Description

The Gli Reporter NIH3T3 Cell Line is designed for monitoring the activity of the hedgehog signaling pathway. This cell line contains the firefly luciferase gene under the control of Gli responsive elements stably integrated into NIH3T3 cells. Luciferase expression correlates with activation of the hedgehog signaling pathway. This cell line has been validated for its response to stimulation with murine Sonic Hedgehog and to treatment with inhibitors of the hedgehog signaling pathway.

Background

The hedgehog pathway controls stem cell growth in embryonic and adult tissues and promotes tumor growth in a number of cancers. The mammalian hedgehog proteins, Sonic Hedgehog (Shh), Indian Hedgehog (Ihh), and Desert Hedgehog (Dhh) activate hedgehog signaling by binding to their membrane receptor "Patched" (PTCH). This binding releases PTCH inhibition of Smoothened (Smo) and allows Smo to activate the Gli family of transcription factors, leading to transcription and expression of hedgehog target genes.

Application

- Monitor Hedgehog signaling pathway activity.
- Screen or characterize activators or inhibitors of the Hedgehog signaling pathway.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains 2 x 10^6 cells in 1 ml of cell freezing medium (BPS Bioscience #79796)

Parental Cell Line

NIH3T3, murine epithelial 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 5	BPS Bioscience #60182
Growth Medium 5B	BPS Bioscience #79541

Materials Used in the Cellular Assay

Name	Ordering Information
Assay Medium 5A	BPS Bioscience #79620
Mouse Sonic Hedgehog (Shh)	BPS Bioscience #91012
Vismodegib (GDC-0449)	BPS Bioscience #27010

Cyclopamine, Free Base	BPS Bioscience #27013
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
White clear-bottom 96-well cell culture-treated plate	
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, it is *highly recommended* to use these validated and optimized media from BPS Bioscience. 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 for maintaining the presence of the transfected gene(s) over passages. Cells should be grown at 37°C with 5% CO_2. BPS Bioscience's cell lines are stable for at least 15 passages when grown under proper conditions.

Media Required for Cell Culture

Thaw Medium 5 (BPS Bioscience #60182):
DMEM medium supplemented with 10% Calf Serum (*not FBS*), 1% Penicillin/Streptomycin.

Growth Medium 5B (BPS Bioscience #79541):
DMEM medium supplemented with 10% Calf Serum (*not FBS*), 1% Penicillin/Streptomycin plus 500 µg/ml of Geneticin.

Media Required for Functional Cellular Assay

Assay Medium 5A (BPS Bioscience #79620):
Opti-MEM™ Reduced Serum medium, 0.5% calf serum, 1% non-essential amino acids, 1 mM Na pyruvate, 10 mM HEPES, 1% Penicillin/Streptomycin.

Cell Culture Protocol

Cell Thawing

1. Swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. As soon as the cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire contents of the vial to a tube containing 10 ml of pre-warmed Thaw Medium 3 **(no Geneticin)**.
   Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.
2. 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 3 **(no Geneticin)**.
3. Transfer the resuspended cells to a T25 flask or T75 flask and incubate at 37°C in a 5% CO_2 incubator.
4. After 24 hours of culture, check for cell attachment and viability. Change medium to fresh Thaw Medium 3 **(no Geneticin)** and continue growing in a 5% CO_2 incubator at 37°C until the cells are ready to passage.
5. Cells should be passaged before they are fully confluent. At first passage and subsequent passages, use Growth Medium 3B **(contains Geneticin)**.

Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS), and detach the cells from the culture vessel with 0.25% Trypsin/EDTA.
2. Once the cells have detached, add Growth Medium 3B and transfer to a tube. Spin down cells at 300 x g for 5 minutes, remove the medium and resuspend the cells in Growth Medium 3B **(contains Geneticin)**. Seed into new culture vessels at the desired sub-cultivation ratio of 1:10 to 1:20 once or twice a week.

Cell Freezing

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS), and detach the cells from the culture vessel with 0.25% Trypsin/EDTA.
2. Once the cells have detached, add Growth Medium 3B 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 Freezing Medium (BPS Bioscience #79796, or 10% DMSO + 90% FBS) at ~2 x 10^6 cells/ml.
4. Dispense 1 ml of cell aliquots into cryogenic vials. 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 storage.

Note: It is recommended to expand the cells and freeze at least 10 vials at an early passage for future use.

Validation Data

A. Dose response of agonist Sonic Hedgehog (mouse Shh) in Gli Luciferase Reporter NIH3T3 cells

- The following assays are designed for 96-well format. To perform the assay in different tissue culture formats, the cell number and reagent volumes should be scaled appropriately.
- The experiments should be performed in triplicate.

1. The day before the experiment, seed 25,000 cells/100 µl/well of Gli Luciferase Reporter NIH3T3 cells in a white clear-bottom 96-well plate in Thaw Medium 5. Keep three wells without cells to determine the background luminescence signal.
2. Incubate cells at 37°C in a 5% CO_2 incubator for 16-20 hours.
3. The next day, the cells should have reached confluency. It is critical for the cells to reach confluency before treatment.

