Description

The SBE Dual-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 reporter driven by multimerized SBE (SMAD binding elements)-responsive element located upstream of the minimal TATA promoter (Figure 1). They also include a Renilla Luciferase-P2A-puromycin sequence downstream of the SBE Luciferase Reporter cassette, driven by a hPGK promoter, to facilitate signal normalization and antibiotic selection. After transduction, activation of the TGFβ/SMAD signaling pathway can be monitored by measuring firefly luciferase activity.

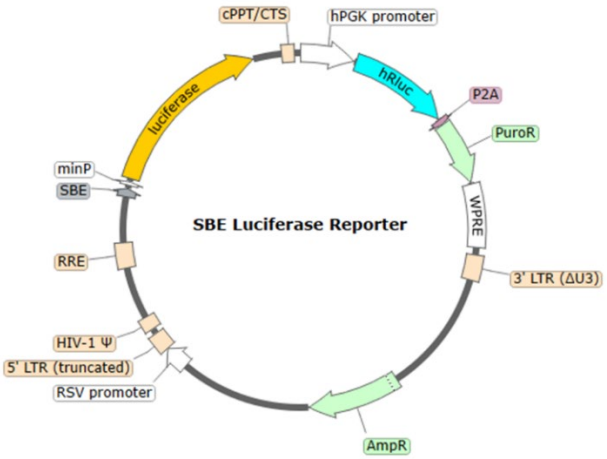

Figure 1. Schematic of the lenti-vector used to generate the SBE Dual-Luciferase Reporter Lentivirus (TGFβ/SMAD Signaling Pathway).

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(s)

- Transduce a conditional firefly luciferase reporter and a constitutive Renilla luciferase reporter in mammalian cells.
- Screen for activators or inhibitors of the TGFβ/SMAD signaling pathway in transduced target cells.
- Generate SBE Dual-Luciferase Reporter stable cell lines.

Formulation

The lentivirus particles were produced from HEK293T cells. They are supplied in cell culture medium containing 90% DMEM + 10% FBS.

Size and Titer

Two vials (500 µl x 2) of lentiviruses at a titer ≥10^7 TU/ml. The titer will vary with each lot; the exact value is provided with each shipment. Virus particles can be packaged in custom formulations by special request, for an additional fee.

Storage

Lentiviruses are shipped with dry ice. For long-term storage, it is recommended to store the lentiviruses at -80°C for up to 12 months from date of receipt. Avoid repeated freeze-thaw cycles. Titers can drop significantly with each freeze-thaw cycle.

Biosafety

The lentiviruses are produced with a SIN (self-inactivation) lentivector which ensures self-inactivation of the lentiviral construct after transduction and after 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 and are not present in the lentivirus particle. Although the pseudotyped lentiviruses are replication-incompetent, they require the use of a Biosafety Level 2 facility. BPS Bioscience recommends following all local federal, state, and institutional regulations and using all appropriate safety precautions.

Materials Required but Not Supplied

These materials are not supplied with the Lentivirus but are necessary the cellular assays described below. BPS Bioscience's reagents are validated and optimized for use with this lentivirus and are highly recommended for best results.

Name	Ordering Information
Thaw Medium 9	BPS Bioscience #79665
Assay Medium 1B	BPS Bioscience #79617
Lenti-Fuse™ Polybrene Viral Transduction Enhancer	BPS Bioscience #78939
TGFβ1 Recombinant	BPS Bioscience #90900
96-well tissue culture treated white clear-bottom assay plate	Corning #3610
TWO-Step Luciferase (Firefly & Renilla) Assay System	BPS Bioscience #60683
Luminometer	

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.

Media Required for HEK293 Cell Culture and Functional Cellular Assay

Thaw Medium 9 (BPS Bioscience #79665):
MEM with 10% FBS, 0.1 mM non-essential amino acids, 1 mM sodium pyruvate.

Assay Medium 1B (BPS Bioscience #79617):
MEM with 0.5% FBS, 0.1 Mm non-essential amino acids, 1 mM sodium pyruvate, 1% Pen/Strep.

Assay Protocol

- The following protocol is a general guideline for transducing HEK293 cells using SBE Dual-Luciferase Reporter Lentivirus (#83059). The optimal transduction conditions (e.g. MOI, concentration of Lenti-Fuse™ Polybrene Viral Transduction Enhancer, time of assay development) should be optimized according to the cell type and the assay requirement. In most cell types, the expression of the reporter 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 with zeocin prior to carrying out the reporter assays.
- The assay should include "Stimulated", "Cell-Free Control" and "Untreated Control" conditions.

Day 1:

1. Seed HEK293 cells at a density of ~8,000 cells per well in 90 µl of Thaw Medium 1 into a white, clear bottom 96-well microplate.

