



TCF/LEF Dual Reporter Vector
 (Wnt/ β -catenin Signaling Pathway)
(TDRV)

Catalog #VT001

100 transfections 96-well

Product Description

The Wnt/ β -catenin signaling pathway (Wingless/Int, a family of secreted glycoproteins that activate cell surface receptors) regulates key cellular processes such as proliferation, differentiation, and tissue homeostasis, with dysregulation implicated in cancer and other diseases. Wnt ligands stabilize β -catenin, which translocates to the nucleus and partners with TCF/LEF transcription factors (T-cell factor/Lymphoid enhancer-binding factor) to activate target gene expression. The TCF/LEF Dual Reporter Vector (TDRV) is a DNA-based dual-luciferase system engineered to monitor this Wnt/ β -catenin-dependent transcriptional activity in mammalian cells. It comprises a TCF/LEF Reporter Mix, in which Firefly luciferase is driven by multimerized TCF/LEF response elements and co-formulated with a constitutive Renilla luciferase plasmid for internal normalization, and a Negative Control Reporter Mix lacking TCF/LEF binding sites to define non-inducible background and pathway specificity. Firefly luciferase provides a strong ATP-dependent signal proportional to β -catenin/TCF-driven transcription, while Renilla luciferase offers an ATP-independent internal control, enabling accurate quantification of Wnt/ β -catenin pathway activity suitable for pathway studies, genetic perturbation experiments, and screening of small-molecule or biologic modulators [1-3].

Kit Components

Cat #	Component	Quantity	Storage
VT001	TCF/LEF Reporter Vector Mix (TCF/LEF Firefly Luciferase Reporter Vector + constitutively Renilla Luciferase Vector), lyophilized	1 vial	-20°C
VTNEG-001	Negative Control Vector Mix (Non-Inducible Firefly Luciferase Vector + constitutively Renilla Luciferase Vector), lyophilized	1 vial	-20°C
GQ100-0.5	Nuclease-free H ₂ O (0.5 mL)	1vial	4°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., UniFectagen, Cat#0983)

Nuclease-free H ₂ O
Cell culture plates (e.g., 6-well, 12-well, 24-well or 96-well plates)
Serum-free or reduced for transfection (e.g., Transfection Max Medium, TMM - Cat#9051)
White or opaque 96-well plate for luminescence reading
Luciferase Assay Kit (LAK, Cat#9048)
Recombinant Human Wnt-3a Protein Fragment 198-223 (Cat#109-03AEF11)
Lithium chloride (LiCl)
Luminometer

Quality Control

Each batch of TCF/LEF Dual Reporter Vector (TDRV) undergoes rigorous quality control to optimal performance. Plasmid DNA purity is verified by spectrophotometric, key regulatory elements and luciferase genes are sequence-validated by Sanger sequencing, and functional activity is confirmed via transient transfection in HEK293T cells and $\pm$ Wnt pathway activation (LiCl 10 mM or Wnt-3a 400 ng/mL, 6h), followed by Luciferase Assay Kit (LAK, Cat#9048) to verify expected Firefly and Renilla luminescence signals (Figure 1).

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 1 α . *Nature Chemical Biology*, 6(11), 829–836.

Procedure

Important Notes:

- *Protocol is optimized for HEK293T cells in a 96-well plate format using UniFectagen transfection reagent (Cat# 0983).*
- *Different reagent: optimize DNA: reagent ratio, incubation time, and complex formation per manufacturer instructions.*
- *Different plate formats: scale volumes and cell numbers by surface area ratios.*
- *Set up ≥ 3 technical triplicate per condition; prepare transfection cocktails for multiple wells to minimize pipetting errors.*

1. Cell Seeding

Seed approximately 30,000 cells/well in 180 μ L complete growth medium in a 96-well plate. Incubate overnight at 37°C in a 5% CO₂.

2. Plasmid Reconstitution

- 2.1. Add 200 µl nuclease-free H₂O (Cat#GQ100-0.5) to **TCF/LEF Reporter Vector Mix** (lyophilized, Cat#VT001) to make a work solution. Aliquot and store at -20°C (manual defrost freezer). Avoid repeated freeze-and-thaw cycles.
- 2.2. Add 200 µl nuclease-free H₂O (Cat#GQ100-0.5) to **Negative Control Vector Mix** (lyophilized, Cat#VTNEG-001) to make a work solution. Aliquot and store at -20°C (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 (Cat#VT001)

Prepare one well by mixing 2 µL of the reporter plasmid with 5 µL of nuclease-free H₂O or serum free medium (TMM, Cat#9051).

