

## Description

The TGFβ/Activin A-Responsive Reporter HEK293 Cell Line is a HEK293 cell line expressing the Firefly luciferase reporter under the control of SMAD-responsive elements (SMAD binding elements, SBE). This cell line monitors the activity of the TGFβ (transforming growth factor beta)/SMAD signaling pathway.

This cell line has been validated by activation in response to human TGFβ1 and to other cytokines of the TGFβ1 superfamily such as Activin A, GDF-8/Myostatin, and GDF-11/BMP-11. Luciferase activity induced by human TGFβ1 was decreased by SB525334, an inhibitor of the TGFβ1-receptor.

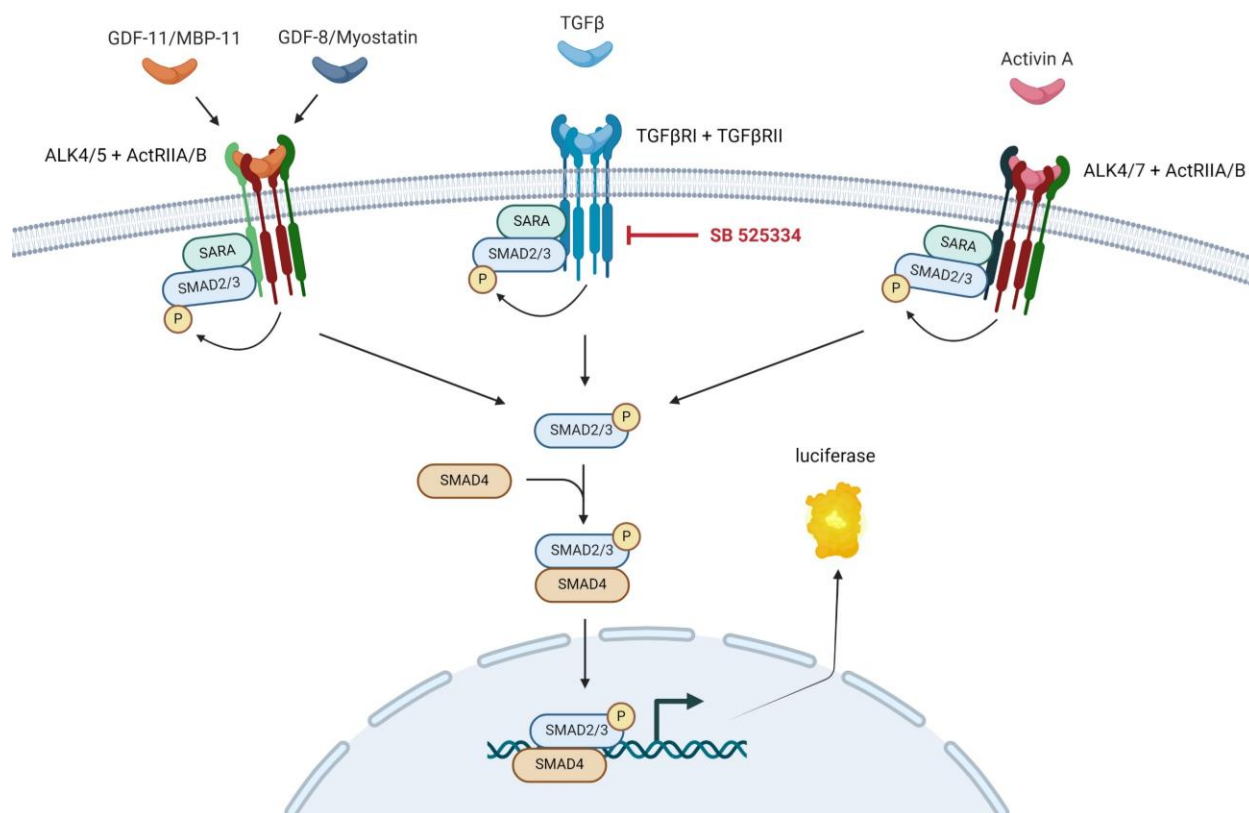


Figure 1: illustration of mechanism of action of the TGFβ/Activin A-Responsive Reporter HEK293 Cell Line.

