Description

The transforming growth factor beta (TGFβ) / SMAD Signaling Pathway 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 is designed to monitor the activity of the TGFβ/SMAD Signaling Pathway. It has been validated for its response to human TGFβ1 stimulation and to treatment with an inhibitor of the TGFβ/SMAD signaling pathway.

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 signaling proteins SMAD2 and SMAD3, which then form a complex with SMAD4. The SMAD complex translocates 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.

Application

- Monitor TGFβ signaling pathway activity
- Screen activators or inhibitors of TGFβ/SMAD 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

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
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, 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 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 **(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 1 **(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 1 **(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 1B **(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 0.05% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 1B 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 1B **(contains Geneticin)**. Seed into new culture vessels at the desired sub-cultivation ratio of 1/5 to 1/10 weekly or twice per week.

   Note: Just after thawing and at low density, the cells may grow at a slower rate. It is recommended to split the cells at a 1/4 ratio to start with. 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) 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 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

- 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.
- Perform the experiment in triplicates.
- Dose-response of agonist and antagonist is best performed in 3-fold serial dilutions.

A. Reporter activation by human TGFβ1 (agonist)

1. Seed SBE Reporter HEK293 cells in a clear-bottom, white 96-well plate at a density of ~35,000 cells per well in 100 µl of Thaw Medium 1. Leave 3 wells empty as cell-free control wells ("Background"). Incubate the plate at 37°C in a 5% CO_2 incubator.

2. 24 hours later, carefully remove the medium from all wells and replace it with 90 µl of fresh Assay Medium 1B. Incubate cells at 37°C in a 5% CO_2 incubator for ~4-5 hours.

3. Prepare serial dilutions of the agonist (we used human TGFβ1) in Assay Medium 1B at concentrations 10-fold higher than the desired final concentrations.
   a. Add 10 µl of each agonist dilution to the wells labeled as "Stimulated cells"
   b. Add 10 µl of Assay Medium 1B to the wells labeled as "Unstimulated control".
   c. Add 100 µl of Assay Medium 1B to the wells not containing cells labeled as "Background".

4. Incubate the plate at 37°C in a CO_2 incubator overnight (~18 hours).

5. Prepare the reagents of the ONE-Step™ Luciferase Detection System according to the corresponding protocol. Add 100 µl of ONE-Step™ Luciferase reagent to each well and rock at room temperature for ~15 to 30 minutes. Measure luminescence using a luminometer.

6. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells. The fold induction of SBE luciferase reporter expression is the average background-subtracted luminescence of TGFβ1-stimulated wells divided by the average background-subtracted luminescence of unstimulated control wells.

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

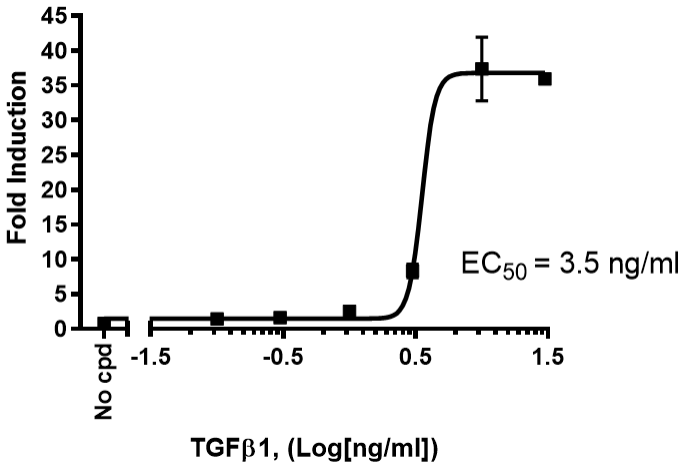

Figure 1: Reporter activation by agonist TGFβ1.
TGFβ/SMAD Signaling Pathway SBE Reporter HEK293 Cells were incubated with increasing concentrations of human TGFβ1 (BPS Bioscience #90900) for 18 hours. Luciferase activity was measured using the ONE-Step™ Luciferase Detection System (BPS Bioscience #60690). The results are shown as fold induction of SBE luciferase reporter expression and the EC_{50} was calculated.

