Data Sheet

Hedgehog Signaling Pathway
Gli Reporter – NIH3T3 Cell Line
Catalog #: 60409

Product Description
The *Gli Reporter – NIH3T3 Cell Line* is designed for monitoring the activity of the hedgehog signaling pathway. The hedgehog pathway controls stem cell growth in embryonic and adult tissues and promotes tumor growth in a number of human 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 signal target genes.

The *Gli Reporter – NIH3T3 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 is validated for its response to stimulation with murine Sonic Hedgehog and to treatment with inhibitors of the hedgehog signaling pathway.

Application
- Monitor hedgehog signaling pathway activity.
- Screen for activators or inhibitors of the hedgehog signaling pathway.

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

Storage
Immediately upon receipt, store 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
- Recombinant Mouse Sonic Hedgehog (mShh) (R&D Systems # 461-SH-025)
- Cyclopamine (BPS Bioscience # 27013): inhibitor of hedgehog pathway (Smo inhibitor)
- Vismodegib (GDC-0449) (BPS Bioscience #27010): inhibitor of hedgehog pathway (Smo inhibitor)

AMSBIO | www.amsbio.com | info@amsbio.com

UK & Rest of the World
184 Park Drive, Milton Park
Abingdon, UK
T: +44 (0)1235 828 200
F: +44 (0) 1235 820 482

North America
1035 Cambridge Street,
Cambridge, MA 02141
T: +1 (617) 945-5033 or
T: +1 (800) 987-0985
F: +1 (617) 945-8218

Germany
Bockenheimer Landstr. 17/19
60325 Frankfurt/Main
T: +49 (0) 69 779099
F: +49 (0) 69 13376880

Switzerland
Centro Nord-Sud 2E
CH-6934 Bioggio-Lugano
T: +41(0) 91 604 55 22
F: +41(0) 91 605 17 85

- Assay medium: Opti-MEM Reduced Serum Medium (Invitrogen #31985-062) with 0.5% calf serum, 1% non-essential amino acids, 1 mM Na-pyruvate, 10 mM HEPES, and 1% Pen/Strep
- 96-well tissue culture plate or 96-well tissue culture-treated white clear-bottom assay plate
- Luciferase reagents for measuring firefly luciferase activity (We use the ONE-Glo™ luciferase assay system, Promega #E6110. Other luciferase assay systems are also suitable).
- Luminometer

A. Dose response of Gli Reporter – NIH3T3 cells to mouse Sonic Hedgehog (mShh)

1. Harvest Gli Reporter – NIH3T3 cells from culture in growth medium and seed cells at a density of 25,000 cells per well into white clear-bottom 96-well microplate in 100 µl of growth medium without Geneticin.

2. Incubate cells at 37°C in a CO_2 incubator for 16-20 hours.

3. The next day, the cells should reach confluency. It is critical for the cells to reach confluency in the wells before treatment.

   Carefully remove the medium from the wells and avoid disrupting the cell monolayer. The cells are prone to detach at this stage. We recommend using a pipettor, not an aspirator, to remove the medium.
   Add 50 µl of threefold serial dilution of mShh in assay medium to stimulated wells.
   Add 50 µl of assay medium to the unstimulated control wells.
   Add 50 µl of assay medium to cell-free control wells (for determining background luminescence).
   Set up each treatment in at least triplicate.

