ScienCell™ Research Laboratories

TCF/LEF Dual Reporter Vector

(Wnt/β-catenin Signaling Pathway)

(TDRV)

Catalog #9038

100 transfections 96-well

Product Description

The TCF/LEF Dual Reporter Vector (TDRV) is a DNA-based reporter system specifically engineered to monitor the transcriptional activity of the Wnt/β-catenin signaling pathway in cultured mammalian cells. TDRV includes two separate plasmid DNA mixtures. The **TCF/LEF Reporter Mix** contains a plasmid encoding Firefly luciferase under the control of multimerized TCF/LEF response elements, co-formulated with a second plasmid expressing Renilla luciferase driven by a constitutive promoter. This allows direct normalization of Firefly signal relative to Renilla luminescence in the same sample. The **Negative Control Reporter Mix** contains a Firefly luciferase vector lacking TCF/LEF binding sites, also co-formulated with the same constitutive Renilla luciferase vector, serves as a non-inducible background control to determine baseline signal and pathway specificity. The Firefly luciferase provides a strong, ATP-dependent luminescent signal (peak ~560 nm) directly proportional to transcriptional activity downstream of β-catenin/TCF. The Renilla luciferase, with an ATP-independent bioluminescent signal (peak ~480 nm), allows internal normalization and reduces assay variability caused by differences in transfection efficiency or cell viability [1-3].

This dual-reporter configuration allows accurate and robust quantification of Wnt/β-catenin activity. The system is ideal for studying TCF/LEF-mediated transcriptional regulation, screening for small molecules or biologics that modulate Wnt signaling, validating gene editing or knockdown experiments, and high-throughput drug screening in cell-based models.

Kit Components

Cat #	Component	Quantity	Storage
9038a	TCF/LEF Reporter Vector Mix (TCF/LEF Firefly Luciferase Reporter Vector + constitutively Renilla Luciferase Vector), lyophilized	1 vial	-20°C
9038b	Negative Control Vector Mix (Non-Inducible Firefly Luciferase Vector +constitutively Renilla Luciferase Vector), lyophilized	1 vial	-20°C

Note: These vectors are ready for transient transfection. They are NOT MEANT for transformation and amplification in bacteria.

Additional Materials Required (Materials Not Included in Kit)

Product Name
Mammalian cell line of interest
Appropriate cell culture media
Transfection reagents suitable for your cell type (e.g., EpiFectagen I, Fugene 4K)
Cell culture plates (e.g., 6-well, 12-well, 24-well or 96-well plates)
Serum-reduced medium or completed medium for transfection
White or opaque 96-well plate with clear bottom for luminescence reading.
Luciferase Assay Kit (LAK, Cat#9048)
Recombinant Human Wnt-3a Protein, (R&D Systems 1324-WN)
Luminometer capable of measuring dual-luciferase signals

Quality Control

Each batch of TCF/LEF Dual Reporter Vector (TDRV) undergoes stringent quality control to ensure optimal performance and reliability. Plasmid DNA purity is confirmed by spectrophotometric analysis (A260/A280 ratio between 1.7 and 2.1), while integrity and size of plasmids are verified by agarose gel electrophoresis. The sequences of key regulatory elements and luciferase genes are validated by Sanger sequencing, and functional activity is confirmed by transient transfection and treatment (LiCl 10mM) in HEK293T, followed by Luciferase Assay Kit (LAK, Cat#9048) to verify expected Firefly and Renilla luminescence signals (Figure 1). This rigorous quality control guarantees reproducible and reliable results in your gene expression and signaling pathway studies.

Product Use

TDRV is for research use only and is not approved for human or animal use, or application in clinical or *in vitro* diagnostic procedures.

Shipping and Storage

The product is shipped at room temperature. Upon receipt, store at -20°C.

References

[1] Chen, B., et al. (2009). *Small molecule-mediated disruption of Wnt-dependent signaling in tissue regeneration and cancer*. Nature Chemical Biology, 5(2), 100–107.

