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**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/RBPJk/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 Notch1 $\Delta$ E (NOTCH1 that has a deletion of the entire extracellular domain) stably integrated into HEK293 cells. Inside the cells, the Notch1 $\Delta$ E can be cleaved by  $\gamma$ -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  $\sim 1.5 \times 10^6$  cells in 1 ml of 10% DMSO.

**Storage**

Immediately upon receipt, store cells in liquid nitrogen.

**General Culture Conditions**

**Thaw Medium 1 (BPS Cat. #60187):** 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)

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**Growth Medium 1A (BPS Cat. #79528):** Thaw Medium 1 (BPS Cat. #60187) and 400 µg/ml of Geneticin (Invitrogen #11811031) and 100 µg/ml of Hygromycin B (Hyclone #SV30070.01).

Cells should be grown at 37°C with 5% CO<sub>2</sub> Growth Medium 1A (Thaw Medium 1 BPS Cat. #60187 plus geneticin and hygromycin). It may be necessary to adjust the percentage of CO<sub>2</sub> in the incubator depending on the NaHCO<sub>3</sub> level in the basal medium.

**To thaw cells,** 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 Thaw Medium 1 (**no geneticin or hygromycin**) spin down cells, and resuspend cells in pre-warmed Thaw Medium 1 (**no geneticin or hygromycin**). Transfer resuspended cells to a T25 flask and culture in a CO<sub>2</sub> incubator at 37°C overnight. The next day, replace the medium with fresh Thaw Medium 1 (**no geneticin or hygromycin**) and continue growing culture in a CO<sub>2</sub> incubator at 37°C until the cells are ready to be split. At first passage, switch to Growth Medium 1A (**contains 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 Growth Medium 1A 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 Growth Medium 1A and transfer to a tube, spin down cells, and resuspend in freezing medium (10% DMSO + 90% FBS). Place at -80°C 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

- Assay Medium: Thaw Medium 1 (BPS Cat. #60187)
- Growth Medium 1A (BPS Cat. #79528)
- 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
- ONE-Step™ Luciferase Assay System (BPS, Cat. #60690)
- Luminometer

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### **Mycoplasma testing**

The cell line has been screened using the PCR-based Venor<sup>®</sup> 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 1A and seed cells at a density of 35,000 cells per well into white clear-bottom 96-well microplate in 90  $\mu$ l of assay medium.
2. Dilute the inhibitor (DAPT) stock in assay medium. Add 10  $\mu$ l of diluted inhibitor to the test wells. The final concentration of DMSO in assay medium can be up to 0.5%.  
Add 10  $\mu$ l of assay medium with same concentration of DMSO without inhibitor to control wells.  
Add 100  $\mu$ l 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<sub>2</sub> 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  $\mu$ l 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.**

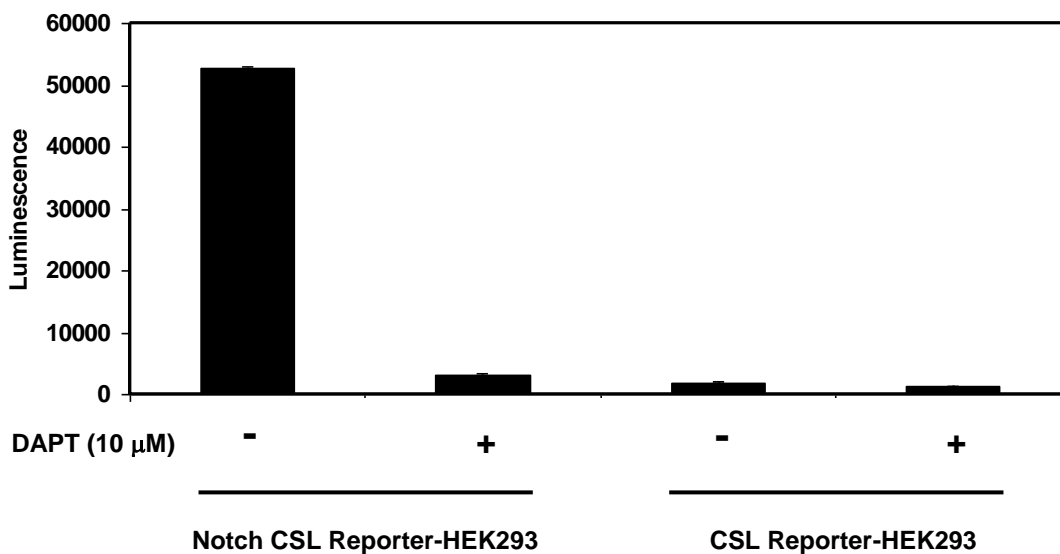
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**A. DAPT Blocked Notch Reporter Activity.** The CSL Reporter-HEK293 cells contain the firefly luciferase gene under the control of Notch-response elements (CSL-responsive elements) stably integrated into HEK293 cells, but not the expression construct for Notch1 $\Delta$ E, 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%.

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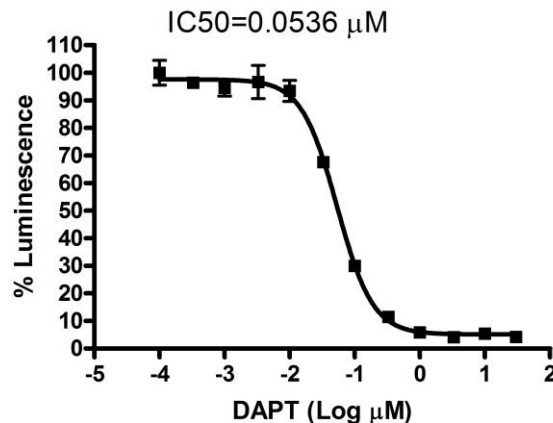
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#### References

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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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
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ONE-Step™ Luciferase Assay System	60690-2	100 ml

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