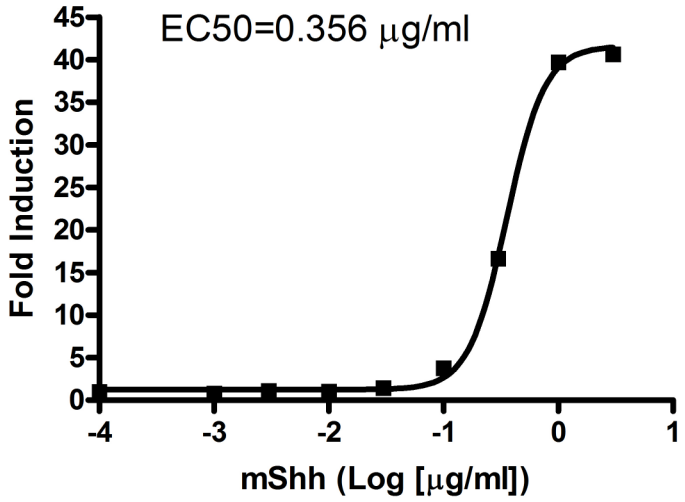
4. Incubate the plate at 37°C in a CO_2 incubator for 24 to 30 hours.

5. Perform luciferase assay using the ONE-Glo luciferase assay system: Add 50 µl of ONE-Glo Luciferase reagent per well and rock at room temperature for ~20 minutes. Measure luminescence using a luminometer.
   If using luciferase reagents from other vendors, follow the manufacturer's assay protocol.

6. 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 = background-subtracted luminescence of mShh-stimulated well / average background-subtracted luminescence of unstimulated control wells

Figure 1. Dose Response of Gli Reporter – NIH3T3 Cells to mShh.
The results are shown as fold induction of Gli luciferase reporter expression.

The EC50 of mShh is ~ 0.356 µg/ml



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

1. Harvest Gli Reporter – NIH3T3 cells from culture in growth medium and seed cells at a density of 25,000 cells per well into white clear-bottom 96-well microplate in 100 µl of growth medium without Geneticin.

2. Incubate cells at 37°C in a CO_2 incubator for 16-20 hours.

3. The next day, the cells should reach confluency. It is critical for the cells to reach confluency in the wells before treatment.

   Carefully remove the medium from wells and avoid disrupting the cell monolayer. The cells are prone to detach at this stage. We recommend using a pipettor, not an aspirator, to remove the medium.

   Prepare stock solution of hedgehog pathway inhibitor (Cyclopamine or GDC-0449) in DMSO.
   Dilute the inhibitor stock in assay medium. Add 45 µl of diluted inhibitor in assay medium to the wells. The final concentration of DMSO in assay medium can be up to 0.5%.

Add 45 µl of assay medium with same concentration of DMSO without inhibitor to inhibitor control wells.
Add 45 µl of assay medium with DMSO to cell-free control wells (for determining background luminescence).

4. Incubate the plate at 37°C in a CO_2 incubator for 1-2 hours.

5. Add 5 µl of diluted mShh in assay medium to stimulated wells (final [mShh] = 1 µg/ml).
Add 5 µl of assay medium to the unstimulated control wells (cells without inhibitor and mShh treatment for determining the basal activity).
Add 5 µl of assay medium to cell-free control wells.
Set up each treatment in at least triplicate.

Treatment Reference Guide

	Stimulated Wells		**Unstimulated Control Wells**	**Cell-free Control Wells**
	With inhibitor	Without inhibitor (control well)		
Step 3	45 µl diluted inhibitor in assay medium	45 µl assay medium with DMSO only	45 µl assay medium with DMSO only	45 µl assay medium with DMSO only
Step 5	5 µl mShh in assay medium (final [mShh] = 1 µg/ml)	5 µl mShh in assay medium (final [mShh] = 1 µg/ml)	5 µl assay medium	5 µl assay medium

6. Incubate the plate at 37°C in a CO_2 incubator for 24-30 hours.

7. Perform luciferase assay using the ONE-Glo luciferase assay system: Add 50 µl of ONE-Glo Luciferase reagent per well and rock at room temperature for ~20 minutes. Measure luminescence using a luminometer.
If using luciferase reagents from other vendors follow the manufacturer's assay protocol.