[2] Brennan, K., et al. (2004). *Inhibition of Wnt/β-catenin signaling by a soluble receptor-1 in a colorectal cancer model*. Cancer Research, 64(13), 5034-5042

[3] Thorne, C. A., et al. (2010). *Small-molecule inhibition of Wnt signaling through activation of casein kinase 1α*. Nature Chemical Biology, 6(11), 829–836.

Procedure

Important Notes:

- *This protocol is optimized for HEK293T cells in a 96-well plate format using EpiFectagen I transfection reagent (Cat# 0923).*
- *If using a different transfection reagent, optimize DNA: reagent ratio, incubation time, and complex formation conditions according to the manufacturer's instructions.*
- *Adjust volumes and cell numbers if using a different plate format, based on surface area ratios.*
- *We recommend setting up at least triplicate assays for each condition and preparing transfection cocktails for multiple wells to minimize pipetting errors.*

1. **Cell Seeding**
   Seed approximately 30,000 cells per well in 180 µL of appropriate complete growth medium in a 96-well plate and incubate overnight at 37°C in a 5% CO_2 incubator.

2. **Plasmid Reconstitution**
   2.1. Add 200 µl nuclease-free H2O to TCF/LEF Reporter Vector Mix (lyophilized, Cat #9048a) to make a work solution. Aliquot and store at -20°C in a manual defrost freezer. Avoid repeated freeze-and-thaw cycles.
   2.2. Add 200 µl nuclease-free H2O to Negative Control Vector Mix (lyophilized, Cat #9048b) to make a work solution. Aliquot as needed. Store at -20°C in a manual defrost freezer. Avoid repeated freeze-and-thaw cycles.

3. **Preparation of DNA Complexes and Cell Transfection**
   3.1. Prepare Plasmid (per well):
   A. TCF/LEF Reporter
   For each well, add 2 µL of #9038a in 5 µL of deionized H_2O.
   B. Negative Control
   For each well, add 2 µL of #9038b in 5 µL of deionized H_2O.

4. **Prepare Transfection Reagent Mix**
   4.1. Add 0.5ul EpiFectagen reagent B into each plasmid prepared into a 1.5mL sterile plastic tube. Vortex gently and spin down.
   4.2. Then add 2 µL EpiFectagen reagent a to make the total volume of the transfection mixture to be 16.5 µL, vortex for 5 seconds and spin down.
   4.3. Incubate at room temperature for 20-30min.
   Note: The amount may vary depending on the reagent and cell type; optimization is recommended.

5. **Transfection**
   5.1 Add the 16.5 µL of transfection mixture to each well containing 100 µL fresh culture medium
   5.2. Gently mix in the medium, avoid to disturber cells seeding.
   5.3. Incubate the cells for 24 hours at 37°C, 5% CO_2 for downstream analysis.

Notes: Depending on the cytotoxicity of the transfection reagent and the sensitivity of the cells,

you may perform a medium change 4–6 hours post-transfection.

6. **Luciferase Assay Kit**
   6.1. At the chosen time point post-transfection and/or treatment, measure luciferase activity using the Luciferase Assay Kit (LAK, Cat#9048).
   6.2. Follow the instructions provided in the kit manual for cell lysis, reagent preparation, and luminescence detection.
   Be sure to:
   - Use a compatible luminometer.
   - Normalized luciferase activity, subtract the background luminescence, then calculate the ratio of Firefly luminescence to Renilla luminescence from the control Renilla luciferase vector, as instructed in the kit protocol.

Validation
Effect of Recombinant Human Wnt3a on TCF/LEF Reporter Activity

Additional materials required in this experiment setup supplied by user (Not provided)
- LiCl (Sigma # L7026)
- Wnt3a (R&D Systems 1324-WN)
- HEK293T cells
- HEK293T growth medium
- 96-well tissue culture treated white opaque or clear-bottom assay plate (Corning # 3610)
- Luciferase Assay Kit (LAK, Cat#9048).

Day 1 – Cell Seeding
A. Plate 30,000 HEK293 cells per well in a white 96-well plate, in 180 µL of complete growth medium.
B. Incubate overnight at 37°C with 5% CO_2.
Note: Use cells in log-phase and low passage (≤15). Plate enough wells for triplicates and controls (e.g., non-transfected cells, untreated cells).

