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Data Sheet

TGFβ/SMAD Signaling Pathway SBE Reporter – HEK293 Cell Line
Catalog #: 60653

Background
The *SBE Reporter – HEK293 Cell Line* is designed for monitoring the activity of the TGFβ/SMAD signaling pathway. The transforming growth factor beta (TGFβ) signaling pathway is involved in a diverse range of cell processes such as differentiation, cell cycle arrest, and immune regulation. TGFβ signaling has been linked to cardiac disease, cancer, Alzheimer's and other human diseases. TGFβ proteins bind to receptors on the cell surface, initiating a signaling cascade that leads to phosphorylation and activation of SMAD2 and SMAD3, which then form a complex with SMAD4. The SMAD complex then translocates to the nucleus and binds to the SMAD binding element (SBE) in the nucleus, leading to transcription and expression of TGFβ/ SMAD responsive genes.

Description
The *TGFβ/SMAD Signaling Pathway SBE Reporter – HEK293 Cell Line* contains a firefly luciferase gene under the control of SMAD-responsive elements stably integrated into HEK293 cells. This cell line is validated for its response to the stimulation of human TGFβ1 and to treatment with an inhibitor of the TGFβ/SMAD signaling pathway.

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

General culture conditions

Thaw Medium 1 (BPS Cat. #60187): MEM medium (Hyclone #SH30024.01) + 10% FBS (Invitrogen #26140-079) + 1% non-essential amino acids (Hyclone #SH30238.01) + 1 mM Na pyruvate (Hyclone #SH30239.01) + 1% Penicillin/Streptomycin (Hyclone SV30010.01)

Growth Medium 1B (BPS Cat. #79531): Thaw Medium 1 (BPS Cat. #60187) plus 400 µg/ml of Geneticin (Invitrogen #11811031).

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Cells should be grown at 37°C with 5% CO_2 using Growth Medium 1B.

If culturing cells in medium from other vendors, it may be required to lower the percentage of CO_2 in the incubator depending on the $NaHCO_3$ level in the basal medium.

To thaw the cells, 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 Thaw Medium 1 (**no Geneticin)**, spin down cells at 1000 rpm, resuspend cells in 5 ml of pre-warmed Thaw Medium 1 (**no Geneticin)**, transfer resuspended cells to T25 flask and culture at 37°C in a 5% CO_2 incubator overnight. The next day, replace the medium with fresh warm Thaw Medium 1 (**no Geneticin)**, and continue growing culture in a CO_2 incubator at 37°C until the cells are ready to be split. Cells should be split before they reach complete confluence. At first passage switch to Growth Medium 1B (**contains Geneticin)**.

To passage the cells, rinse cells with phosphate buffered saline (PBS), detach cells from culture vessel with 0.05% Trypsin/EDTA. After detachment, add Growth Medium 1B (**contains Geneticin)** and transfer to a tube, spin down cells, resuspend cells in Growth Medium 1B (**contains Geneticin)** and seed appropriate aliquots of cell suspension into new culture vessels. Sub cultivation ration: 1:5 to 1:10 weekly or twice a week.

Note: Just after thawing and at low density, the cells may grow at a slower rate. It is recommended to split the cells with ~ 1:4 ratio at the beginning of culturing. After several passages, the cell growth rate increases and the cells can be split with higher ratio.

To freeze down the cells, rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with 0.05% Trypsin/EDTA. After detachment, add Thaw Medium 1 (**no Geneticin)** and count the cells, then transfer to a tube, spin down cells, and resuspend in 4°C Freezing Medium (10% DMSO + 90% FBS) to ~$2x10^6$ cells/ml. Dispense 1 ml of cell aliquots into cryogenic vials. Place vials in an insulated container for slow cooling and store at -80°C overnight. Transfer to liquid nitrogen the next day for storage.
It is recommended to expand the cells and freeze down more than 10 vials of cells for future use at early passage.