B. Negative Control (Cat#VTNEG-001)

Prepare a separate well by mixing 2 µL of the control plasmid with 5 µL of nuclease-free H₂O or serum free medium (TMM, Cat#9051).

4. Prepare Transfection Reagent Mix (per well)

- 4.1. Add 0.5 µL of UniFectagen Reagent B (Cat#0983) to each plasmid prepared in a 1.5mL sterile microcentrifuge tube. Vortex gently and spin down.
- 4.2. Add 2 µL of UniFectagen Reagent A (Cat#0983) to bring the total transfection mixture volume to 9.5 µL. Vortex for 5 seconds and spin down.
- 4.3. Incubate the mixtures at room temperature for 20-30 minutes.
Note: The optimal amount of transfection reagent may vary depending on the reagent and cell type; optimization is recommended.

5. Transfection

- 5.1. Add 9.5 µL of transfection mixture to each well containing 100 µL fresh culture medium.
- 5.2. Gently mix into the medium, avoid to disturb cells seeding.
- 5.3. Incubate the cells for 24 hours at 37°C, 5% CO₂ before downstream analysis.
Note: Depending on the cytotoxicity of the transfection reagent and the sensitivity of the cells, a medium change 4–6 hours post-transfection may be performed.

6. Treatment

- 6.1. Apply treatments according to experimental groups:
 - **Group 1:** Fresh medium only (Untreated)
 - **Group 2:** Fresh medium + LiCl (10 mM) or Wnt-3a (400 ng/mL)
- 6.2. Incubate for 6 hours at 37°C.

7. Luciferase Assay

- 7.1. At the selected time point post-transfection and/or treatment, measure luciferase activity using the Luciferase Assay Kit (LAK, Cat#9048).
- 7.2. Follow the instructions provided in the kit manual for cell lysis, reagent preparation, and luminescence detection. Be sure to:
 - Use a compatible luminometer.

- Normalize per well: Firefly RLU (Relative Luminescence Units) $\div$ Renilla RLU (same well).
- Use the Negative Control Vector to assess background/non-specific effects
- Calculate fold induction as Treatment $\div$ Untreated for each reporter separately.

Dose response of TCF/LEF Dual Reporter Vector to Wnt-3a and LiCl

Day 1 – Cell Seeding

- A. Plate 30,000 HEK293T cells/well in a 96-well plate in 180 μ L complete growth medium.
- B. Incubate overnight at 37°C, 5% CO₂.

Day 2 – Transfection

- A. Prepare two sets of wells: **TCF/LEF Reporter Mix (Cat#VT001)** and **Negative Control Mix (Cat#VTNEG-001)**.
- B. Transfect using optimized 96-well conditions with UniFectagen (Cat# 0983) as described in Procedure section.
- C. Incubate the cells for 16-24 hours at 37°C.

Day 3 –Treatment

- **Group 1:** Fresh medium only (Untreated)
- **Group 2:** Fresh medium + LiCl (3-10 mM)
- **Group 3:** Fresh medium + Wnt-3a (50-400 ng/mL)
- Incubate 6 hours at 37°C.

Day 4 – Luciferase Assay

- Perform assay using Luciferase Assay Kit (Cat#9048) per kit instructions.
- Normalize Firefly RLU $\div$ Renilla RLU (same well).
- Calculate fold induction: Treatment $\div$ Untreated.

Notes

- *CHIR99021/LiCl: Direct GSK-3 β inhibitors $\rightarrow$ rapid β -catenin accumulation [1-3].*
- *Wnt-3a: Physiological activation via receptor binding [1-3].*
- *Use transfection reagent-compatible medium for optimal efficiency/viability.*

Data Analysis

To obtain normalized luciferase activity, calculate per well: Firefly RLU $\div$ Renilla RLU (same well). Fold induction = Treatment $\div$ Untreated (for each reporter separately).