## Background

The TGFβ signaling pathway participates in diverse cellular processes such as growth and differentiation, cell cycle arrest, and immune regulation. TGFβ signaling has been linked to heart disease, cancer, Alzheimer's disease, among others. TGFβ proteins bind to receptors on the cell surface, initiating a signaling cascade that leads to phosphorylation and activation of the signaling proteins SMAD2 (mothers against decapentaplegic homolog 2) and SMAD3, which then form a complex with SMAD4. The SMAD complex gets translocated to the nucleus and binds to the SMAD binding element (SBE) in the promoter of target genes, leading to transcription and expression of TGFβ/ SMAD responsive genes. The understanding and regulation of this pathway can provide essential clues for the development of therapeutical approaches for cancer treatment.

## Application

- Monitor activation of the TGFβ signaling pathway.
- Screen for compounds that regulate the TGFβ/SMAD signaling pathway.

**Materials Provided**

Components	Format
2 vials of frozen cells	Each vial contains >1 x 10 <sup>6</sup> cells in 1 ml of Cell Freezing Medium (BPS Bioscience #79796)

**Parental Cell Line**

HEK293, Human Embryonic Kidney, epithelial-like 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 1	<a href="#">BPS Bioscience #60187</a>
Growth Medium 1B	<a href="#">BPS Bioscience #79531</a>

*Materials Used in Cellular Assay*

Name	Ordering Information
Assay Medium 1B	<a href="#">BPS Bioscience #79617</a>
Human TGFβ1	<a href="#">BPS Bioscience #90900</a>
Recombinant Human/Murine/Rat Activin A	PeproTech #120-14E
Recombinant Human/Mouse/Rat GDF-8/Myostatin Protein	R&D Systems #788-G8
Recombinant Human/Mouse/Rat GDF-11/BMP-11 Protein	R&D Systems #1958-GD
Inhibitor SB525334	Selleckchem #356559-20-1
Clear-bottom, white 96-well tissue culture-treated plate	Corning #3610
ONE-Step™ Luciferase Assay System	<a href="#">BPS Bioscience #60690</a>
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](mailto:support@bpsbioscience.com) if the cells are not frozen in dry ice upon arrival.

**Media Formulations**

For best results, the use of validated and optimized media from BPS Bioscience is *highly recommended*. 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 to maintain selective pressure on the cell population expressing the gene of interest.

Cells should be grown at 37°C with 5% CO<sub>2</sub>. BPS Bioscience's cell lines are stable for at least 10 passages when grown under proper conditions.

#### *Media Required for Cell Culture*

*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 of Geneticin.

#### *Media Required for Functional Cellular Assay*

*Assay Medium 1B (BPS Bioscience #79617):*

MEM Medium, 0.5% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin.

#### **Cell Culture Protocol**

**Note: HEK293 cells are derived from human material and thus the use of adequate safety precautions is recommended.**

#### *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 1.

**Note: 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 1.
3. Transfer the resuspended cells to a T25 flask or T75 flask and incubate at 37°C in a 5% CO<sub>2</sub> incubator.
4. After 24 hours of culture, check for cell attachment and viability. Change medium to fresh Thaw Medium 1 and continue growing in a 5% CO<sub>2</sub> 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 1B.

#### *Cell Passage*

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS) without Ca<sup>2+</sup>/Mg<sup>2+</sup>, and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.
2. Once the cells have detached, add Growth Medium 1B and transfer to a tube.
3. Spin down cells at 300 x g for 5 minutes, remove the medium and resuspend the cells in Growth Medium 1B.

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:5 to 1:10 once or twice a week.

