Description

TCF/LEF Luciferase Reporter Lentivirus (Wnt/β-catenin Signaling Pathway) 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 a firefly luciferase reporter under the control of TCF (T cell factor)/LEF (lymphoid enhancer factor)-responsive element located upstream of the minimal TATA promoter. The lentiviruses also transduce a puromycin selection gene (Figure 1). After transduction, activation of the Wnt/β-catenin signaling pathway in target cells can be monitored by assessing luciferase expression.

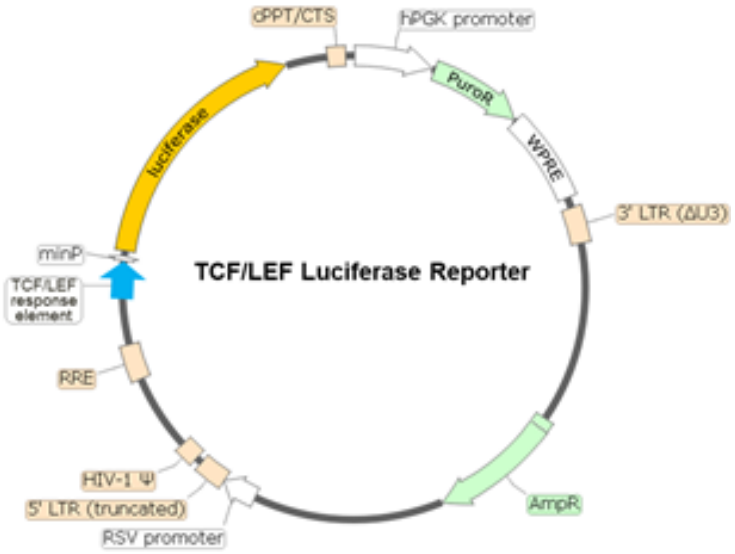

Figure 1. Schematic of the lenti-vector used to generate the TCF/LEF Luciferase Reporter Lentivirus (Wnt/β-catenin Signaling Pathway).

Background

The Wnt signaling pathway is a well described regulator of organism development and cell fate specification. Cellular pluripotency and differentiation of multiple lineages, including cardiomyocytes and neurons are modulated by this pathway. There are 19 Wnt ligands and 10 Frizzled receptors, which stimulate canonical as well as non-canonical Wnt pathways. In the canonical pathway, cell activation by a Wnt ligand leads to the stabilization and nuclear translocation of β-catenin and subsequent activation of TCF (T cell factor)/LEF (lymphoid enhancer factor) transcription factor. Dysfunction of these pathways can result in cancer and also type 2 diabetes. A deeper understanding of the role of this pathway and the development of new therapeutic strategies around it will open new clinical avenues.

Application

- Screen for activators or inhibitors of Wnt signaling pathway in transduced target cells.
- Generate TCF/LEF reporter cell pools or stable cell lines following puromycin 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 TCF/LEF Luciferase Reporter Lentivirus (Wnt/β-catenin Signaling Pathway) 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	
Thaw Medium 9	BPS Bioscience #79665
Lenti-Fuse™ Polybrene Viral Transduction Enhancer	BPS Bioscience #78939
LiCl	Sigma #L7026
Recombinant Mouse Wnt-3a Protein	R&D Systems #1324-WN
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
96-well white clear-bottom assay plate	Corning #3610
Luminometer	

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 puromycin prior to carrying out the assays.
- Conditions should be tested in triplicate.
- The assay should include “Cell-Free Control”, “Untreated Condition” and “Treated Condition” wells.

Day 1:

1. Harvest HEK293 cells from culture by centrifugation and resuspend the cells in fresh Thaw Medium 9.

2. Count cells.

3. Plate HEK293 cells at a density of 5,000-10,000 cells per well in 50 µl of Thaw Medium 9 in a white opaque 96-well plate. Leave a few empty wells as "Cell-Free Control" wells (for determining background luminescence).

4. Incubate cells at 37°C with 5% CO_2 overnight.

Day 2:

1. Add 10 µl of TCF/LEF Luciferase Reporter Lentivirus (Wnt/β-catenin Signaling Pathway) to the cells.

2. Add Lenti-Fuse™ Polybrene Viral Transduction Enhancer to each well to 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 18-24 hours.

