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CSL Reporter – HEK293 Cell line

Notch Signaling Pathway

Catalog #: 79754

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) stably integrated into HEK293 cells. Transfection of this cell line with a Notch expression vector and activation of the Notch pathway leads to expression of luciferase reporter. This cell line is validated with a Notch1DelE expression vector, which is constitutively processed by γ-secretase, leading to the release of NICD and the expression of luciferase.

Application

- Monitor Notch signaling pathway activity.
- Screen for inhibitors of the Notch signaling pathway.
- Test synthetic Notch receptors (synNotch) or other genetic constructs that conditionally activate the CSL promoter

Format

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

Storage

Immediately upon receipt, store cells in liquid nitrogen.

General Culture Conditions

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 1B (BPS Bioscience #79531): MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin, plus 400 µg/ml Geneticin®

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Cells should be grown at 37°C with 5% CO_2 in Growth Medium 1B. If using different medium components, it may be necessary to adjust the percentage of CO_2 in the incubator depending on the $NaHCO_3$ level in the basal medium.

Cell Thawing: Thaw the frozen cells in a 37°C water-bath for 40 seconds, until freezing medium is partially thawed but with some ice remaining. Transfer to a tube containing 10 ml of Thaw Medium 1 **(no geneticin)**, and spin down cells at 200 x g for 5 minutes. Remove supernatant and resuspend cells in pre-warmed Thaw Medium 1 **(no geneticin)**. Transfer resuspended cells to a T25 flask and culture in a 5% CO_2 incubator at 37°C overnight. The next day, replace the medium with fresh Thaw Medium 1 **(no geneticin)** 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 1B **(contains geneticin).** 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 Growth Medium 1B 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: 1:5 to 1:7 twice a week. *Note: This cell line will grow more slowly than wildtype HEK293 cells.*

To freeze down the cells, rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with 0.05% Trypsin/EDTA. Add Growth Medium 1B, transfer to a tube, spin down cells, and resuspend in freezing medium (10% DMSO + 90% FBS). Dispense 1 ml of cell aliquots into cryogenic vials. Place vials in an insulated container for slow cooling (-0.5°C to -1°C per min) and freeze at -80°C overnight. The next day, transfer the cells to liquid nitrogen for long term storage.

Functional Validation and Assay Performance
The following assays are 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.

Materials Required but Not Supplied

- Assay Medium: Thaw Medium 1 (BPS Bioscience #60187)
- Growth Medium 1B (BPS Bioscience #79531)
- Lipofectamine 2000 (Invitrogen #11668027) or other transfection reagent
- DAPT (Selleckchem #S2215): inhibitor of Notch pathway (γ-secretase inhibitor). Prepare 10 mM of stock solution in DMSO.
- 96-well tissue culture plate or 96-well tissue culture-treated white clear-bottom assay plate (Corning #3610)
- ONE-Step™ Luciferase Assay System (BPS Bioscience #60690)
- Luminometer

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Mycoplasma testing
The cell line has been screened using the MycoAlert Mycoplasma Detection kit (Lonza #LT07-318) 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 CSL Reporter – HEK293 cells from culture in Growth Medium 1B and seed cells at a density of 35,000 cells per well into white clear-bottom 96-well microplate in 90 µl Thaw Medium 1. Grow cells overnight at 37°C in a 5% CO_2 incubator. Leave a couple wells empty for use as the control for background luminescence.

2. Using Lipofectamine 2000 or other transfection reagent, transfect cells according to manufacturer's protocol with 50,ng per well of Notch1DelE expression vector, or a similar genetic construct of interest. Grow cells overnight at 37°C in a 5% CO_2 incubator.

3. Remove medium from the cells and replace with 90 µl Thaw Medium 1. Add 10 µl of diluted inhibitor (DAPT) or vehicle control to the test wells. The final concentration of DMSO in assay medium should be below 0.3%.
   Add 100 µl of Thaw Medium 1 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 5% CO_2 incubator for 24 - 48 hours.

4. Perform luciferase assay using the ONE-Step™ Luciferase Assay System according to the protocol provided: Mix component B with component A (1:100 ratio) and add 100 µl master mix per well. Rock at room temperature for ~15 minutes, and 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.

