

Description

The transforming growth factor beta (TGFβ)-Responsive SBE Reporter HEK293 Cell Line contains a stably integrated Firefly luciferase gene under the control of SMAD-responsive elements (SMAD binding elements, SBE). This cell line monitors the activity of the TGFβ/SMAD Signaling Pathway.

It is activated in response to human TGFβ1 and to the agonist Activin A. Luciferase activity induced by human TGFβ1 is decreased by SB525334, an inhibitor of TGFβ1-receptor.

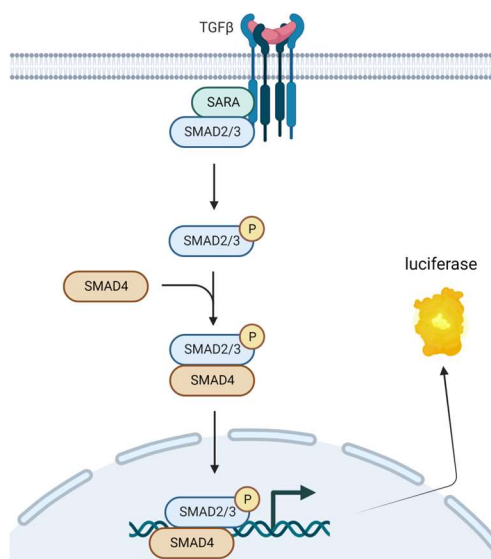


Figure 1: illustration of TGFβ/SMAD Signaling Pathway SBE Reporter HEK293 Cell Line.

Background

The TGFβ signaling pathway participates in diverse cell 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 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 therapeutical approaches in 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 ⁶ 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	BPS Bioscience #60187
Growth Medium 1B	BPS Bioscience #79531

Materials Used in Cellular Assay

Name	Ordering Information
Assay Medium 1B	BPS Bioscience #79617
Human TGFβ1	BPS Bioscience #90900
Recombinant Human/Murine/Rat Activin A	Pepro Tech #120-14E
Inhibitor SB525334	Selleckchem #356559-20-1
Clear-bottom, white 96-well tissue culture-treated plate	Corning #3610
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
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, 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 for maintaining the presence of the transfected gene(s) over passages. Cells should be grown at 37°C with 5% CO₂. 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

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.

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% CO₂ 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% CO₂ 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 Ca²⁺/Mg²⁺, 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 weekly or twice per 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 phosphate buffered saline (PBS) without Ca²⁺/Mg²⁺ 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 10⁶ 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 agonist

- 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” controls.
1. Seed SBE 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 3 wells empty as cell-free control wells (“Luminescence Background Control”). Incubate the plate at 37°C in a 5% CO₂ incubator.
 2. 24 hours later, carefully remove the medium from all wells and replace it with 90 µl of fresh Assay Medium 1B.
 3. Incubate cells at 37°C in a 5% CO₂ incubator for ~4-5 hours.
 4. Prepare serial dilutions of the agonist (for instance, human TGFβ1 or Activin A) in Assay Medium 1B at concentrations 10-fold higher than the desired final concentrations.
 5. Add 10 µl of each agonist dilution to the wells labeled as “Stimulated”.
 6. Add 10 µl of Assay Medium 1B to the wells labeled as “Unstimulated”.
 7. Add 100 µl of Assay Medium 1B to the wells not containing cells and labeled as “Luminescence Background Control”.
 8. Incubate the plate at 37°C in a CO₂ incubator overnight (~18 hours).
 9. Add 100 µl of ONE-Step™ Luciferase reagent to each well and rock at Room Temperature (RT) for ~15 to 30 minutes.
 10. Measure luminescence using a luminometer.

11. 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.

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

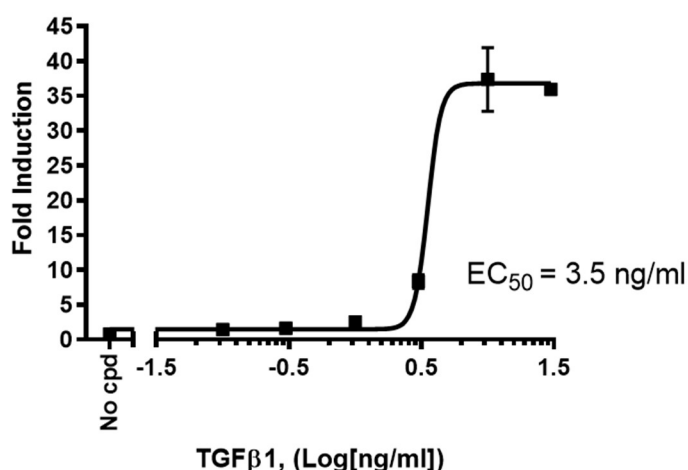


Figure 1: Reporter activation by the agonist TGFβ1.

