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Data Sheet

SBE Luciferase Reporter Lentivirus (TGFβ/SMAD Pathway)

Catalog #: 79806

Background

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 SBE Luciferase Reporter Lentivirus (TGFβ/SMAD signaling pathway) are replication incompetent, HIV-based, VSV-G pseudotyped lentiviral particles that are ready to be transduced into almost all types of mammalian cells, including primary and non-dividing cells. The particles contain a firefly luciferase gene driven by multimerized SBE-responsive element located upstream of the minimal TATA promoter (Figure 1). After transduction, activation of the TGFβ/SMAD signaling pathway can be monitored by measuring the luciferase activity.

Application

- Screen for activators or inhibitors of the TGFβ/SMAD signaling pathway in transduced target cells
- Generation of SBE Luciferase Reporter stable cell line

Formulation

The lentiviruses were produced from HEK293T cells in medium containing 90% DMEM + 10% FBS.

Titer

Two vials (500 µl x 2) of SBE luciferase reporter lentivirus at a titer ≥5 x 10^6 TU/ml. The titer will vary with each lot; the exact value is provided with each shipment.

Storage

Lentiviruses are shipped with dry ice. For long term storage, it is recommended to store the virus at -80°C. Avoid repeated freeze-thaw cycles. Titers can drop significantly with each freeze-thaw cycle.

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Biosafety

The lentiviruses are produced with the third generation SIN (self-inactivation) lentivector which ensures self-inactivation of the lentiviral construct after transduction and integration into the genomic DNA of the target cells. None of the HIV genes (gag, pol, rev) will be expressed in the transduced cells, as they are expressed from packaging plasmids lacking the packing signal.

Although the pseudotyped lentiviruses are replication-incompetent, they require the use of a Biosafety Level 2 facility. BPS recommends following all local federal, state, and institutional regulations and using all appropriate safety precautions.

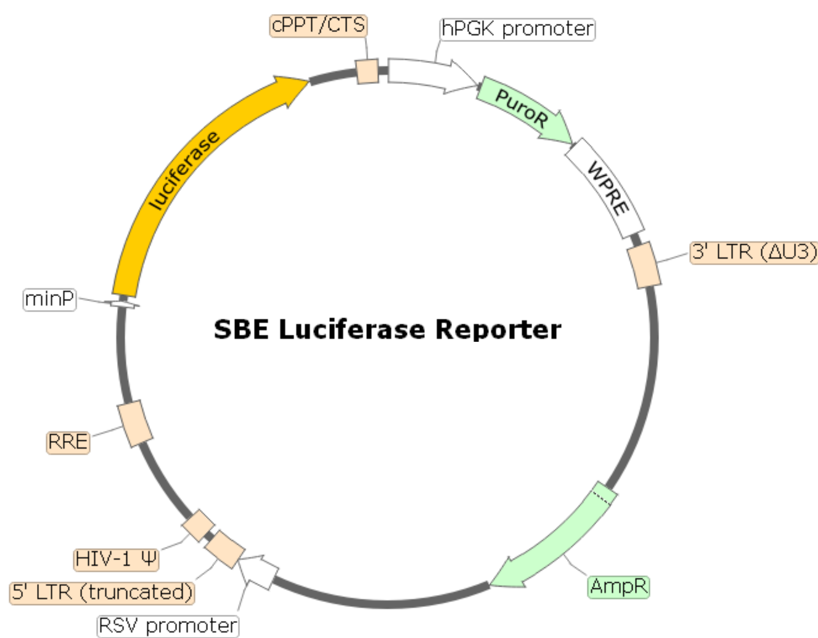

Figure 1. Schematic of the lenti-vector used to generate the SBE luciferase reporter lentivirus

Materials Required but Not Supplied

- Human TGFβ1 (BPS Bioscience, #90900-1)
- HEK293 growth medium
  Thaw Medium 9 (BPS Bioscience, #79665): MEM with 10% FBS, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate
- Assay Medium 1B (BPS Bioscience, #79617): MEM with 0.5% FBS, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 1% Pen/Strep
- Polybrene (Millipore, #TR-1003-G)
- 96-well tissue culture treated white clear-bottom assay plate (Corning, #3610)
- ONE-Step™ luciferase assay system (BPS Bioscience, #60690)
- Luminometer

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Assay Protocol

The following protocol is a general guideline for transducing HEK293 cells using SBE luciferase reporter lentivirus. The optimal transduction conditions (e.g. MOI, concentration of polybrene, time of assay development) should be optimized according to the cell type and the assay requirements. In most cell types, the expression of the reporter gene can be measured approximately 72 hours after transduction. For cell types with low transduction efficacy, it may be necessary to select the cells stably expressing the reporter gene with puromycin prior to carrying out the reporter assays.