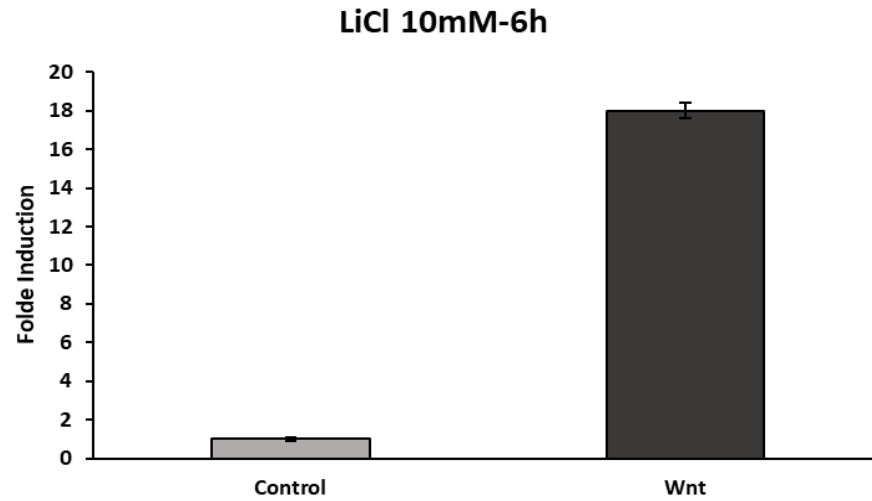


Figure 1. HEK293T cells transiently transfected with TCF/LEF Dual Reporter Vector, treated 6h with LiCl (10 mM). Firefly/Renilla normalized luciferase activity (mean ± SD, triplicates).

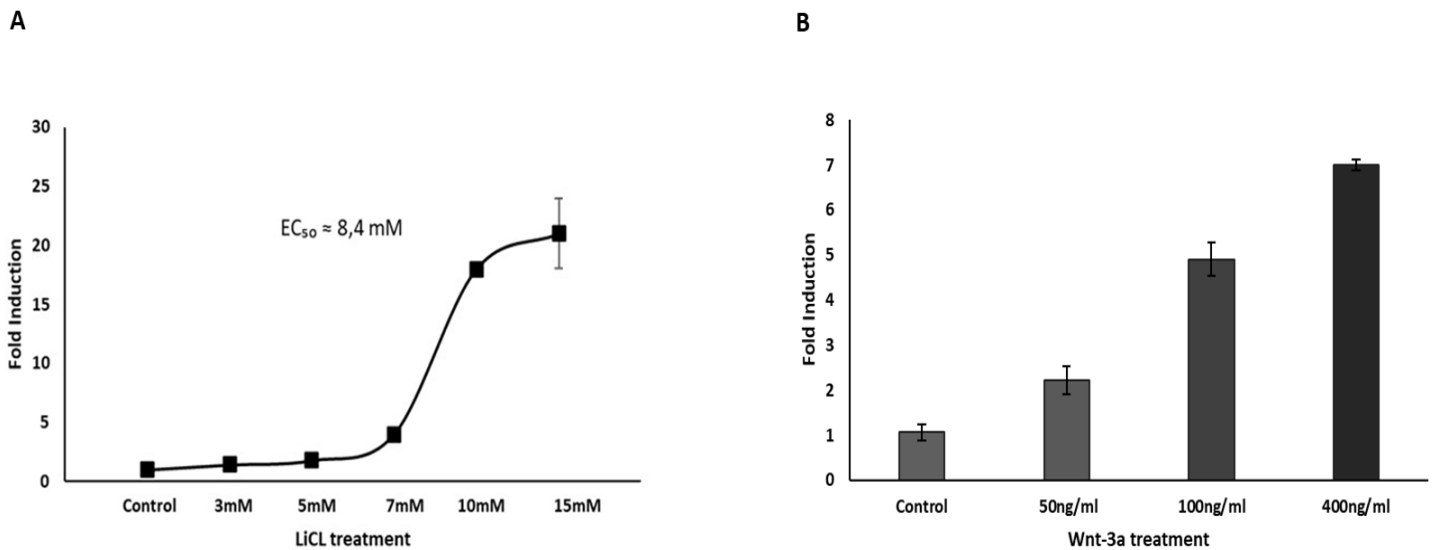


Figure 2. HEK293T cells transfected with TCF/LEF Dual Reporter Vector, treated 6h with indicated concentrations of LiCl (**A**) and Wnt-3a (**B**). Firefly/Renilla normalized luciferase activity shows dose-dependent TCF/LEF reporter induction by both agonists (mean ± SD, triplicates).