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

UK & Rest of the World
184 Park Drive, Milton Park
Abingdon, UK
T: +44 (0)1235 828 200
F: +44 (0) 1235 820 482

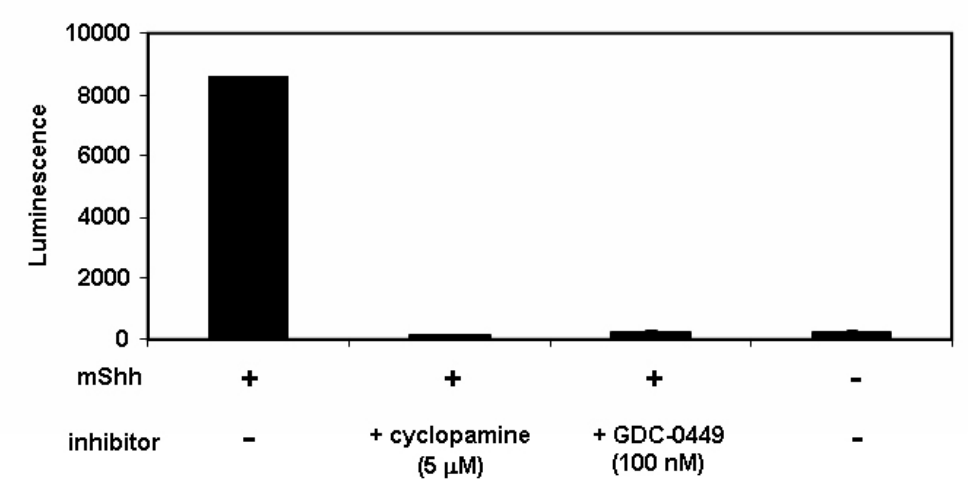
North America
1035 Cambridge Street,
Cambridge, MA 02141
T: +1 (617) 945-5033 or
T: +1 (800) 987-0985
F: +1 (617) 945-8218

Germany
Bockenheimer Landstr. 17/19
60325 Frankfurt/Main
T: +49 (0) 69 779099
F: +49 (0) 69 13376880

Switzerland
Centro Nord-Sud 2E
CH-6934 Bioggio-Lugano
T: +41(0) 91 604 55 22
F: +41(0) 91 605 17 85

Figure 2. Inhibition of mShh-induced Reporter Activity by Hedgehog Pathway Inhibitors in Gli Reporter – NIH3T3 Cells

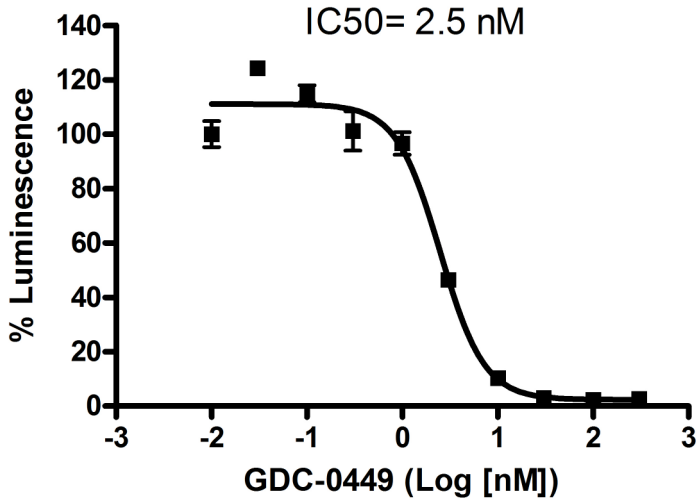
2A. Cyclopamine or GDC-0449 Blocked mShh-induced Gli Reporter Activity.



2B. GDC-0449 Inhibition Dose Response Curve

The results were shown as percentage of luminescence. The background-subtracted luminescence of cells stimulated with mShh in the absence of GDC-0449 was set at 100%.