   Carefully remove the medium from the wells, avoiding disruption of the cell monolayer. The cells are prone to detach when confluent. We recommend using a pipettor, not aspiration, to remove the medium.
4. Prepare a serial dilution of the agonist in Assay Medium and add 100 µl of each dilution to the cells ("stimulated").
   Add 100 µl of Assay Medium to the "unstimulated control" cells.
   Add 100 µl of Assay Medium to cell-free control wells (for determining background luminescence).
5. Incubate the plate at 37°C in a 5% CO_2 incubator for 24 to 30 hours.

6. Perform the luciferase assay using ONE-Step™ Luciferase Assay buffer, prepared according to the recommended instructions. Add 100 µl of the ONE-Step™ Luciferase reagent per well and rock gently at room temperature for ~15 minutes. Measure luminescence using a luminometer.
7. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells. The fold induction of Gli luciferase reporter expression is the background-subtracted luminescence of stimulated cells divided by the average background-subtracted luminescence of unstimulated control wells.

$$Fold\ induction = \frac{average\ Lumin.of\ stimulated\ cells - average\ backgound}{average\ Lumin.of\ unstimulated\ cells - average\ background}$$

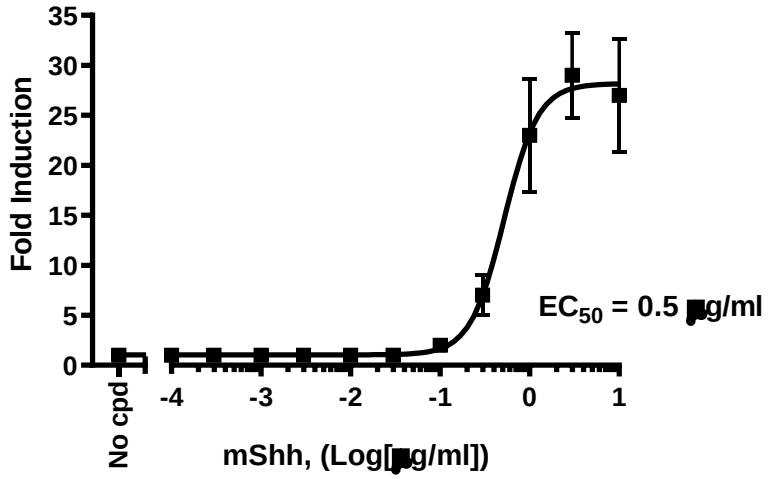

Figure 1. Stimulation of Gli Luciferase Reporter NIH3T3 Cells by agonist mouse Shh.
The experiment was performed as described in the protocol above. Results are expressed as fold induction of luciferase activity relative to unstimulated cells. A dose-dependent increase in luciferase activity was observed in response to mShh treatment.

B: Inhibition of mShh-induced reporter activity by inhibitors of hedgehog signaling pathway in Gli Luciferase Reporter NIH3T3 cells

- The following assays are designed for 96-well format. To perform the assay in different tissue culture formats, the cell number and reagent volumes should be scaled appropriately.
- The experiments should be performed in triplicate.
- The effect of the inhibitor should be tested in the presence of an agonist. Luminescence signals will be very low in the absence of an agonist.
- The positive control is the agonist-stimulated condition without inhibitor. The negative control (measure of basal luciferase activity) is the non-stimulated condition.

1. The day before the experiment, seed 25,000 cells/100 µl/well of Gli Luciferase Reporter NIH3T3 cells in a white clear-bottom 96-well plate in Thaw Medium 5. Keep three wells without cells to determine the background luminescence signal.
2. Incubate cells at 37°C in a 5% CO_2 incubator for 16-20 hours.
3. The next day, the cells should have reached confluency. It is critical for the cells to reach confluency before treatment.

   Carefully remove the medium from the wells, avoiding disruption of the cell monolayer. The cells are prone to detach when confluent. We recommend using a pipettor, not aspiration, to remove the medium.
4. Prepare a stock solution of hedgehog pathway inhibitor Cyclopamine or vismodegib in DMSO. Dilute the inhibitor in Assay Medium 5A at a concentration that is 2-fold higher than the desired final concentration. Add 50 µl of diluted inhibitor to the cells. The final concentration of DMSO in assay medium can be up to 0.5%.

   Add 50 µl of Assay Medium 5A containing the same concentration of DMSO (without inhibitor) to the "positive control" cells.

   Add 50 µl of Assay Medium 5A containing the same concentration of DMSO to cell-free control wells (for determining background luminescence).
5. Incubate the plate at 37°C in a CO_2 incubator for 1-2 hours.
6. Prepare a dilution of the mShh agonist in Assay Medium 5A at a concentration 2-fold higher than the desired final concentration.
7. Add 50 µl of agonist to the cells, including inhibitor-treated and positive control. In the experiments shown below, mShh was used at a final concentration of 1 µg/ml.

   Add 50 µl of Assay Medium to the "unstimulated control" cells (negative control).