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

1. Seed SBE Reporter HEK293 cells in a clear-bottom, white 96-well plate at a density of ~35,000 cells per well in 100 µl of Thaw Medium 1. Include enough control wells to evaluate both the presence ("Positive control") and absence ("Negative control") of agonist. Leave 3 wells empty as cell-free control wells ("Background").

   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_2 incubator.

3. 24 hours later, prepare a serial dilution of the test inhibitor (we used TGFβ inhibitor SB525334) in 90 µl/well using Assay Medium 1B.

   Note: Keep final DMSO concentration at or below 0.5%.

4. Carefully remove the medium from all wells.
   a. Add 90 µl of each inhibitor dilution to the wells labeled as "Inhibitor".
   b. Add 90 µl of Assay Medium 1B (without SB525334) to the wells labeled as "Positive control" and "Negative Control"
   c. Incubate the cells at 37°C in a 5% CO_2 incubator for ~4-5 hours.

5. Prepare the desired agonist at a concentration 10-fold higher than the desired final concentration using Assay Medium 1B (we used human TGFβ1 at a final concentration of 20 ng/ml).
   a. Add 10 µl of agonist dilution to the wells labeled as "Inhibitor" and "Positive control".
   b. Add 10 µl of Assay Medium 1B to the wells labeled as "Negative control".
   c. Add 100 µl of Assay Medium 1B to the wells not containing cells labeled as "Background".

6. Incubate the cells at 37°C in a 5% CO_2 incubator overnight (~18 hours).

7. Prepare the reagents of the ONE-Step™ Luciferase Detection System according to the corresponding protocol. Add 100 µl of ONE-Step™ Luciferase reagent to each well and rock at room temperature for ~15 to 30 minutes. Measure luminescence using a luminometer.

8. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells. The percent luminescence of SBE Luciferase Reporter Activity is the background-subtracted luminescence of the treated wells divided by the average background-subtracted luminescence of the untreated wells, multiplied by 100.

$$Percent\ luminescence = \left(\frac{Luminescence\ of\ Treated\ Wells - avg.background}{Avg.Luminescence\ of\ Untreated\ Wells - avg.background}\right) x100$$

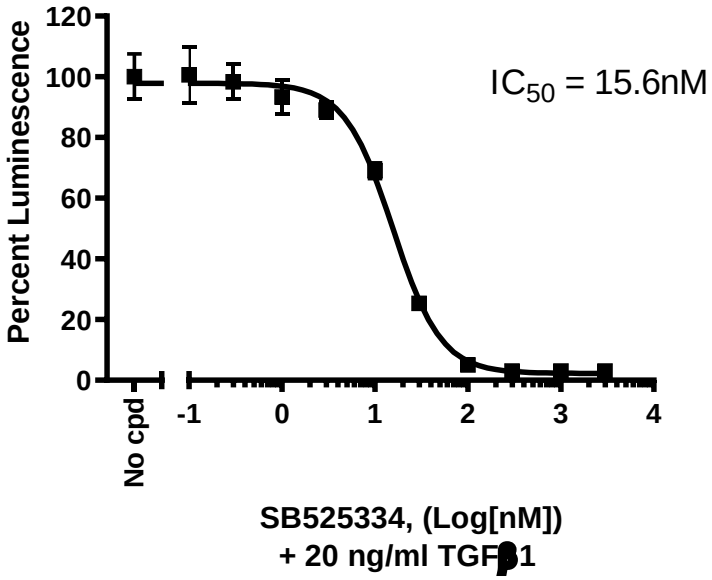

Figure 2: Inhibition of TGFβ1-induced reporter activity by SB525334 in SBE reporter HEK293 cells. The cells were incubated with increasing doses of SB525334 prior to stimulation with human TGFβ1. The results are shown as percentage of luminescence in which the background-subtracted luminescence of cells stimulated with TGFβ1 in the absence of SB525334 was set at 100%.

References

Moustakas A, *et al.* (2001) Smad regulation in TGF-beta signal transduction. *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

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