*Note: Just after thawing and when cells are at low density, the cells may grow at a slower rate. It is recommended to split the cells at a 1:4 ratio in those cases. After several passages, the cell growth rate increases, and the cells can be split using a higher ratio.*

### Cell Freezing

1. Aspirate the medium, wash the cells with PBS without Ca<sup>2+</sup>/Mg<sup>2+</sup> and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.
2. Once the cells have detached, add Growth Medium 1B 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 Cell Freezing Medium (BPS Bioscience #79796) at ~2 x 10<sup>6</sup> cells/ml.
4. Dispense 1 ml of cell suspension into each cryogenic vial. 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 long term storage.



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

### Validation Data

- The following assays were 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.
- Dose-response of agonist and antagonist is best performed using 3-fold serial dilutions.

### A. Reporter activation by cytokines

- This experiment measures the effect of an agonist on reporter activation.
  - All conditions should be performed in triplicate.
  - The assay should include “Stimulated Cells”, with “Luminescence Background Control” and “Unstimulated Control” conditions.
1. Seed TGFβ/Activin A-Responsive Luciferase Reporter HEK293 cells at a density of ~35,000 cells/well in 100 µl of Thaw Medium 1 into a clear-bottom, white 96-well plate. Leave a few empty wells as cell-free control wells (“Luminescence Background Control”).
  2. Incubate the plate at 37°C in a 5% CO<sub>2</sub> incubator for 24 hours.

3. 24 hours later, carefully remove the medium from all wells and replace it with 90 µl of fresh Assay Medium 1B.
4. Incubate cells at 37°C in a 5% CO<sub>2</sub> incubator for ~4-5 hours.
5. Prepare serial dilutions of the agonist in Assay Medium 1B at concentrations 10-fold higher than the desired final concentrations.
6. Add 10 µl of each agonist dilution to the wells labeled as “Stimulated Cells”.
7. Add 10 µl of Assay Medium 1B to the wells labeled as “Unstimulated Control”.
8. Add 100 µl of Assay Medium 1B to the wells not containing cells and labeled as “Luminescence Background Control”.
9. Incubate the plate at 37°C in a CO<sub>2</sub> incubator overnight (~18 hours).
10. Add 100 µl of ONE-Step™ Luciferase reagent to each well and rock at Room Temperature (RT) for ~15 to 30 minutes.
11. Measure luminescence using a luminometer.
12. Data Analysis: Subtract the average background luminescence from the luminescence reading of all other wells. The fold induction of SBE luciferase reporter expression is the average background-subtracted luminescence of stimulated wells divided by the average background-subtracted luminescence of unstimulated control wells.

$$\text{Fold induction} = \frac{\text{Luminescence of Stimulated Wells} - \text{avg. background}}{\text{Avg. Luminescence of Unstimulated Wells} - \text{avg. background}}$$

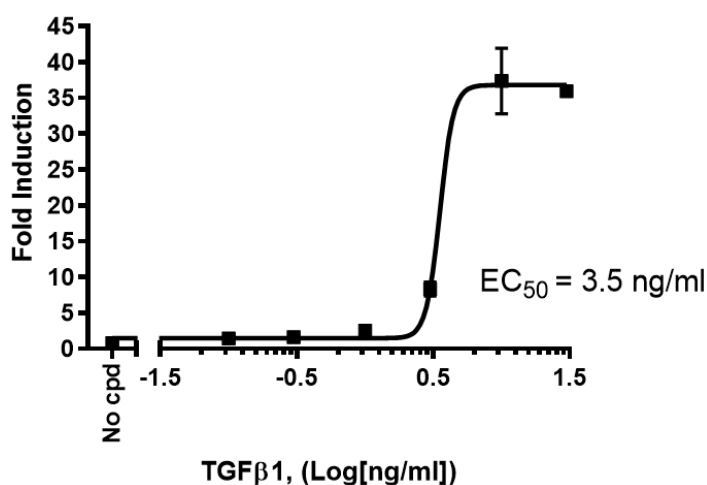


Figure 2: Reporter activation in the TGFβ/Activin A-Responsive Luciferase Reporter HEK293 Cell Line by the agonist TGFβ1.