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

- Human TGFβ1 (BPS Bioscience #90900-1)
- SB525334 (Selleckchem #356559-20-1): inhibitor of TGFβ pathway. Prepare stock solution in DMSO.
- Thaw Medium 1 (BPS Bioscience #60187)
- Assay Medium 1B (BPS Bioscience #79617)

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- Growth Medium 1B (BPS Bioscience #79531)
- 96-well tissue culture-treated white clear-bottom assay plate (Corning # 3610)
- ONE-Step™ Luciferase Assay System (BPS, Cat. #60690)
- Luminometer

A. Dose response of *SBE Reporter – HEK293* cells to human TGFβ1.

1. Harvest SBE reporter-HEK293 cells from culture in Growth Medium 1B and seed cells at a density of ~35,000 cells per well into a white clear-bottom 96-well microplate in 100 µl of Thaw Medium 1 (BPS Bioscience #60187). Incubate the plate at 37°C in a CO_2 incubator.

2. 24 hours after seeding, change all wells to 90 µl of fresh Assay Medium 1B. Incubate cells at 37°C in a CO_2 incubator for ~ 4-5 hours.

   Assay Medium 1B (BPS Bioscience #79617): MEM Medium, + 0.5% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin

3. Set up each treatment in at least triplicate.
   a. Add 10 µl of threefold serial dilution of human TGFβ1 in Assay Medium 1B to stimulated wells.
   b. Add 10 µl of Assay Medium 1B to the unstimulated control wells.
   c. Add 100 µl of Assay Medium 1B to cell-free control wells (for determining background luminescence).

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

5. Perform luciferase assay using ONE-Step™ Luciferase Assay System according to the protocol provided: Add 100 µl of ONE-Step™ Luciferase reagent per well and rock at room temperature for ~15 to 30 minutes. Measure luminescence using a luminometer. *If using other luciferase reagents from other vendors, follow the manufacturer's assay protocol.*

6. Data Analysis: Subtract average background luminescence (cell-free control wells) from the luminescence reading of all wells.

Note: The fold induction of SBE luciferase reporter expression = average background-subtracted luminescence of TGFβ1-stimulated wells / average background-subtracted luminescence of unstimulated control wells

Figure 1. Dose response of SBE reporter-HEK293 cells to human TGFβ1.
The results are shown as fold induction of SBE luciferase reporter expression. The EC50 of hTGFβ1 is ~ 3.5 ng/ml.

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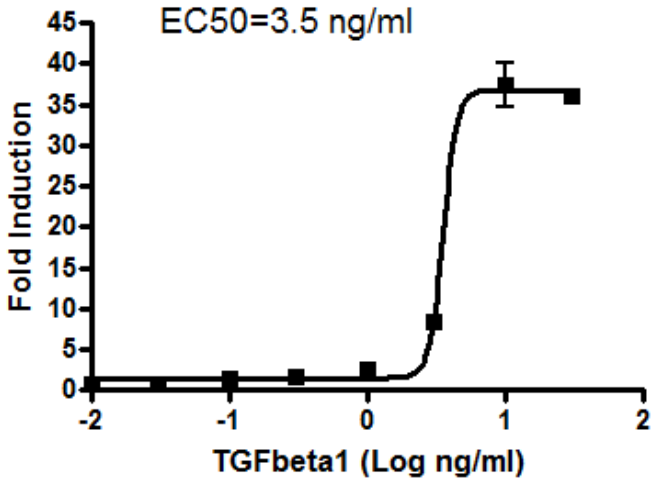

B. Inhibition of TGFβ1-induced reporter activity by an inhibitor of TGFβ signaling pathway in SBE reporter-HEK293 cells

1. Harvest SBE reporter-HEK293 cells from culture in Growth Medium 1B and seed cells at a density of ~35,000 cells per well into a white clear-bottom 96-well microplate in 100 µl of Thaw Medium 1 (BPS Bioscience #60187). Incubate the plate at 37°C in a CO_2 incubator.