   Add 50 µl of Assay Medium to cell-free control wells (background luminescence).

	Positive control	**Inhibitor-treated**	**Negative Control**
Inhibitor in Assay Medium	-	50 µl	-
Agonist in Assay Medium	50 µl	50 µl	-
Assay Medium	50 µl	-	100 µl
Total	**100 µl**	**100 µl**	**100 µl**

8. Incubate the plate at 37°C in a 5% CO_2 incubator for 24 to 30 hours.
9. Perform the luciferase assay using ONE-Step™ Luciferase Assay buffer, prepared according to the recommended instructions. Add 100 µl of the ONE-Step™ Luciferase reagent per well and rock gently at room temperature for ~15 minutes. Measure luminescence using a luminometer.
10. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells. The % luminescence is the background-subtracted luminescence of inhibitor-treated cells divided by the average background-subtracted luminescence of positive control wells multiplied by 100.

$$\%\ Lumin. = \left(\frac{lumin.\,inhibitor\ treated\ cells - average\ background}{lumin.\,no\ inhibitor\ cells - average\ background}\right) x100$$

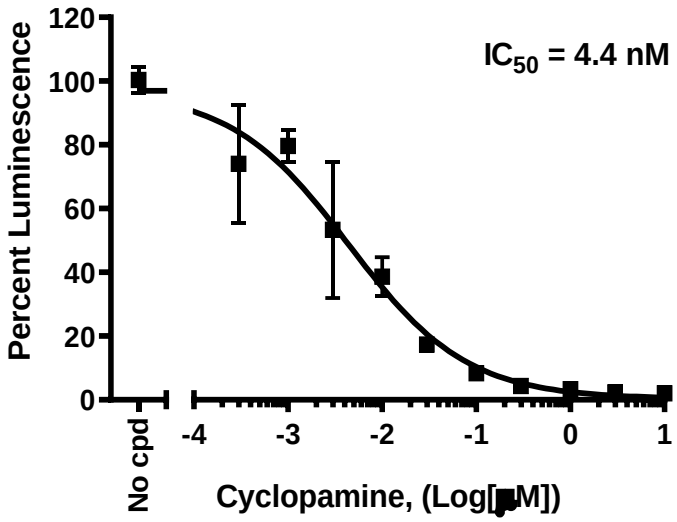

Figure 2: Inhibition of mShh stimulation by cyclopamine.
Cells were incubated with increasing concentrations of cyclopamine before stimulation with 1 µg/ml of mShh agonist. Results are expressed as percent of luminescence, where the positive control (agonist without cyclopamine) is set at 100%.

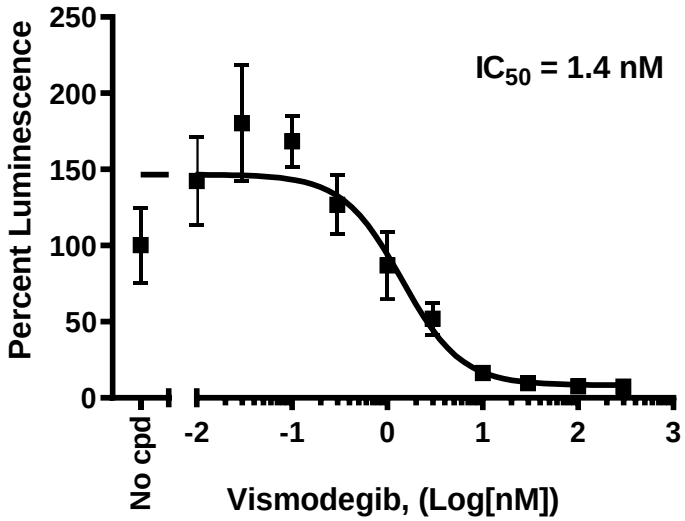

Figure 3: Inhibition of mShh stimulation by vismodegib (GDC-0449).
Cells were incubated with increasing concentrations of vismodegib before stimulation with 1 µg/ml of mShh agonist. Results are expressed as percent of luminescence, where the positive control (agonist without vismodegib) is set at 100%.

References

- Kinzler KW *et al*. (1990) The GLI gene encodes a nuclear protein which binds specific sequences in the human genome. *Mol Cell Biol*. **10(2)**: 634-642.

- Mullor JL *et al*. (2002) Pathways and consequences: Hedgehog signaling in human disease. *Trends Cell Biol*. **12(12)**: 562-569.
- Peukert S *et al*. (2010) Small-molecule inhibitors of the hedgehog signaling pathway as cancer therapeutics. *ChemMedChem*. **5(4)**: 500-512.

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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*
Mouse Sonic Hedgehog (Shh), His-tag Recombinant	91012	25 µg
Cyclopamine, Free Base	27013	10 mg
Vismodegib (GDC-04491)	27015	50 mg
Wnt Signaling Pathway TCF/LEF Reporter HEK293 Cell Line	60501	2 vials
TCF/LEF Reporter Kit (Wnt/β-catenin Signaling Pathway)	60500	500 reactions