTCH (NICD). NICD translocates to the nucleus and associates with transcription factors CSL (CBF1/RBPJκ/Suppressor of Hairless/Lag-1) and coactivator Mastermind to turn on transcription of Notch-responsive genes.

Description

The Notch CSL Reporter – HEK293 cell line contains the firefly luciferase gene under the control of Notch-response elements (CSL responsive elements) alone with expression construct for Notch1ΔE (NOTCH1 that has a deletion of the entire extracellular domain) stably integrated into HEK293 cells. Inside the cells, the Notch1ΔE can be cleaved by γ-secretase. The active Notch1 NICD is released to nucleus and induces the constitutive expression of 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.

Application

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

Format

Each vial contains ~1.5 X 10^6 cells in 1 ml of 10% DMSO.

Storage

Immediately upon receipt, store cells in liquid nitrogen.

General culture conditions

Cells should be grown at 37°C with 5% CO_2 using 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), plus 400 µg/ml of Geneticin (invitrogen #11811031) and 100 µg/ml of

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Hygromycin B (Hyclone #SV30070.01). It may be necessary to adjust the percentage of CO_2 in the incubator depending on the $NaHCO_3$ level in the basal medium.

It is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, transfer to a tube containing 10 ml of growth medium without Geneticin and Hygromycin, spin down cells, and resuspend cells in pre-warmed growth medium without Geneticin and Hygromycin. Transfer resuspended cells to a T25 flask and culture in a CO_2 incubator at 37°C overnight. The next day, replace the growth medium with fresh growth medium without Geneticin and Hygromycin, and continue growing culture in a CO_2 incubator at 37°C until the cells are ready to be split. At first passage, switch to growth medium containing Geneticin and Hygromycin. Cells should be split before they reach complete confluence.

To passage the cells, rinse cells with phosphate buffered saline (PBS), and detach cells from the culture vessel with 0.05% Trypsin/EDTA. Add complete growth medium and transfer to a tube, spin down the cells, then resuspend cells and seed appropriate aliquots of cell suspension into new culture vessels. Subcultivation ration: 1:10 to 1:20 weekly or twice a week.

To freeze down the cells, rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with Trypsin/EDTA. Add complete growth medium and transfer to a tube, spin down cells, and resuspend in freezing medium (10% DMSO + 90% FBS). Place at -80℃ overnight and place in liquid nitrogen the next day. Alternatively, vials may be placed directly in liquid nitrogen.

Functional Validation and Assay Performance
The following assays are designed for 96-well format. To perform the assay in different tissue culture formats, the cell number and reagent volume should be scaled appropriately.

Materials Required but Not Supplied
- DAPT (Selleckchem # S2215): inhibitor of Notch pathway (γ-secretase inhibitor). Prepare 10 mM of stock solution in DMSO.
- Assay medium: growth medium without Geneticin and Hygromycin
- 96-well tissue culture plate or 96-well tissue culture-treated white clear-bottom assay plate
- ONE-Step™ Luciferase Assay System (Cat. #60690)
- Luminometer

Mycoplasma testing
The cell line has been screened using the PCR-based Venor® GeM Mycoplasma Detection kit (Sigma-Aldrich) to confirm the absence of Mycoplasma species.

Inhibition of Notch reporter activity by inhibitor of Notch signaling pathway in Notch CSL Reporter – HEK293 cells

1. Harvest Notch CSL Reporter – HEK293 cells from culture in growth medium and seed cells at a density of 35,000 cells per well into white clear-bottom 96-well microplate in 45 µl of assay medium.

2. Dilute the inhibitor (DAPT) stock in assay medium. Add 5 µl of diluted inhibitor to the test wells. The final concentration of DMSO in assay medium can be up to 0.5%.
   Add 5 µl of assay medium with same concentration of DMSO without inhibitor to control wells.
   Add 50 µl of assay medium with DMSO to cell-free control wells (for determining background luminescence).
   Set up each treatment in at least triplicate.