TGF $\beta$ /Activin A-Responsive Luciferase Reporter HEK293 cells were incubated with increasing concentrations of human TGF $\beta$ 1 (#90900) for 18 hours. Luciferase activity was measured using ONE-Step™ Luciferase Assay System (#60690). The results are shown as fold induction of SBE luciferase reporter expression in relation to the activity of cells in the absence of agonist.

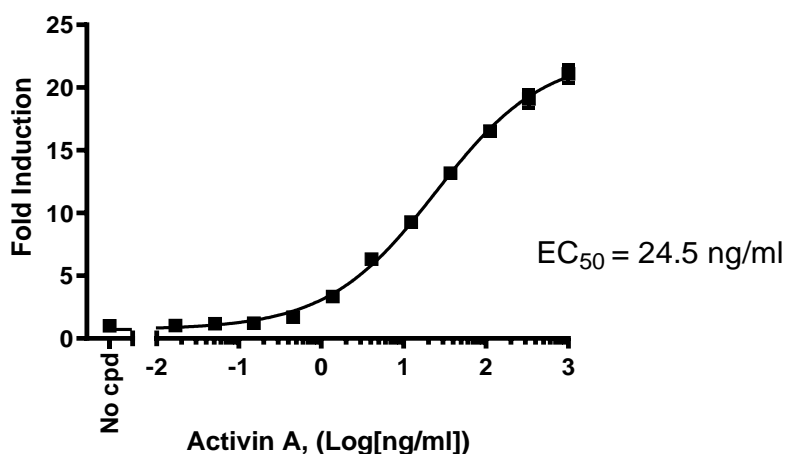


Figure 3: Reporter activation in the TGF $\beta$ /Activin A-Responsive Luciferase Reporter HEK293 Cell Line by the agonist Activin A.

TGF $\beta$ /Activin A-Responsive Luciferase Reporter HEK293 cells were incubated with increasing concentrations of Activin A (PeproTech #120-14E) for 18 hours. Luciferase activity was measured using ONE-Step™ Luciferase Assay System (BPS Bioscience #60690). The results are shown as fold induction of SBE luciferase reporter expression in relation to the activity of cells in the absence of agonist.

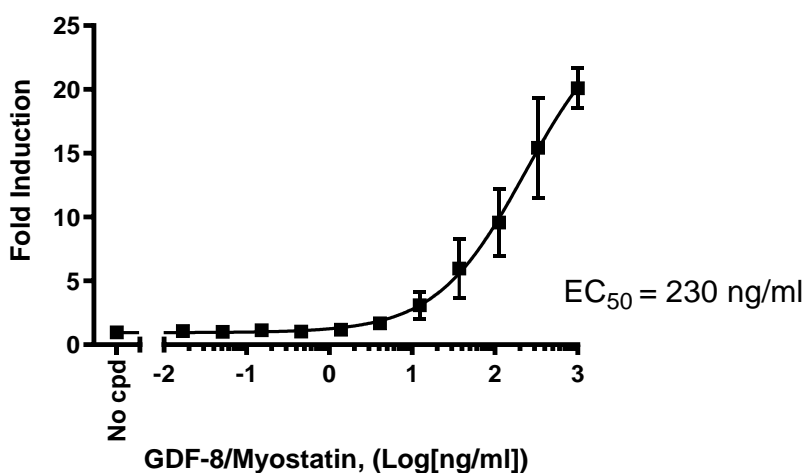


Figure 4: Reporter activation in the TGF $\beta$ /Activin A-Responsive Luciferase Reporter HEK293 Cell Line by the agonist GDF-8/Myostatin.

TGF $\beta$ /Activin A-Responsive Luciferase Reporter HEK293 cells were incubated with increasing concentrations of GDF-8/Myostatin for 18 hours. Luciferase activity was measured using ONE-Step™ Luciferase Assay System (#60690). The results are shown as fold induction of SBE luciferase reporter expression in relation to the activity of cells in the absence of agonist.