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

Day 3:

1. Remove media and add 100 µl of fresh Thaw Medium 9.

   Note: If neither the polybrene nor the lentivirus adversely affects the target cells, it is not necessary to change the medium on Day 3. The target cells can be incubated with the virus for 48-72 hours before changing the medium.

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

Day 4:

1. Prepare a 10 mM solution of LiCl in Thaw Medium 9 (90 µl/well).

2. Add 90 µl of 10 mM LiCl to each of the "Treated Condition" wells.

3. Add 90 µl of Thaw Medium 9 to the "Untreated Condition" wells.

4. Incubate cells at 37°C with 5% CO_2 for 16 hours.

Day 5:

1. Prepare a diluted solution of mouse Wnt3a in Thaw Medium 9 (10 µl/well).

2. Add 10 µl of diluted Wnt3a to each of the "Treated Condition".

3. Add 10 µl of Thaw Medium 9 to the "Untreated Condition" wells.

4. Add 100 µl of Thaw Medium 9 to the "Cell-Free Control" wells.

5. Add 100 µl of ONE-Step™ Luciferase reagent per well.

6. Rock at room temperature for ~15-30 minutes.

7. Measure luminescence using a luminometer.

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

   The fold induction is the average background-subtracted luminescence of treated wells divided by the average background-subtracted luminescence of untreated control wells.

$$Fold\ Induction\ = \left(\frac{luminescence\ of\ Treated\ cells - background}{luminescence\ of\ Untreated\ \ cells - background}\right)$$

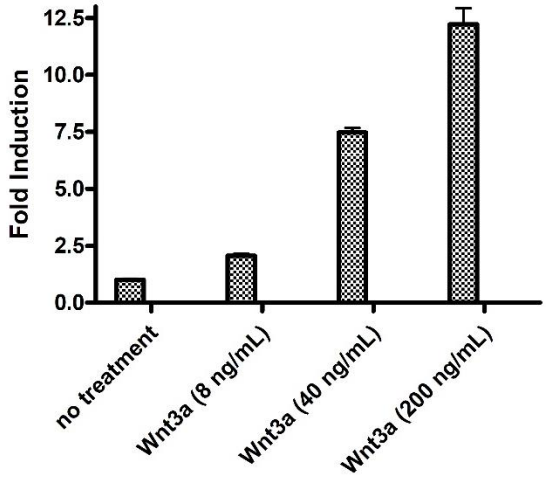

Figure 2: TCF/LEF luciferase reporter activity stimulated by mouse Wnt3a in HEK293 cell transduced with TCF/LEF Luciferase Reporter Lentivirus (Wnt/β-catenin Signaling Pathway). Approximately 10,000 HEK293 cells/well were transduced with 100,000 TU/well TCF/LEF luciferase reporter lentivirus.48 hours post-transduction, the cells were treated with 10 mM of LiCl for 16 hours. The cells were then treated with mouse Wnt3a for 5 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 Wnt3a treatment.

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

Notes

1. To generate a TCF/LEF luciferase reporter-expressing stable 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, https://bpsbioscience.com/cell-line-faq), for antibiotic selection of transduced cells, followed by clonal selection.
2. The following Lentivirus Reporter Controls are available from BPS Bioscience to meet your experimental needs:
    a. Negative Control Luciferase Lentivirus (#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.
    b. Renilla Luciferase Lentivirus (#79565): Ready-to-transduce lentiviral particles expressing Renilla luciferase under the control of a 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 (#79692-G, #79692-H, #79692-P): Ready-to-transduce lentiviral particles expressing firefly luciferase under the control of a CMV promoter. It can serve as a positive control for transduction optimization studies.

Troubleshooting Guide

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

References

Tian S., *et al.,* 1994 *Blood* 84(6):1760-1764.
Zhong, Z., *et al.,* 1994 *Science* 264(5155):95-98.

Related Products

Products	*Catalog #*	*Size*
TFC/LEF Reporter – HEK293 Cell Line (Wnt Singaling Pathway)	60501	2 vials
TFC/LEF Reporter Kit (Wnt Singaling Pathway)	60500	500 reactions
Transfection Collection™: TCF/LEF Transient Pack Wnt/ β-catenin Signaling Pathway	79273	100 reactions

Version 042224