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Data Sheet

TCF/LEF Reporter Kit (Wnt / β-catenin signaling pathway)
Catalog #: 60500

Background

The Wnt / β-catenin signaling pathway controls a large and diverse set of cell fate decisions in embryonic development, adult organ maintenance and disease. Wnt proteins bind to receptors on the cell surface, initiating a signaling cascade that leads to stabilization and nuclear translocation of β-catenin. β-catenin then binds to TCF/LEF transcription factors in the nucleus, leading to transcription and expression of Wnt-responsive genes.

Description

The TCF/LEF Reporter kit is designed for monitoring the activity of Wnt / β-catenin signaling pathway in the cultured cells. The kit contains transfection-ready TCF/LEF luciferase reporter vector, which is a Wnt pathway-responsive reporter. This reporter contains a firefly luciferase gene under the control of multimerized TCF/LEF responsive element located upstream of a minimal promoter. The TCF/LEF reporter is premixed with constitutively expressing Renilla luciferase vector that serves as internal control for transfection efficiency.
The kit also includes a non-inducible firefly luciferase vector premixed with constitutively expressing Renilla luciferase vector as negative control. The non-inducible luciferase vector contains a firefly luciferase gene under the control of a minimal promoter, without any additional response elements. The negative control is critical to determining pathway specific effects and background luciferase activity.

Applications

- Monitor Wnt signaling pathway activity.
- Screen activators or inhibitors of Wnt / β-catenin signaling pathway.
- Study effects of RNAi or gene overexpression on the activity of Wnt pathway.

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Components

Component	Specification	Amount	Storage
Reporter (Component A)	TCF/LEF luciferase reporter vector + constitutively expressing Renilla luciferase vector	*500 µl (60 ng DNA/ µl)*	-20°C
Negative Contol Reporter (Component B)	Non-inducible luciferase vector + constitutively expressing Renilla luciferase vector	*500 µl (60 ng DNA/ µl)*	-20°C

These vectors are ready for transient transfection. They are NOT MEANT for transformation and amplication in bacteria.

Materials Required but Not Supplied

- Mammalian cell line and appropriate cell culture medium
- 96-well tissue culture plate or 96-well tissue culture treated white clear-bottom assay plate (Corning # 3610)
- Transfection reagent for mammalian cell line [We use Lipofectamine™ 2000 (Invitrogen # 11668027). However, other transfection reagents work equally well.
- Opti-MEM I Reduced Serum Medium (Invitrogen #31985-062)
- Dual luciferase assay system:
  Dual-Glo® Luciferase Assay System (Promega #E2920): This system assay cells directly in growth medium. It can be used with any luminometer. Automated injectors are not required.
  OR
  Dual-Luciferase® Reporter Assay System (Promega #E1910): This system reqired cell lysis step. It is ideal for luminometer with automated injectors.
- Luminometer

Generalized Transfection and Assay Protocols

The following procedure is designed to transfect the reporter to HEK293 cells using Lipofectamine 2000 in a 96-well format. To transfect cells in different tissue culture formats, adjust the amounts of reagents and cell number in proportion to the relative surface area. If using a transfection reagent other than Lipofectamine 2000 follow the manufacture's transfection protocol. Transfection condition should be optimized according to the cell type and study requirement.

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All amounts and volumes in the following setup are given on a per well basis.

1. One day before transfection, seed HEK293 cells at a density of 30,000 cells per well in 100 µl of growth medium so that cells will be 90% confluent at the time of transfection.

2. Next day, for each well, prepare complexes as follows:

   a. Dilute DNA mixtures in 15 µl of Opti-MEM I medium (antibiotic-free). Mix gently.
   Depending upon the experimental design, the DNA mixtures may be any of following combinations:
   - **1 µl of Reporter** (component A); in this experiment, the control transfection is **1 µl of Negative Control Reporter** (component B).
   - **1 µl of Reporter** (component A) + experimental vector expressing gene of interest; in this experiment, the control transfection is: **1 µl of Reporte**r (component A) + negative control expression vector, **1 µl of Negative Control Reporter** (component B) + experimental vector expressing gene of interest, and **1 µl of Negative Control Reporter** (component B) + negative control expression vector.
   - **1 µl of Reporter** (component A) + specific siRNA; in this experiment, the control transfection is: **1 µl of Reporte**r (component A) + negative control siRNA, **1 µl of Negative Control Reporter** (component B) + specific siRNA, and **1 µl of Negative Control Reporter** (component B) + negative control siRNA.

   Note: we recommend setting up at least triplicates for each condition, and prepare transfection cocktail for multiple wells.

   b. Mix Lipofectamine 2000 gently before use, then dilute 0.35 µl of Lipofectamine 2000 in 15 µl of Opti-MEM I medium (antibiotic-free). Incubate for 5 minutes at room temperature.

   Note: Prepare this dilution cocktail in volumes sufficient for the whole experiment.

   c. After the 5 minute incubation, combine the diluted DNA with diluted Lipofectamine 2000. Mix gently and incubate for 25 minutes at room temperature.