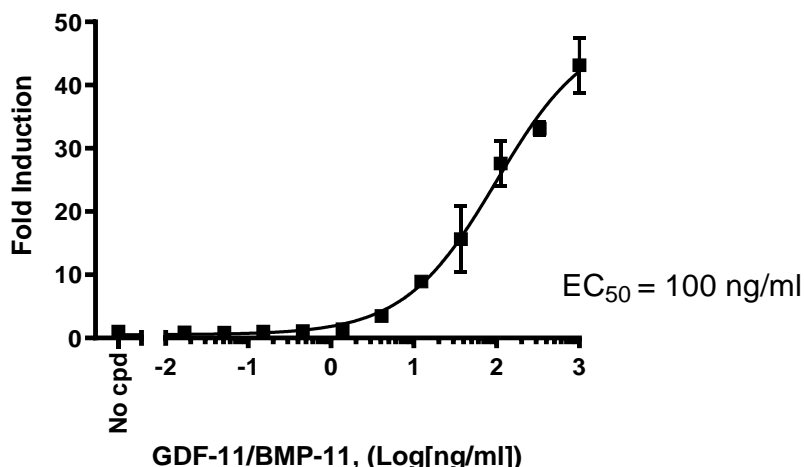


Figure 5: Reporter activation in the TGF $\beta$ /Activin A-Responsive Luciferase Reporter HEK293 Cell Line by the agonist GDF-11/BMP-11.

TGF $\beta$ /Activin A-Responsive Luciferase Reporter HEK293 cells were incubated with increasing concentrations of GDF-11/BMP-11 for 18 hours. Luciferase activity was measured using ONE-Step™ Luciferase Assay System (#60690). The results are shown as fold induction of SBE luciferase reporter expression in relation to the activity of cells in the absence of agonist.

#### B. Inhibition of agonist-induced reporter activity in the TGF $\beta$ /Activin A-Responsive Luciferase Reporter HEK293 Cell Line by an inhibitor of the TGF $\beta$ signaling pathway

- This experiment measures the effect of inhibitors of the TGF $\beta$  signaling pathway on agonist induced SBE reporter activation.
  - All conditions should be performed in triplicate.
  - The assay should include “Luminescence Background Control”, “Positive Control”, “Negative Control” and “Test Inhibitor” conditions.
1. Seed TGF $\beta$ /Activin A-Responsive Luciferase Reporter HEK293 cells at a density of ~35,000 cells/well in 100  $\mu$ l of Thaw Medium 1 into a clear-bottom, white 96-well plate. Include wells for a “Positive Control” (effect of the presence of agonist) and “Negative Control” (basal SBE reporter activity in the absence of agonist). Leave a few cell-free wells as “Luminescence Background Control”.

*Note: The cells must be treated with an agonist to measure the effect of an inhibitor.*

2. Incubate the plate at 37°C in a 5% CO<sub>2</sub> incubator for 24 hours.
3. 24 hours later, prepare a serial dilution of the test inhibitor (for example, TGF $\beta$  inhibitor SB525334) (90  $\mu$ l/ well).

3.1 For a small molecule inhibitor soluble in media, prepare a serial dilution in Thaw Medium 1B. For controls use Assay Medium 1B (Diluent Solution).

**OR**

3.2 For a small molecule inhibitor soluble in DMSO, prepare a stock solution in 100% DMSO at a concentration 1,000x higher than the highest desired final concentration, then dilute 1,000-fold with Thaw Medium 1B to prepare the highest concentration of the serial dilution. The concentration of DMSO is now 0.1% DMSO.

Use Assay Medium 1B + 0.1% DMSO to prepare the serial dilution and keep the concentration of DMSO constant.

For controls use Assay Medium 1B + 0.1% DMSO (Diluent Solution).

