Description

The SRE (Serum Response Element) Luciferase Reporter Lentiviruses 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 the Serum Response Element located upstream of the minimal TATA promoter (Figure 1). After transduction, activation of the MAPK/ERK signaling pathway in the target cells can be monitored by measuring the luciferase activity.

Background

The MAPK/ERK signaling pathway is a major participant in the regulation of cell growth and differentiation. It can be activated by various extracellular stimuli including mitogens, growth factors, and cytokines. Upon stimulation, MEK1/2 phosphorylate and activate ERK1/2. The activated ERK1/2 translocate to the nucleus where they phosphorylate and activate transcription factors. The TCFs (Ternary Complex Factors), including transcription factor Elk1, are among the best-characterized substrates of ERK. When phosphorylated by ERK, Elk1 forms a complex with Serum Response Factor (SRF) and binds to the Serum Response Element (SRE), resulting in the expression of numerous mitogen-inducible genes.

Application

1. Screen for activators or inhibitors of MAPK/ERK signaling pathway in transduced target cells
2. Generate SRE Luciferase Reporter stable cell lines (puromycin resistant)

Formulation

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

Titer

Two vials (500 µl x 2) of lentivirus at a titer ≥10^7 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 lentiviruses at -80°C. Avoid repeated freeze-thaw cycles. Titers can drop significantly with each freeze-thaw cycle.

Biosafety

The lentiviruses are produced with a third generation 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 this lentivirus but are necessary to follow the protocol described in the "Validation Data" section. Media, reagents, and luciferase assay buffers used at BPS Bioscience are all validated and optimized for use with this lentivirus and are highly recommended for best results.

Name	Ordering Information
HEK293 cells	ATCC #CRL-1573
Thaw Medium 1	BPS Bioscience #60187
Assay Medium 1B	BPS Bioscience #79617
Recombinant human EGF	BPS Bioscience #90201-1
FBS	Invitrogen #26140-079
Polybrene	Millipore, #TR-1003-G
96-well tissue culture, clear-bottom, white plate	Corning, #3610
One-Step luciferase assay system	BPS Bioscience #60690
Luminometer	

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.

Media Required for the Proposed Assay

Thaw Medium 1 (BPS Bioscience, #60187):

MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin.

Assay Medium 1B (BPS Bioscience, #79617):

MEM medium supplemented with 0.5% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin.

Assay Protocol

The following protocol is a general guideline for transducing HEK293 cells using the SRE 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 the appropriate antibiotic 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, clear bottom 96-well microplate in 90 µl of Thaw Medium 1 (BPS Bioscience #60187).

   To each well, add 5 µl of SRE luciferase reporter lentivirus. *Optional: Add polybrene to each well to a final concentration of 5 µg/ml.*

   Gently swirl the plate to mix. Incubate the plate at 37°C with 5% CO_2 for 24 hours.

2. Day 2: Remove the medium containing the lentivirus from the wells. Add 50 µl of Assay Medium 1B to each well. Incubate the plate at 37°C with 5% CO_2 overnight.

3. Day 3: Prepare diluted human EGF or FBS in Assay Medium 1B.
   Add 50 µl of diluted EGF or FBS to stimulated wells.
   Add 50 µl of Assay Medium 1B to the unstimulated control wells.
   Add 100 µl of Assay Medium 1B to cell-free control wells (for determine background luminescence).

   Incubate the plate at 37°C with 5% CO_2 for 5-6 hours. Perform the ONE-Step™ Luciferase reagent per recommended protocol (100 µl/well). Incubate the plate at room temperature for ~15 to 30 minutes and measure luminescence using a luminometer.

Figures and Validation Data

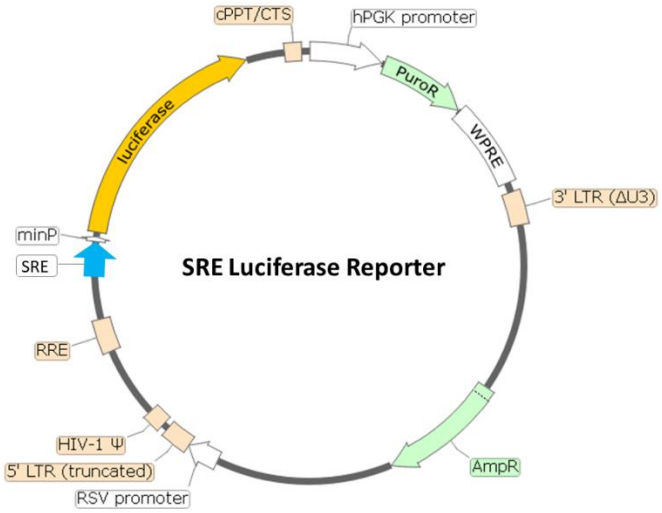

Figure 1. Schematic of the lenti-vector used to generate the SRE Luciferase Reporter Lentivirus.