3. Add the 30 µl of complexes to each well containing cells and medium. Mix gently by tapping the plate.

4. Incubate cells at 37°C in a CO2 incubator. After ~24 hours of transfection, change medium to fresh growth medium. ~48 hours after transfection perform dual luciferase assay following manufacturer's protocol.
   To study the effect of activators / inhibitors on the Wnt pathway, treat the cells with tested activator/inhibitor after ~24 hours or ~ 42 hours of transfection. Perform dual luciferase assay ~48 hours after transfection.

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Sample protocol to determine the dose response of HEK293 cells transfected with TCF/LEF reporter to mouse Wnt3a

Additional materials required in this experiment setup

- LiCl (Sigma # L7026)
- Mouse Wnt3a (R&D Systems 1324-WN)
- HEK293 growth medium: MEM/EBSS (with L-glutamine) (Hyclone #SH30024.01) + 10% FBS, 1% non-essential amino acid, 1mM Na-pyruvate + 1% Pen/Strep
- 96-well tissue culture treated white clear-bottom assay plate (Corning # 3610)
- Dual-Glo® Luciferase Assay System (Promega #E2920)

1. One day before transfection, seed HEK293 cells at a density of 30,000 cells per well into white clear-bottom 96-well plate in 100 µl of growth medium. Incubate cells at 37° in a CO2 incubator for overnight.

2. Next day, transfect 1 µl of TCF/LEF luciferase reporter (component A) into cells fowllowing the procedure in **Generalized Transfection and Assay Protocols.**

3. After ~24 hours of transfection, treat transfected cells with LiCl (10mM) in 50 µl of fresh growth medium. Incubate cells at 37° in a CO2 incubator for ~ 16 hours.

4. After ~40 hours of transfection, add threefold serial dilution of mouse Wnt3a in 5µl of growth medium to stimulated wells; add 5µl of growth medium to unstimulated control wells; add 55µl of growth medium to cell-free control wells (for determining background luminescence). Set up each treatment in at least triplicate.

5. Incubate at 37° in a CO2 incubator for 5-6 hours.

6. Perform dual luciferase assay using Dual-Glo® Luciferase Assay System: Add 55 µl of Luciferase reagent per well and rocking at room temperature for ~15 minutes and measure firefly luminescence using a luminometer. Add 55 µl of Stop & Glo reagent per well and rocking at room temperature for ~15 minutes and measure Renilla luminescence.

7. To obtain the normalized luciferase activity for TCF/LEF reporter, subtract background luminescence then calculate the ratio of firefly luminescence from the TCF/LEF reporter to Renilla luminescence from the control Renilla luciferase vector.

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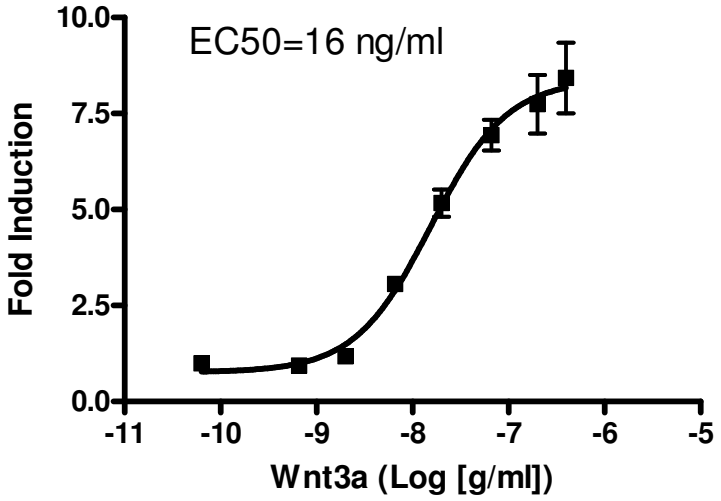
Figure 1. Dose response of TCF/LEF reporter activity to mouse Wnt3a. The results were shown as fold induction of normalized TCF/LEF luciferase reporter activity. Fold induction was determined by comparing values against the mean value for control cells without Wnt3a treatment.

The EC50 of mWnt3a is ~16 ng/ml.



Sample protocol to determine the effect of antagonists of Wnt signaling pathway on Wnt3a-induced TCF/LEF reporter activity in HEK293 cells

Additional materials required in this experiment setup

- IWR-1-endo (Santa Cruz biotechnology # sc-295215): inhibitor of Wnt pathway
- LiCl (Sigma # L7026)
- Mouse Wnt3a (R&D Systems 1324-WN)
- HEK293 growth medium: MEM/EBSS (with L-glutamine) (Hyclone #SH30024.01) + 10% FBS, 1% non-essential amino acid, 1mM Na-pyruvate + 1% Pen/Strep
- 96-well tissue culture treated white clear-bottom assay plate (Corning # 3610)
- Dual-Glo® Luciferase Assay System (Promega #E2920)

1. One day before transfection, seed HEK293 cells at a density of 30,000 cells per well into white clear-bottom 96-well plate in 100 µl of growth medium. Incubate cells at 37° in a CO2 incubator for overnight.