2. Incubate the plate at 37°C with 5% CO_2 for 18-24 hours.

Day 2:

1. To each well, add 10 µl of SBE Luciferase Reporter lentiviruses.

2. Add of Lenti-Fuse™ Polybrene Viral Transduction Enhancer to each well at a final concentration of 5 µg/ml.

3. Gently swirl the plate to mix.

4. Incubate the plate at 37°C with 5% CO_2 for 48 hours.

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

Day 3/4:

1. Remove the medium containing the lentiviruses from the wells.

2. Add 90 µl of fresh Assay Medium 1B (BPS Bioscience #79617) to each well.

3. Incubate cells at 37°C with 5% CO_2 for ~4-5 hours.

4. Prepare diluted TGFβ1 in Assay Medium 1B (10 µl/ well) at 10x the final desired concentration.

5. Add 10 µl of diluted TGFβ1 to the "Stimulated" wells.

6. Add 10 µl of Assay Medium 1B to the "Untreated Control" wells (for measuring the uninduced level of SBE reporter activity).

7. Incubate the plate at 37°C with 5% CO_2 overnight.

Day 4/5:

1. To measure Luciferase activity, use TWO-Step Luciferase (Firefly & Renilla) Assay System (60683) following the recommended protocol.

Notes

To generate a stable SBE luciferase reporter cell line, remove the growth medium 48 hours after transduction and replace it with fresh growth medium containing the appropriate amount of puromycin (as pre-determined from a killing curve, Kill Curve Protocol), for antibiotic selection of transduced cells.

Validation Data

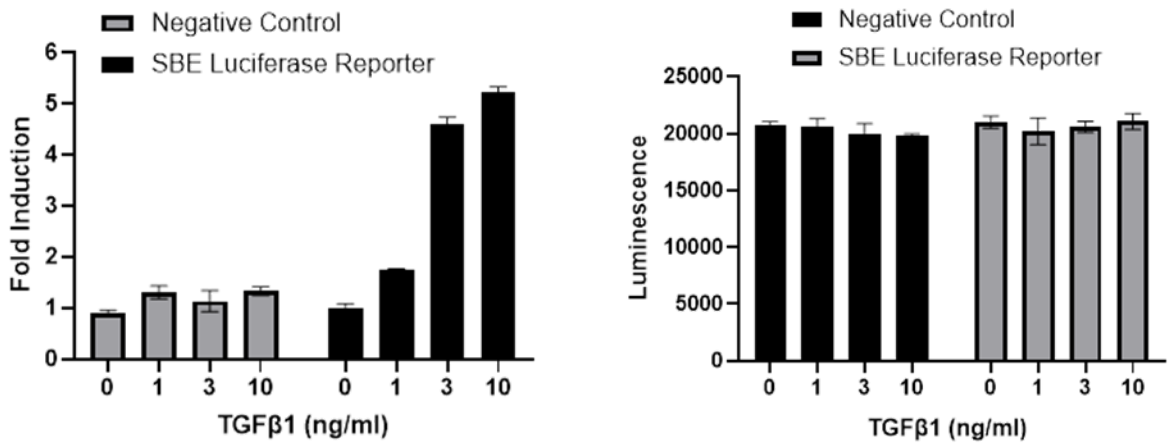

Figure 2. Luciferase reporter activity stimulated by human TGFβ1 in HEK293 cells transduced with SBE Dual-Luciferase Reporter Lentivirus (TGFβ/SMAD Signaling Pathway).
Approximately 10,000 HEK293 cells/well were transduced with 100,000 TU/well of SBE Dual-Luciferase Reporter Lentivirus (TGFβ/SMAD Signaling Pathway) or Negative control Dual-Luciferase Lentivirus (#83058). 48 hours post-transduction, the medium was changed to Assay Medium 1B. 54 hours post-transduction, cells were treated with TGFβ1 for 18 hours. Left panel: Fold induction of firefly luciferase reporter expression. Fold induction was determined by comparing values against the mean value for control cells without TGFβ1 treatment. Right Panel: Renilla luciferase reporter expression was determined in the same cells.

Data shown is representative.

References

Moustakas A,. *et al*., 2001 *J. Cell Sci*.114(Pt 24): 4359-69

Troubleshooting Guide

Visit bpsbioscience.com/lentivirus-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit https://bpsbioscience.com/contact.

Related Products

Products	*Catalog #*	*Size*
TGFβ/Activin A/Myostatin-Responsive Reporter HEK293 Cell Line	60653	2 vials
Transfection Collection™ : SBE Transient Pack (TGFβ/SMAD Signaling Pathway)	79272	500 µl x 2
SBE Luciferase Reporter Lentivirus (TGFβ/SMAD Pathway)	79806	500 µl x 2
Negative control Dual-Luciferase Lentivirus	83058	500 µl x 2

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