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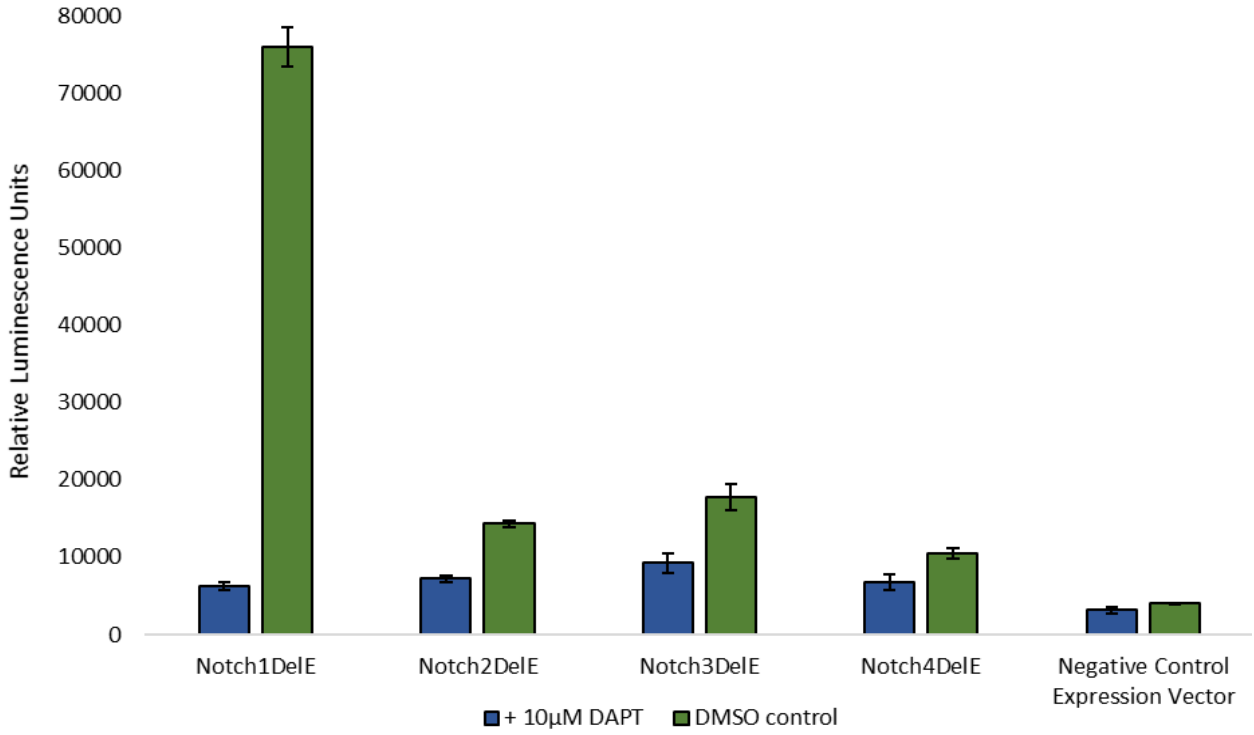
Figure 1. Response of Notch CSL Reporter – HEK293 cells to Notch pathway inhibitor.

A. DAPT Blocked Notch Reporter Activity. CSL-HEK293 cells transfected with Notch1DelE, Notch2DelE, Notch3DelE, Notch4DelE, or empty vector were treated with 10 µM DAPT or DMSO control. DAPT inhibited γ-secretase processing of Notch, resulting in decreased luciferase expression.



References

Lu, F.M., *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, J., *et al* (2008) The Notch signaling inhibitor DAPT down-regulates cdk5 activity and modulates the distribution of neuronal cytoskeletal proteins. *J. Neurochem*. **106:** 2236-2248.

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Related Products

Product name	Catalog#	Size
Notch1 / CSL Reporter – HEK293 Cell line	60652	2 vials
Human Notch1 Pathway Reporter Kit	79503	500 rxns
Mouse Notch1 Pathway Reporter Kit	60509	500 rxns
Mouse NICD Notch1 Pathway Reporter Kit	79504	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
Dual Luciferase (Firefly-Renilla) Assay System	60683-1	10 mL
Dual Luciferase (Firefly-Renilla) Assay System	60683-2	100 mL
Wnt Signaling Pathway TCF/LEF (Luc) Reporter HEK293	60501	2 vials
Hedgehog Signaling Pathway Gli 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
Thaw Medium 1	60187-1	100 ml
Thaw Medium 1	60187-2	500 ml
Growth Medium 1B	79531	500 ml