2. Next day, transfect 1 µl of TCF/LEF luciferase reporter (component A) into cells fowllowing the procedure in **Generalized Transfection and Assay Protocols.**

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3. After ~24 hours of transfection, treat transfected cells with threefold serial dilution of IWR-1-endo plus LiCl (10mM) in 50 µl of fresh growth medium. Incubate cells at 37° in a CO2 incubator for ~ 16 hours. For wells without IWR-1-endo, treat cells with LiCl only.

4. After ~40 hours of transfection, add mouse Wnt3a (final concentration 40 ng/ml) in 5µl of growth medium to stimulated wells (cells treated with Wnt3a+LiCl, and with or without IWR-1-endo); add 5µl of growth medium to the unstimulated control wells (cells treated with LiCl only for determining the basal activity); add 55µl of growth medium to cell-free control wells (for determining background luminescence). Set up each treatment in at least triplicate.

5. Incubate at 37° in a CO2 incubator for 5-6 hours.

6. Perform dual luciferase assay using Dual-Glo® Luciferase Assay System: Add 55 µl of Luciferase reagent per well and rocking at room temperature for ~15 minutes and measure firefly luminescence using a luminometer. Add 55 µl of Stop & Glo reagent per well and rocking at room temperature for ~15 minutes and measure Renilla luminescence.

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

Figure 2. Inhibition of Wnt3a-induced TCF/LEF reporter activity by IWR-1-endo.

Figure 2a. IWR-1-endo completely blocked Wnt3a-induced TCF/LEF reporter activity.

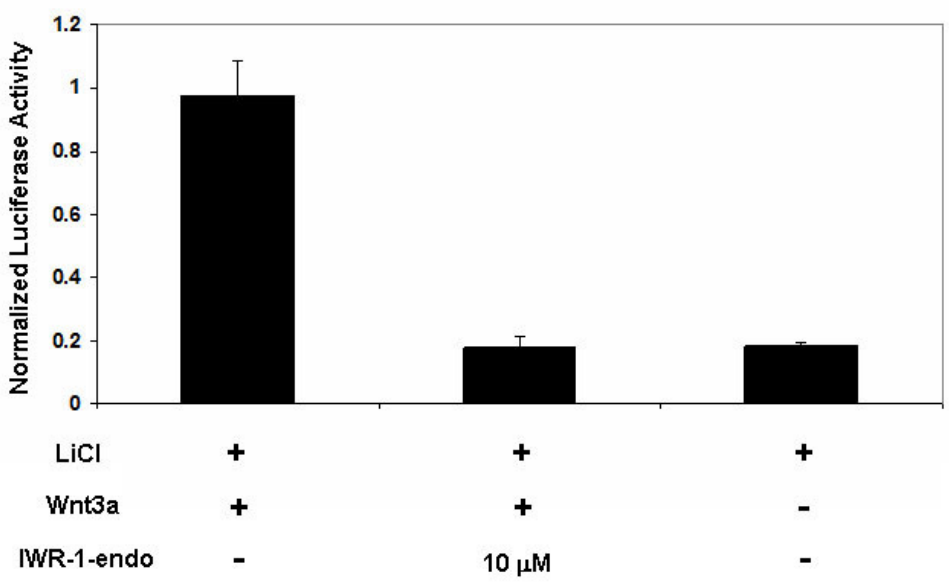

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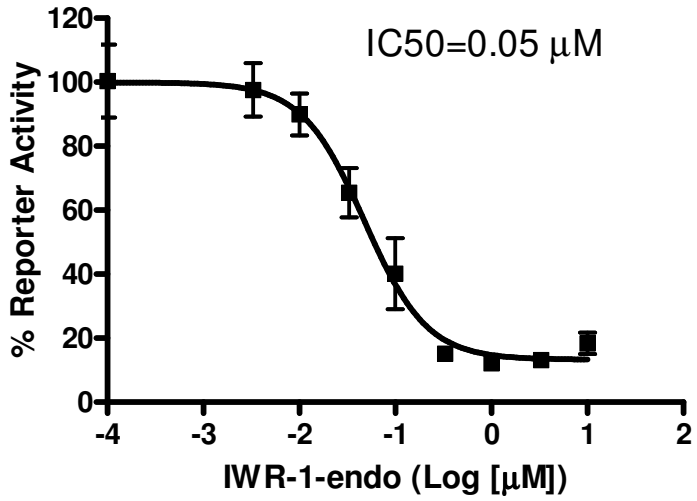
Figure 2b. Dose response of Wnt3a-induced TCF/LEF reporter activity to IWR-1-endo. The results were shown as percentage of TCF/LEF reporter activity. The normalized luciferase activity for cells stimulated with Wnt3a in the absence of IWR-1-endo was set at 100%.

The IC50 of IWR-1-endo is ~ 0.05 µM.



Reference

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.