Description

SRE TurboRFP Reporter Lentivirus are replication incompetent, HIV-based, VSV-G pseudotyped lentiviral particles that are ready to transduce almost all types of mammalian cells, including primary and non-dividing cells. The particles contain an TurboRFP (red fluorescent protein) reporter driven by the SRE (Serum Response Element) located upstream of the minimal TATA promoter. The lentiviruses also transduce a hygromycin selection gene (Figure 1). After transduction, activation of the MAPK (mitogen-activated protein kinase)/ERK (extracellular regulated kinase) signaling pathway in target cells can be monitored by assessing RFP expression.

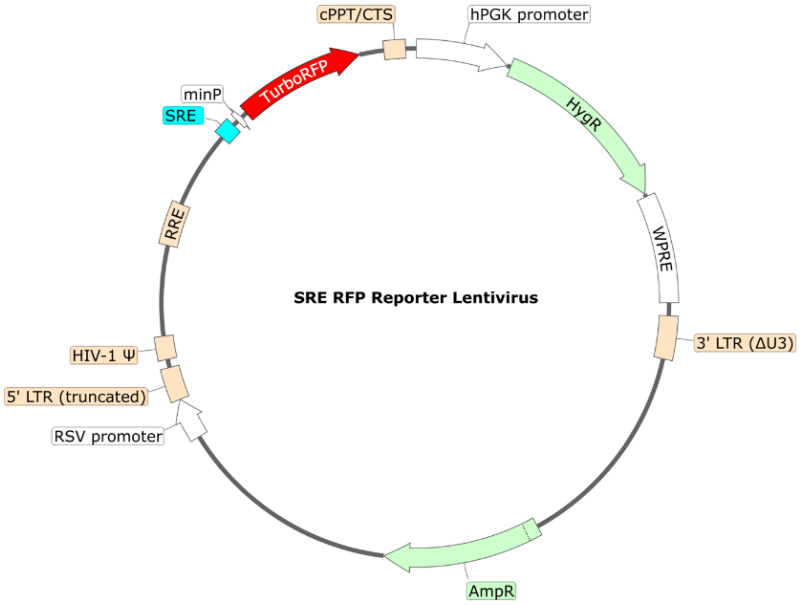

Figure 1. Schematic of the lenti-vector used to generate the SRE TurboRFP Reporter Lentivirus.

Background

The MAPK (mitogen-activated protein kinase)/ERK (extracellular regulated kinase) 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 (MAP kinase kinase 1/2) 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 (ETS like-1 protein), 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. TurboRFP, a red (orange) fluorescent protein derived from the sea anemone *Entacmaea quadricolor*, has high photostability and pH stability allowing for easy assay readouts.

Application

- Screen for activators or inhibitors of MAPK/ERK signaling pathway in transduced cells.
- Generate SRE TurboRFP reporter cell pools or stable cell lines following hygromycin selection.

Formulation

The lentiviruses were produced from HEK293T cells. Supplied in medium containing 90% DMEM + 10% FBS. Virus particles can be packaged in custom formulations and produced at higher titers by special request, for an additional fee.

Size and Titer

Two vials (500 µl x 2) of SRE TurboRFP Reporter Lentivirus at ≥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 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 the 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.

Materials Required but Not Supplied

These materials are not supplied with this lentivirus but are necessary to follow the designed protocol. BPS Bioscience media, reagents, and luciferase assay systems 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
EGF Recombinant	BPS Bioscience #90201
FBS (Fetal Bovine Serum)	Invitrogen #26140-079
Lenti-Fuse™ Polybrene Viral Transduction Enhancer	BPS Bioscience #78939
96-well tissue culture, clear-bottom, white plate	Corning, #3610

Assay Protocol

- The following protocol is a general guideline for transducing HEK293 cells. 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 target gene can be measured approximately 48-72 hours after transduction. For cell types with low transduction efficacy, it may be necessary to select the cells stably expressing the target with Hygromycin prior to carrying out the assays.
- The assay should include “Stimulated” and “Unstimulated” wells.