TGFβ-Responsive SBE Reporter HEK293 Cells were incubated with increasing concentrations of human TGFβ1 (BPS Bioscience #90900) for 18 hours. Luciferase activity was measured using the 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.

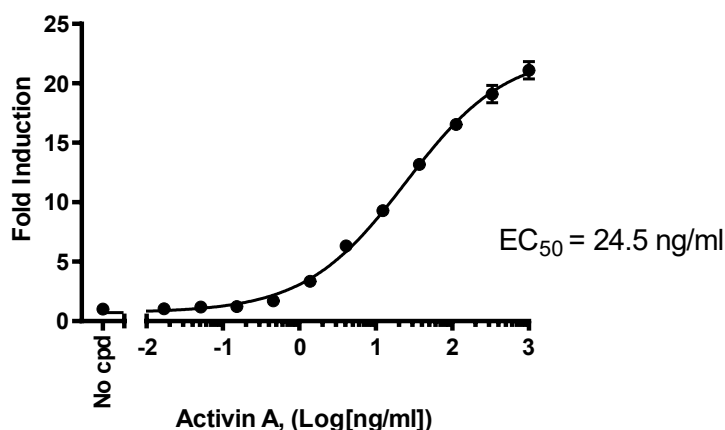


Figure 2: Reporter activation by the agonist Activin A.

TGFβ-Responsive SBE Reporter HEK293 Cells were incubated with increasing concentrations of Activin A (PeproTech #120-14E) for 18 hours. Luciferase activity was measured using the 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.

B. Inhibition of agonist-induced reporter activity by an inhibitor of the TGF β signaling pathway in SBE reporter HEK293 cells

- This experiment measures the effect of inhibitors of the TGF β signaling pathway on the 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”.
1. Seed SBE Reporter HEK293 cells at a density of ~35,000 cells/well in 100 μ 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 3 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% CO₂ incubator for 24 hours.
3. 24 hours later, prepare a serial dilution of the test inhibitor (for example, TGF β inhibitor SB525334) in 90 μ l/well using Assay Medium 1B.
 - a. For a small molecule inhibitor soluble in DMSO, prepare a stock solution in 100% DMSO at a concentration 220x higher than the highest desired final concentration, then dilute 200-fold with Thaw Medium 1B to prepare the highest concentration of the serial dilution. The concentration of DMSO is now 0.5% DMSO.

Use Thaw Medium 1B + 0.5% DMSO to prepare the serial dilution and keep the concentration of DMSO constant.

For controls use Thaw Medium 1B + 0.5% DMSO (Diluent Solution). The concentration of DMSO should not exceed 0.5% in the final reaction.
 - b. For a small molecule inhibitor soluble in media, prepare a serial dilution in Thaw Medium 1B. For controls use Thaw Media 1B (Diluent Solution).

4. Carefully remove the medium from all wells.
5. Add 90 μ l of each inhibitor dilution to the wells labeled as “Test Inhibitor” (the inhibitor is now at 1.1x).
6. Add 90 μ l of Diluent Solution to the wells labeled as “Positive Control” and “Negative Control”.
7. Incubate the cells at 37°C in a 5% CO₂ 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 TGF β 1 at 200ng/ml for a final concentration of 20 ng/ml).
9. Add 10 μ l 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% CO₂ 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.

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

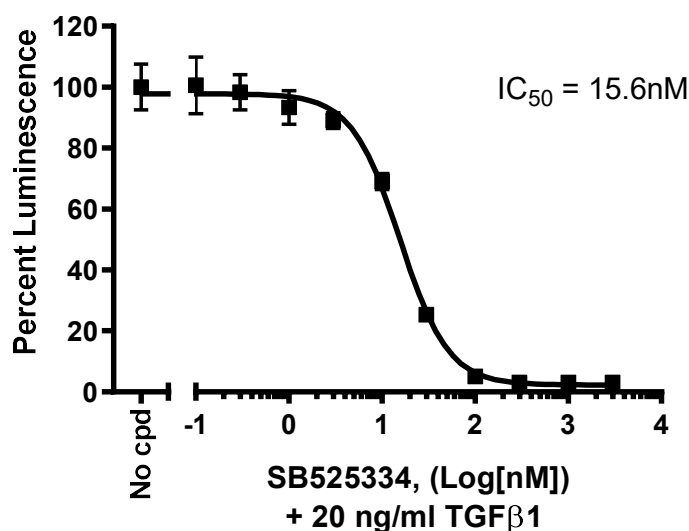


Figure 3: Inhibition of TGFβ1-induced reporter activity by SB525334 in TGFβ-Responsive SBE reporter HEK293 cells.

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.

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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

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
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