*Note: The concentration of DMSO should not exceed 0.1% in the final reaction.*

4. Carefully remove the medium from all wells.
5. Add 90 µl of each inhibitor dilution to the wells labeled as “Test Inhibitor” (the inhibitor is now at 1.1x).
6. Add 90 µl of Diluent Solution to the wells labeled as “Positive Control” and “Negative Control”.
7. Incubate the cells at 37°C in a 5% CO<sub>2</sub> incubator for ~4-5 hours.
8. Prepare the desired agonist at a concentration 10-fold higher than the desired final concentration using Assay Medium 1B (for example, prepare human TGFβ1 at 200 ng/ml for a final concentration of 20 ng/ml).
9. Add 10 µl of agonist dilution to the wells labeled as “Test Inhibitor” and “Positive Control”.
10. Add 10 µl of Assay Medium 1B to the wells labeled as “Negative Control”.
11. Add 100 µl of Assay Medium 1B to the “Luminescence Background Control”.
12. Incubate the cells at 37°C in 5% CO<sub>2</sub> overnight (~18 hours).
13. Add 100 µl of ONE-Step™ Luciferase reagent to each well and rock at room temperature for ~15 to 30 minutes.
14. Measure luminescence using a luminometer.
15. Data Analysis: Subtract the average background luminescence from the luminescence reading of all other wells. The percent luminescence of SBE Luciferase Reporter Activity is the background-subtracted luminescence of the inhibitor-treated wells divided by the average background-subtracted luminescence of the untreated wells (negative control without inhibitor), multiplied by 100.

$$\text{Percent luminescence} = \left( \frac{\text{Luminescence of Inhibitor Treated Wells} - \text{avg.background}}{\text{Avg.Luminescence of Untreated Wells} - \text{avg.background}} \right) \times 100$$

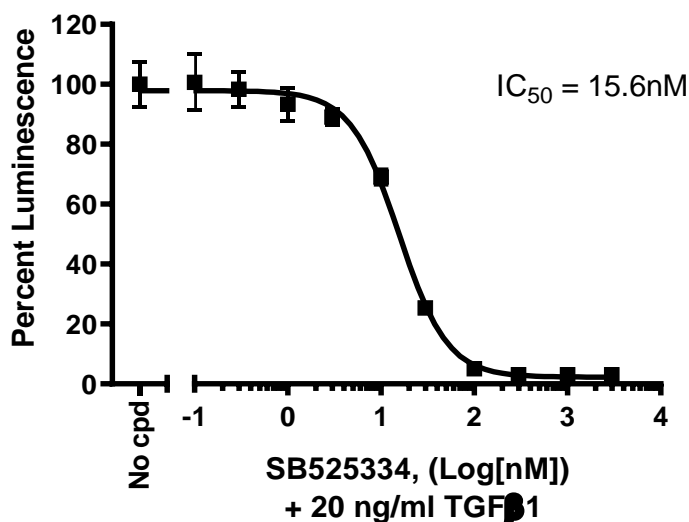


Figure 6: Inhibition of TGFβ1-induced reporter activity by SB525334 in the TGFβ/Activin A-Responsive Luciferase Reporter HEK293 Cell Line.

The cells were incubated with increasing doses of SB525334 prior to stimulation with human TGFβ1. The results are shown as percentage of luminescence in which cells stimulated with TGFβ1 in the absence of SB525334 was set at 100%.

## References

Moustakas A, *et al.*, 2001 *J. Cell Science*. 114: 4359-69.

## License Disclosure

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## Troubleshooting Guide

Visit [bpsbioscience.com/cell-line-faq](https://bpsbioscience.com/cell-line-faq) for detailed troubleshooting instructions. For all further questions, please email [support@bpsbioscience.com](mailto:support@bpsbioscience.com).

## Related Products

Products	Catalog #	Size
TGFβ1, Latent	90901	5 µg/25 µg/1 mg
TGFβR1, Fc fusion, Avi-Tag Recombinant	100644	20 µg
TGFβR2, GST-Tag Recombinant	40707	50 µg
PAI-1 (Luc) Reporter Mv1-Lu Cell Line	60544	2 vials
SBE Luciferase Reporter Lentivirus (TGFβ/SMAD Pathway)	79806	500 µl x 2
TGFBR2 CRISPR/Cas9 Lentivirus (Non-Integrating)	78536	500 µl x 2
TGFBR2 CRISPR/Cas9 Lentivirus (Integrating)	78535	500 µl x 2

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