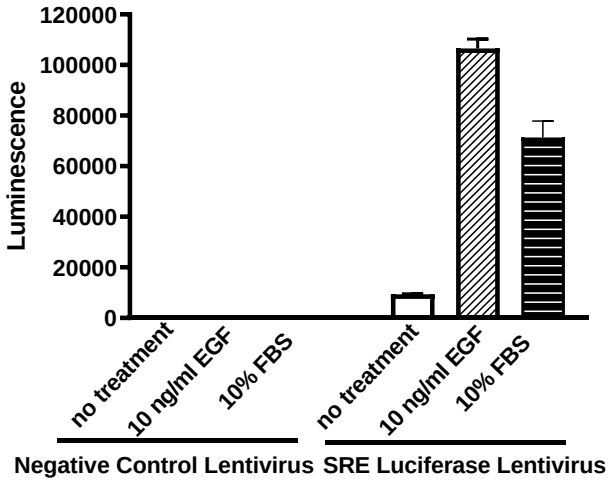

Figure 2. SRE luciferase reporter activity stimulated by human EGF or FBS in HEK293 cells. Approximately 10,000 HEK293 cells/well were transduced with 100,000 TU SRE Luciferase Reporter Lentivirus. After 24 hours of transduction, the medium was changed to Assay Medium 1B. After 48 hours of transduction, cells were stimulated with 10 ng/ml of human EGF or 10% FBS for 6 hours. Results are shown as the raw luminescence reading. The Negative Control Luciferase Lentivirus (BPS Bioscience #79578) was performed in in parallel as control.

Notes

1. To generate the SRE luciferase reporter stable cell line, remove the growth medium 48 hours after transduction and replace it with fresh growth medium containing the appropriate amount of puromycin for antibiotic selection of transduced cells.
2. The following Lentivirus Reporter Controls are available from BPS Bioscience to meet your experimental needs:
    a. Negative Control Luciferase 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 treatment and to determine the background reporter activity.
    b. Renilla Luciferase 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.
    c. Firefly Luciferase Lentivirus (BPS Bioscience #79692-G, #79692-H, #79692-P): Ready-to-transduce lentiviral particles expressing firefly luciferase under the CMV promoter. It serves as a positive control for transduction optimization studies.

Reading Luminescence

Luminescence is the emission of light resulting from a chemical reaction. The detection of luminescence requires no wavelength selection because the method used is emission photometry and not emission spectrophotometry.

To properly read luminescence, make sure the plate reader is set for LUMINESCENCE mode. Typical integration time is 1 second, delay after plate movement is 100 msec. Do not use a filter when measuring light emission. Typical settings for the Synergy 2 BioTek plate reader: use the "hole" position on the filter wheel; Optics position: Top; Read type: endpoint. Sensitivity may be adjusted based on the luminescence of a control assay.

References

1. Wong, K.K. Recent developments in anti-cancer agents targeting the Ras/Raf/ MEK/ERK pathway. *Recent Pat Anticancer Drug Discov*. 2009; 4(1): 28-35.
2. Treisman, R. The serum response element. *Trends Biochem Sci. 1992;* 17(10): 423-426.

Troubleshooting Guide

Visit bpsbioscience.com/lentivirus-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

Related Products

Products	*Catalog #*	*Size*
SRE Reporter Kit (MAPK/ERK Signaling Pathway)	60511	500 reactions
SRE Reporter - HEK293 Recombinant Cell Line (ERK Pathway)	60406	2 vials
SRE eGPF Reporter - HEK293 Cell Line (ERK Pathway)	78327	2 vials
Transfection Collection™: SRE Transient Pack (MAPK/ERK Signaling Pathway)	79271	500 reactions
CSF1R / SRE – Reporter HEK293 Recombinant Cell Line	79380	2 vials