1. Day 1: Harvest HEK293 cells from culture and seed cells at a density of 5,000-10,000 cells per well into white opaque 96-well microplate in 50 µl of Thaw Medium 9 (BPS Bioscience #79665). Incubate cells at 37°C with 5% CO_2 overnight.

2. Day 2: To each well add 10 µl of SBE luciferase reporter lentivirus. Add polybrene to each well at a final concentration of 5 µg/ml. Gently swirl the plate to mix. Incubate the plate at 37°C with 5% CO_2 for 40-48 hours.

   Alternatively, seeding cells and the transduction can be performed on the same day.

3. Day 4 morning: Remove the medium containing the lentivirus from the wells. Add 90 µl of fresh assay medium 1B (BPS Bioscience #79617) to each well. Incubate cells at 37°C with 5% CO_2 for ~4-5 hours.

   Day 4 afternoon: Prepare diluted TGFβ1 in assay medium 1B. Add 10 µl of diluted TGFβ1 to the TGFβ1-stimulated wells. Add 10 µl of assay medium 1B to the unstimulated control wells (for measuring the uninduced level of SBE reporter activity).

4. Incubate at 37°C with 5% CO_2 overnight (~18 hours).

5. Prepare the ONE-Step™ Luciferase reagent per recommended protocol. Add 100 µl of ONE-Step™ Luciferase Assay reagent per well. Incubate at room temperature for ~15 to 30 minutes and measure luminescence using a luminometer.

Important Notes:

1. To generate the SBE luciferase reporter stable cell line, on day 4 remove Thaw Medium 9 (BPS Bioscience #79665) and replace it with fresh growth medium containing the appropriate amount of puromycin for antibiotic selection of transduced cells.

2. The following Lenti Reporter Controls are also available from BPS Bioscience to meet your experimental needs:

   1) Negative Control Lentivirus (BPS Bioscience, #79578): Ready-to-transduce lentiviral particles expressing firefly luciferase under the control of a minimal promoter. The negative control is important to establish the specificity of any treatments and to determine the background reporter activity.

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2) Renilla Luciferase (Rluc) Lentivirus (BPS Bioscience, #79565): Ready-to-transduce lentiviral particles expressing Renilla luciferase under the CMV promoter. The RLuc lentivirus can serve as an internal control to overcome sample-to-sample variability when performing dual-luciferase reporter assays.

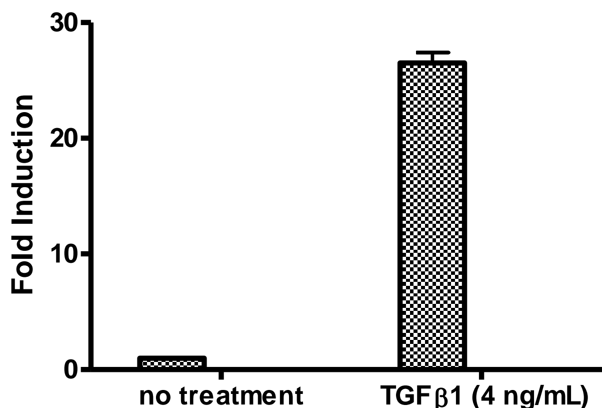

Figure 2. SBE luciferase reporter activity stimulated by human TGFβ1 in HEK293 cells. Approximately 10,000 HEK293 cells/well were transduced with 100,000 TU/well SBE luciferase reporter lentivirus. After 48 hours of transduction, medium was changed to assay medium 1B. After 54 hours of transduction, cells were treated with 4 ng/mL of TGFβ1 for 18 hours. The results are shown as fold induction of luciferase reporter expression. Fold induction was determined by comparing values against the mean value for control cells without TGFβ1 treatment.

Related Products

Product	Cat. #	Size
TGFβ/SMAD Signaling Pathway SBE Reporter-HEK293	60653	2 vials
SBE Reporter Kit (TGFβ/SMAD Signaling Pathway	60654	500 rxns
NFκB Luciferase Reporter Lentivirus	79564	500 µl x2
CRE Luciferase Reporter Lentivirus	79580	500 µl x2
STAT3 Luciferase Reporter Lentivirus	79744	500 µl x2
Negative Control Lentivirus	79578	500 µl x2
Renilla Luciferase (Rluc) Lentivirus	79565	500 µl x2
Firefly Luciferase Lentivirus (G418 and Hygromycin)	79692	500 µl x2
Human TGFβ1	90900-10	10 µg
Thaw Medium 9	79665	100 ml
Assay Medium 1B	79617-1	100 ml

References

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