Day 1:

1. Harvest HEK293 cells from culture and seed cells at a density of 5,000-10,000 cells per well in 50 µl of Thaw Medium 1 into a 96-well cell culture plate.

2. Add 10 µl of SRE TurboRFP Reporter Lentivirus into each well.

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

4. Gently swirl the plate to mix.

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

Day 2:

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

2. Add 100 µl of Assay Medium 1B to each well.

3. Incubate the plate at 37°C with 5% CO_2 for 24 hours.

Day 3:

1. Prepare diluted human EGF or FBS in Assay Medium 1B.

2. Add 100 µl of diluted human EGF or FBS or both to the "Stimulated" wells.

3. Add 100 µl of Assay Medium 1B to the "Unstimulated" control wells (for measuring the uninduced level of SRE TurboRFP reporter activity).

4. Incubate at 37°C with 5% CO_2 for 24 hours.

Day 4:

1. The expression of TurboRFP can be analyzed by microscopy or flow cytometry, or another method of interest.

Important Notes

To generate an SRE-TurboRFP reporter cell line, remove the growth medium 48 hours after transduction and replace it with fresh growth medium containing the appropriate amount of hygromycin (as pre-determined from a killing curve, https://bpsbioscience.com/cell-line-faq), for antibiotic selection of transduced cells, followed by clonal selection.

Figures and Validation Data

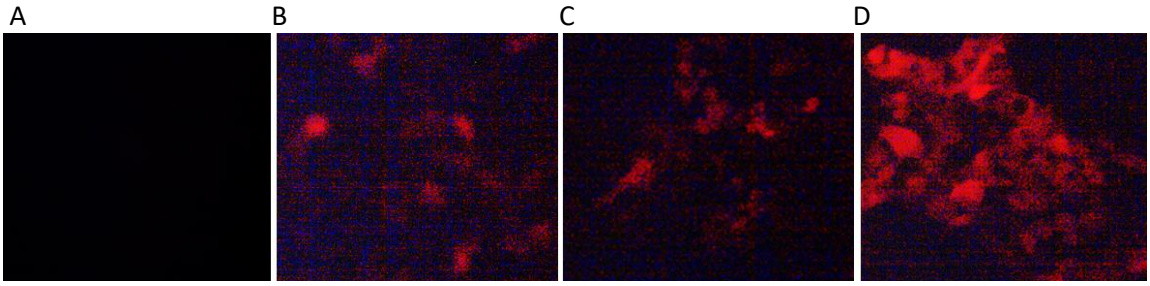

Figure 2. SRE TurboRFP reporter activity stimulated by human EGF or FBS in HEK293 cells transduced with SRE TurboRFP Reporter Lentivirus.
Approximately 8,000 HEK293 cells/well were transduced with 100,000 TU/well of SRE TurboRFP Reporter Lentivirus. 24 hours post-transduction the medium was changed to Assay Medium 1B. 48 hours post-transduction, cells were left unstimulated (A) or were stimulated with 10 ng/ml of human EGF (B), 10% FBS (C) or both (D), for 24 hours. Expression of TurboRFP was observed under a fluorescence microscope.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

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

References
Wong K.K., 2009 *Recent Pat Anticancer Drug Discov.* 4(1):28-35.
Treisman R., 1992 *Trends Biochem Sci.* 17(10): 423-426.

Related Products

Products	*Catalog #*	*Size*
SRE Luciferase Reporter Lentivirus	78627	500 µl x 2
CSF1R/ SRE – Reporter HEK293 Recombinant Cell LIne	79380	2 Vials
TrkA/ SRE Reporter – HEK293 Recombinant Cell LIne	79798	2 vials
SRE eGFP Reporter – HEK293 Cell Line (ERK Pathway)	78327	2 vials
SRE Reporter – HEK293 Recombinant Cell Line (ERK Pathway)	60406	2 vials

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