The IC50 of GDC-0449 is ~ 2.5 nM



AMSBIO| www.amsbio.com | info@amsbio.com

UK & Rest of the World
184 Park Drive, Milton Park
Abingdon, UK
T: +44 (0)1235 828 200
F: +44 (0) 1235 820 482

North America
1035 Cambridge Street,
Cambridge, MA 02141
T: +1 (617) 945-5033 or
T: +1 (800) 987-0985
F: +1 (617) 945-8218

Germany
Bockenheimer Landstr. 17/19
60325 Frankfurt/Main
T: +49 (0) 69 779099
F: +49 (0) 69 13376880

Switzerland
Centro Nord-Sud 2E
CH-6934 Bioggio-Lugano
T: +41(0) 91 604 55 22
F: +41(0) 91 605 17 85

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

Culture conditions
Cells should be grown at 37° with 5% CO_2 using DMEM medium supplemented with 10% Calf Serum, 1% Penicillin/Streptomycin, and 500 µg/ml of Geneticin (Life Technologies #11811031). 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, spin down cells, resuspend cells in pre-warmed growth medium without Geneticin, transfer resuspended cells to a T25 flask and culture in a CO_2 incubator at 37°C. At first passage, switch to growth medium containing 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 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.

References

1. 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.
2. Mullor JL *et al.* (2002) Pathways and consequences: Hedgehog signaling in human disease. *Trends Cell Biol*. **12(12):**562-569.
3. Peukert S *et al.* (2010) Small-molecule inhibitors of the hedgehog signaling pathway as cancer therapeutics. *ChemMedChem.* **5(4):**500-512.

Related Products

Product Name	Catalog #	Size
Cyclopamine	27013	5 mg
Vismodegib (GDC-0449)	27010	10 mg
Wnt Signaling Pathway TCF/LEF Reporter – HEK293 Cell Line	60501	1 Vial
TCF/LEF Reporter Kit (Wnt/β-catenin Signaling Pathway)	60500	500 Reactions

AMSBIO| www.amsbio.com | info@amsbio.com

UK & Rest of the World
184 Park Drive, Milton Park
Abingdon, UK
T: +44 (0)1235 828 200
F: +44 (0) 1235 820 482

North America
1035 Cambridge Street,
Cambridge, MA 02141
T: +1 (617) 945-5033 or
T: +1 (800) 987-0985
F: +1 (617) 945-8218

Germany
Bockenheimer Landstr. 17/19
60325 Frankfurt/Main
T: +49 (0) 69 779099
F: +49 (0) 69 13376880

Switzerland
Centro Nord-Sud 2E
CH-6934 Bioggio-Lugano
T: +41(0) 91 604 55 22
F: +41(0) 91 605 17 85

License Disclosure

Purchase of this cell line grants you with a 10-year license to use this cell line in your immediate laboratory, for research use only. This license does not permit you to share, distribute, sell, sublicense, or otherwise make the cell line available for use to other laboratories, departments, research institutions, hospitals, universities, or biotech companies. The license does not permit the use of this cell line in humans or for therapeutic or drug use. Inappropriate use or distribution of this cell line will result in revocation of the license and result in an immediate cease of sales and distribution of BPS products to your laboratory. Publications using this cell line should reference BPS Bioscience, Inc., San Diego, CA.

AMSBIO| www.amsbio.com | info@amsbio.com


UK & Rest of the World
184 Park Drive, Milton Park
Abingdon, UK
T: +44 (0)1235 828 200
F: +44 (0) 1235 820 482


North America
1035 Cambridge Street,
Cambridge, MA 02141
T: +1 (617) 945-5033 or
T: +1 (800) 987-0985
F: +1 (617) 945-8218


Germany
Bockenheimer Landstr. 17/19
60325 Frankfurt/Main
T: +49 (0) 69 779099
F: +49 (0) 69 13376880


Switzerland
Centro Nord-Sud 2E
CH-6934 Bioggio-Lugano
T: +41(0) 91 604 55 22
F: +41(0) 91 605 17 85