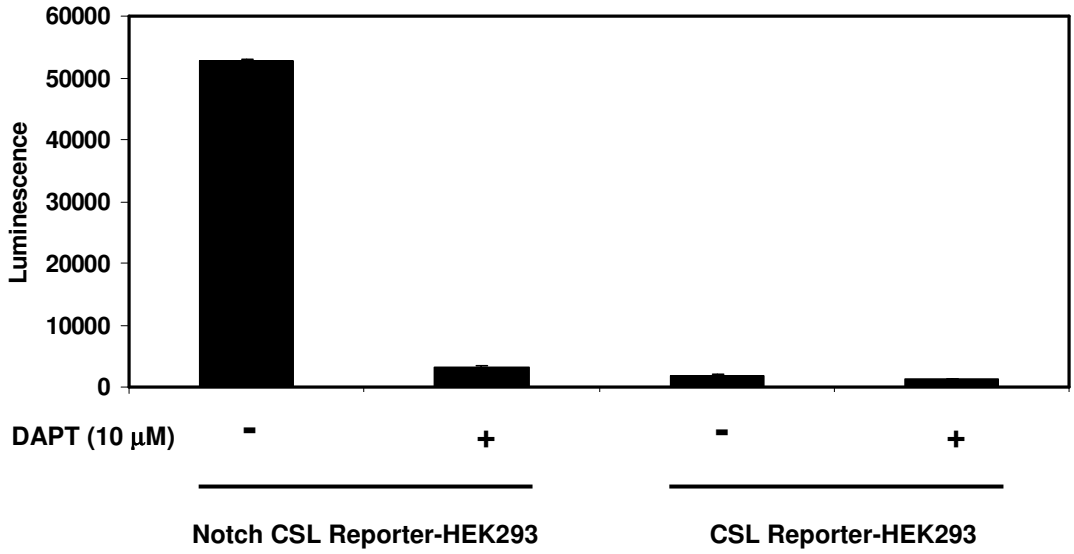
3. Incubate the plate at 37°C in a CO_2 incubator for 24 hours.

4. The next day, perform luciferase assay using the ONE-Step™ Luciferase Assay System according to the protocol provided: Add 100 µl of ONE-Step™ Luciferase reagent per well and rock at room temperature for ~15 minutes. Measure luminescence using a luminometer. *If using other luciferase reagents from other vendors, follow the manufacturer's assay protocol.*

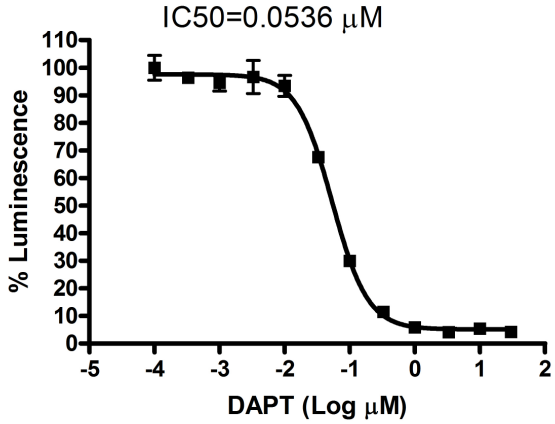
5. Data Analysis: Obtain the background-subtracted luminescence by subtracting the average background luminescence (cell-free control wells) from the luminescence reading of all wells.

Figure 1. Response of Notch CSL Reporter – HEK293 cells to Notch pathway inhibitor.

A. DAPT Blocked Notch Reporter Activity. The CSL Reporter-HEK293 cells contain the firefly luciferase gene under the control of Notch-response elements (CSL-responsive elements) stably integrated into HEK293 cells, but not the expression construct for Notch1ΔE, so it is unable to express the Notch luciferase reporter.



B. DAPT Inhibition Dose Response Curve. The results were shown as percentage of luminescence. The background-subtracted luminescence of cells in the absence of DAPT was set at 100%.



References

Lu FM *et al*. (1996) Constitutively active human Notch1 binds to the transcription factor CBF1 and stimulates transcription through a promoter containing a CBF1-responsive element. *Proc. Natl. Acad. Sci. USA* **93(11):** 5663-5667.

Kanungo *et al* (2008) The Notch signaling inhibitor DAPT down-regulates cdk5 activity and modulates the distribution of neuronal cytoskeletal proteins. *J. Neurochem*. **106:** 2236

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Related Products

Product name	Catalog#	Size
Notch Pathway Reporter Kit	60509	500 rxns
TCF/LEF Reporter Kit (Wnt / β-catenin signaling Pathway)	60500	500 rxns
SRE Reporter Kit (MAPK/ERK signaling Pathway)	60511	500 rxns
CRE/CREB Kit (cAMP/PKA signaling Pathway)	60611	500 rxns
Wnt Signaling Pathway TCF/LEF (Luc) Reproter HEK293	60501	2 vials
Hedgehog Signaling PathwayGli Reporter-NIH3T3 Cell Line	60409	2 vials
ERK Signaling Pathway SRE Reporter-HEK293 Cell Line	60406	2 vials
JNK Signaling Pathway AP1 Reporter-HEK293 Cell Line	60405	2 vials
JAK/STAT Signaling Pathway ISRE Reporter-HEK293	60409	2 vials
ONE-Step™ Luciferase Assay System	60690-1	10 ml
ONE-Step™ Luciferase Assay System	60690-2	100 ml

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