2. 24 hours after seeding, treat cells with threefold serial dilution of SB525334 in 90 µl Assay Medium 1B. Incubate cells at 37°C in a CO_2 incubator for ~4-5 hours. For control wells without SB525334, change to 90 µl Assay Medium 1B with no treatment.

   Assay Medium 1B (BPS Bioscience #79617): MEM Medium, + 0.5% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin

3. Set up each treatment in at least triplicate.
   a. Add 10 µl of diluted human TGFβ1 in Assay Medium 1B to stimulated wells (final TGFβ1 concentration = 20 ng/ml).
   b. Add 10 µl of Assay Medium 1B to the unstimulated control wells (for determining the basal activity).
   c. Add 100 µl of Assay Medium 1B to cell-free control wells (for determining background luminescence).

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

5. Perform luciferase assay using ONE-Step™ Luciferase Assay System according to the protocol provided: Add 100 µl of ONE-Step™ Luciferase reagent per well and rock at room

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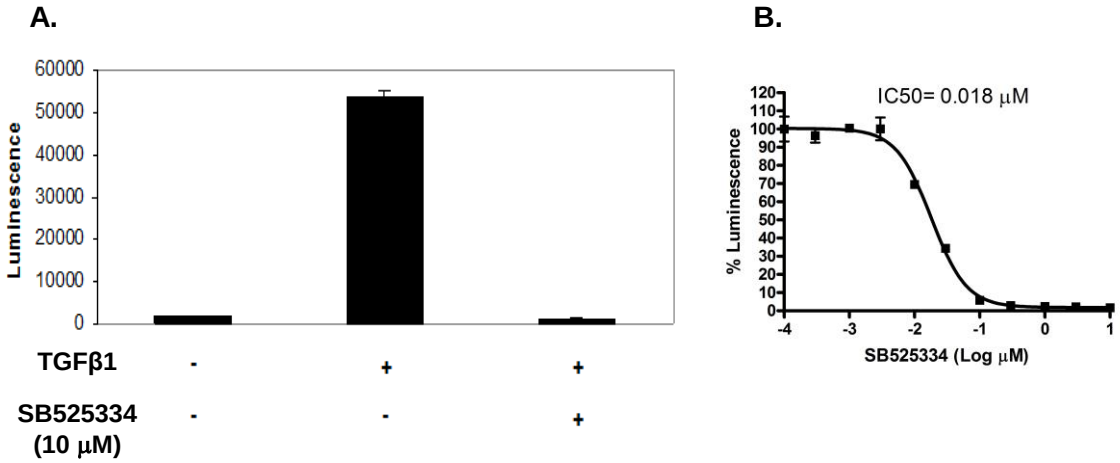
temperature for ~15 to 30 minutes. Measure luminescence using a luminometer. *If using other luciferase reagents from other vendors follow the manufacturer's assay protocol.*

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

Figure 2. Inhibition of TGFβ1-induced reporter activity by SB525334 in SBE reporter-HEK293 cells. (A) SB525334 blocks TGFβ1-induced SBE reporter activity. **(B)** SB525334 inhibition dose response curve. The results are shown as percentage of luminescence. The background-subtracted luminescence of cells stimulated with TGFβ1 in the absence of SB525334 was set at 100%.



Reference

1. Moustakas, A., *et al.* (2001) Smad regulation in TGF-beta signal transduction. *J. Cell Science.* **114(Pt 24):** 4359-69.

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Related Products

Product	Cat. #	Size
ONE-Step™ Luciferase Assay System	60690-1	10 ml
ONE-Step™ Luciferase Assay System	60690-2	100 ml
TGFβ1	90900-1	1 µg
TGFβ1	90900-2	5 µg
TGFβ1	90900-10	10 µg
TGFβ1	90900-3	1 mg
TGFβ1, Latent	90901-1	5 µg
TGFβ1, Latent	90901-2	25 µg
TGFβ1, Latent	90901-3	1 mg
TGFβR2, GST-tag	40707	50 µg
PAI-1 Reporter (Luc) – Mv1 Lu Cell Line	60544	2 vials

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