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Data Sheet

TCF/LEF Luciferase Reporter Lentivirus (Wnt/β-catenin Signaling Pathway)

Catalog #: 79787

Product Description

The TCF/LEF Luciferase Reporter Lentivirus 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 under the control of TCF/LEF-responsive element located upstream of the minimal TATA promoter (Figure 1). After transduction, activation of the Wnt/β-catenin signaling pathway in the target cells can be monitored by measuring the luciferase activity.

Applications

- Screen for activators or inhibitors of Wnt signaling pathway in the transduced target cells
- Generation of TCF/LEF luciferase reporter stable cell line

Formulation

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

Titer

Two vials (500 µl x 2) of TCF/LEF luciferase reporter lentivirus at a titer ≥1 x 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 virus at -80°C. Avoid repeated freeze-thaw cycles. Titers can drop significantly with each freeze-thaw cycle.

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.

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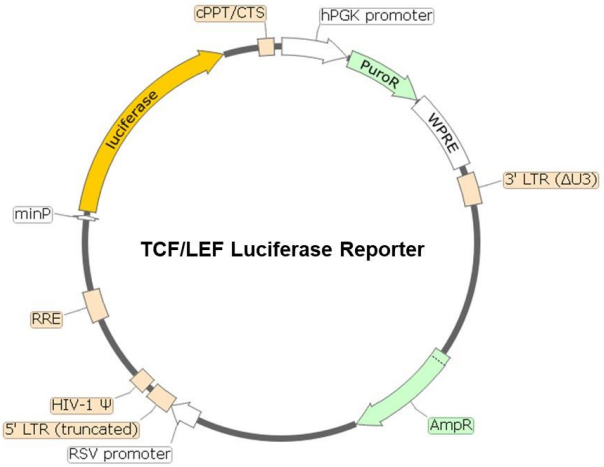

Figure 1. Schematic of the lenti-vector used to generate the TCF/LEF luciferase reporter lentivirus

Materials Required but Not Supplied

- LiCl (Sigma, #L7026)
- Mouse Wnt3a (R&D Systems 1324-WN)
- HEK293 growth medium or use Thaw Medium 9 (BPS Bioscience, #79665): MEM with 10% FBS, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate
- 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

Assay Protocol

The following protocol is a general guideline for transducing HEK293 cells using TCF/LEF 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 HEK growth medium. Incubate cells at 37°C with 5% CO_2 overnight.

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2. Day 2: To each well add 10 µl of TCF/LEF 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 18-24 hours.
   Alternatively, seeding cells and the transduction can be performed at the same day.

3. Day 3: Remove the medium containing the lentivirus from the wells. Add 100 µl of fresh growth medium to each well.
   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.

4. Day 4: Treat transduced cells with LiCl (final concentration 10 mM) in 90 µl fresh growth medium. Incubate the cells at 37°C with 5% CO_2 for 16 hours.

5. Day 5: Prepare diluted mouse Wnt3a in HEK growth medium. Add 10 µl diluted Wnt3a to stimulated cells; add 10 µl growth medium to unstimulated control cells; add 100 µl growth medium to cell-free control wells (for determining background luminescence). Set up each treatment in at least triplicate. Incubate at 37°C with 5% CO_2 for 5-6 hours.

6. 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 TCF/LEF luciferase reporter stable cell line, on day 4 remove HEK growth medium and replaced 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.

   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.

   3) Firefly Luciferase (Fluc) Lentivirus (BPS Bioscience, #79692-G, #79692-H): Ready-to-transduce lentiviral particles expressing firefly luciferase under the CMV promoter. The Fluc lentivirus can serve as a positive control for transduction optimization studies.

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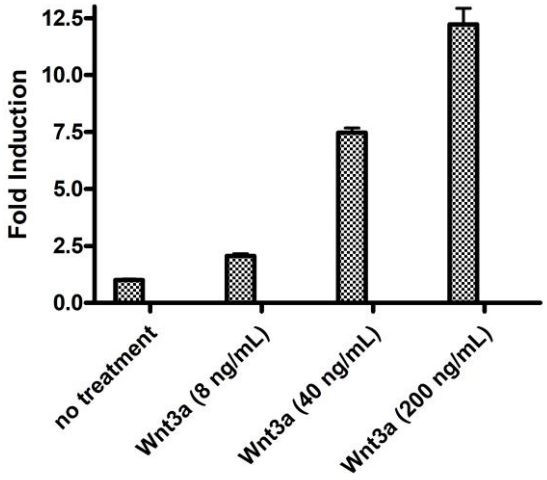

Figure 2. TCF/LEF luciferase reporter activity stimulated by mouse Wnt3a in HEK293 cells. Approximately 10,000 HEK293 cells/well were transduced with 100,000 TU/well TCF/LEF luciferase reporter lentivirus. After 48 hours of transduction, the cells were treated with LiCl (10 mM) for 16 hours. The cells 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.

Related Products

Product	Cat. #	Size
NF-κB Luciferase Reporter Lentivirus	79564	500 µl x2
CRE Luciferase Reporter Lentivirus	79580	500 µl x2
NFAT Luciferase Reporter Lentivirus	79579	500 µl x2
STAT3 Luciferase Reporter Lentivirus	79744	500 µl x2
STAT5 Luciferase Reporter Lentivirus	79745	500 µl x2
SBE Luciferase Reporter Lentivirus	79806	500 µl x2
Negative Control Lentivirus	79578	500 µl x2
Renilla Luciferase (Rluc) Lentivirus	79565	500 µl x2
Firefly Luciferase (Fluc) Lentivirus (G418)	79692-G	500 µl x2
Firefly Luciferase (Fluc) Lentivirus (Hygromycin)	79692-H	500 µl x2
TCF/LEF Reporter Kit (Wnt Signaling Pathway)	60500	500 rxns
TCF/LEF Reporter (Luc)-HEK293 cell line	60501	2 vials
ONE-Step™ Luciferase Assay System	60690-1	10 ml
ONE-Step™ Luciferase Assay System	60690-2	100 ml
Dual Luciferase (Firefly-Renilla) Assay System	60683	10 ml
Thaw Medium 9	79665	100 ml

References

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