Day 2 – Transfection
A. Transfect each well with:
- TCF/LEF Dual Reporter Vector Mix
- Negative Control Vector Mix

B. Use an optimized transfection reagent and conditions suitable for 96-well format.
C. Incubate the cells for 16-24 hours at 37°C.

Day 3 – Medium Change and Treatment
A. Apply treatments according to experimental groups:
- Group 1: Fresh medium only (no treatment)
- Group 2: Fresh medium + LiCl (3-10 mM)
- Group 3: Fresh medium + Wnt3a (50-400 ng/mL)

B. Incubate for 6 hours at 37°C.

Day 4 – Luciferase Assay Kit (96-well reading)
A. Perform the luciferase assay following the instructions provided in the Luciferase

Assay Kit product sheet (Cat #9048).
B. Remove medium, lyse cells, and measure Firefly and Renilla luminescence using a compatible luminometer.
C. Record RLU values and normalize Firefly to Renilla as described in the kit instructions.

Notes

- CHIR99021 and LiCl acts by directly inhibiting GSK-3β, causing rapid and strong β-catenin accumulation.
- Wnt3a activates the Wnt pathway physiologically through receptor binding.
- The culture medium used during transfection should follow the transfection reagent's manufacturer instructions for optimal efficiency

Data Analysis

To obtain the normalized luciferase activity, subtract the background luminescence, then calculate the ratio of firefly luminescence to Renilla luminescence from the control Renilla luciferase vector.

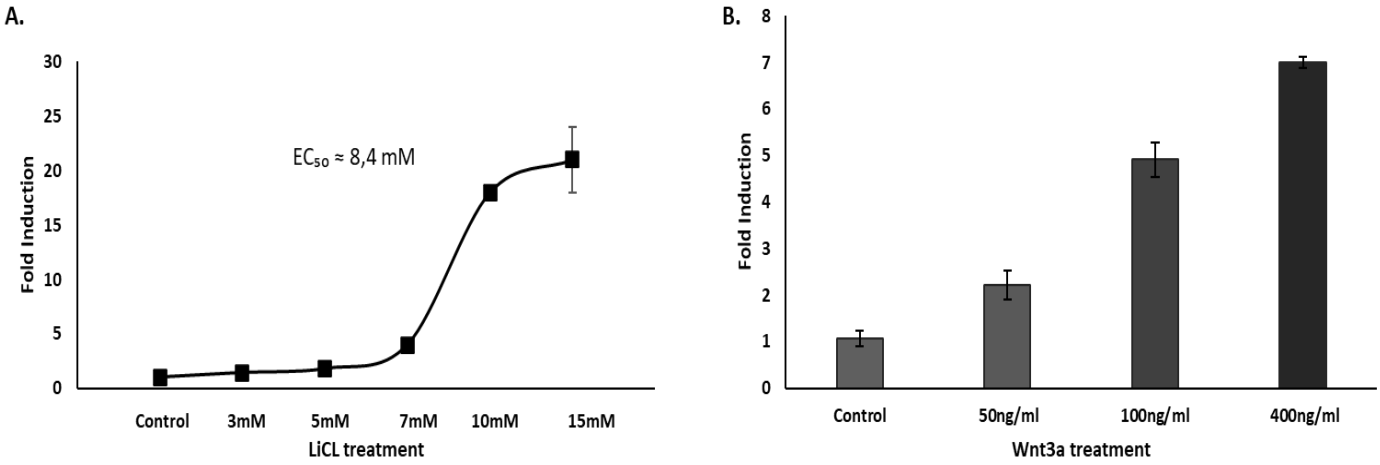

Figure 1. Dose-dependent activation of TCF/LEF reporter by LiCl, and Wnt3. HEK 293T cells transfected with the TCF/LEF Firefly-Renilla Dual Reporter Vector were treated for 6 h with the indicated concentrations. Firefly and Renilla luciferase activities were measured and normalized (Firefly/Renilla). LiCl and induces TCF/LEF reporter activity in a dose-dependent manner, with calculated EC_{50} demonstrating assay sensitivity (**A**). Wnt3a (**B**) show at representative concentrations for comparison.