ScienCell™ Research Laboratories

TCF/LEF GFP Reporter Vector

(Wnt/β-catenin Signaling Pathway)

(TL-GFP)

Catalog #VT007

100 transfections 96-well

Product Description

The Wnt/β-catenin pathway controls proliferation, differentiation, stem-cell maintenance, and tissue homeostasis, and its dysregulation is linked to cancer and developmental disorders. Upon Wnt activation, β-catenin accumulates in the cytoplasm and translocates to the nucleus, where it binds TCF/LEF (T cell factor/lymphoid enhancer factor family) transcription factors to activate target genes. The TCF/LEF GFP reporter vector (TL-GFP) is a plasmid-based fluorescent reporter for real-time monitoring of Wnt/β-catenin–dependent transcription in mammalian cells; it contains multimerized TCF/LEF response elements upstream of GFP so pathway activation induces GFP expression, enabling direct visualization of activity in living cells without lysis or extra detection reagents [1-3].

Kit Components

Cat #	Component	Quantity	Storage
VT007	TCF/LEF GFP Reporter Vector, lyophilized	1 vial	-20°C
GQ100-0.5	Nuclease-free H_2O	1vial (0.5mL)	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)
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)
Recombinant Human Wnt-3a Protein Fragment 198-223 (Cat#109-03AEF11)
Lithium chloride (LiCl)
Fluorescence microscope or fluorescence plate reader

Quality Control

Each batch of TCF/LEF GFP Reporter Vector (TL-GFP) is verified for plasmid DNA purity and

sequence integrity by spectrophotometry and Sanger sequencing. Functional validation is performed by transient transfection in HEK293T cells followed by Wnt pathway activation using LiCl or Wnt-3a (LiCl 10 mM or Wnt-3a 400 ng/mL, 24h). Inducible GFP expression is confirmed by fluorescence microscopy. (Figure 1).

Product Use

TL-GFP 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] 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.

[3] Wesslowski. J, et al. (2020). eGFP-tagged Wnt-3a enables functional analysis of Wnt trafficking and signaling and kinetic assessment of Wnt binding to full-length Frizzled. *J Biol Chem,* 295(26):8759–8774.

Procedure

Important Notes:

- *Protocol is optimized for HEK293T cells in a 6-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 500,000 cells/well in 2 mL complete growth medium in a 6-well plate. Incubate overnight at 37°C in a 5% CO_2.

2. **Plasmid Reconstitution**
   2.1. Add 200 µl nuclease-free H_2O (Cat#GQ100-0.5) to **TCF/LEF GFP Reporter Vector** (lyophilized, Cat#VT007) to make a work solution. Aliquot and store at -20°C (manual defrost freezer). Avoid repeated freeze-and-thaw cycles.

3. **Transfection Mix (per well):**
   3.1. Prepare one well by mixing 40 µL of the reporter plasmid with 36 µL of nuclease-free H_2O or serum free medium (TMM, Cat#9051).
   3.2. Add 18 µL UniFectagen Reagent B (Cat#0983). Vortex gently and spin down.
   4.1. Add 48 µL of UniFectagen Reagent A (Cat#0983) to bring the total transfection mixture volume to 142 µL. Vortex for 5 seconds and spin down.
   4.2. Incubate the mixtures at room temperature for 20-30 minutes.

5. **Transfection**
   - **5.1.** Add 142 µL of transfection mixture to each well containing 2 mL fresh culture medium**.**
   - **5.2.** Gently mix into the medium, avoid to disturber cells seeding.
   - **5.3.** Incubate the cells for 24 hours at 37°C, 5% CO_2 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 24 hours at 37°C.
     Notes: *LiCl: Direct GSK-3β inhibitors → rapid β-catenin accumulation [1-3].*
     Wnt-3a: Physiological activation via receptor binding [1-3].

7. **Fluorescence Detection**
   - **7.1.** Monitor GFP expression using fluorescence microscopy or a fluorescence plate reader. GFP expression reflects Wnt/β-catenin pathway activation.

Data Analysis

Increased GFP fluorescence relative to untreated cells indicates activation of Wnt/β-catenin signaling.

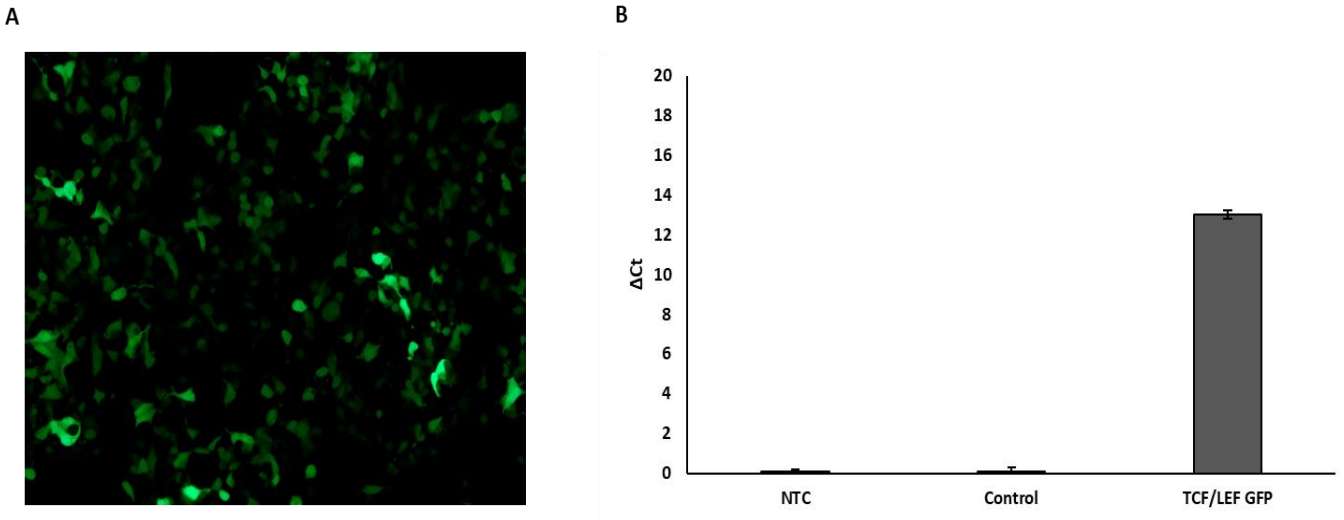

Figure 1. A. Representative fluorescence images of HEK293T cells transfected with the TCF/LEF GFP Reporter Vector showing Wnt/β-catenin-dependent GFP expression following pathway activation (100x). **B.** GFP expression was detected by RT-qPCR in transfected HEK293T cells (Ct ~26), with no signal in non-transfected controls. Data were normalized to GAPDH (ΔCt).