Description

The TCF/LEF Reporter Kit (Wnt/ β-catenin Signaling Pathway) is designed for monitoring the activity of Wnt / β-catenin signaling pathway in a cellular model. The kit contains a transfection-ready TCF/LEF (T-cell factor/ lymphoid enhancer factor) Luciferase Reporter Vector, which is a Wnt pathway-responsive reporter. The TCF/LEF Luciferase Reporter Vector contains the firefly luciferase reporter under the control of multimerized TCF/LEF responsive element located upstream of a minimal promoter. The TCF/LEF reporter vectors come premixed with a constitutively expressing sea pansy (Renilla) luciferase vector, which serves as an 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 reporter 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.

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 ((T-cell factor/ lymphoid enhancer factor) transcription factors in the nucleus, leading to transcription and expression of Wnt-responsive genes.

Applications

- Monitor Wnt signaling pathway activity.
- Screen for compounds that affect Wnt/β-catenin signaling pathway activity.
- Study effects of RNAi or gene overexpression on the activity of the Wnt/β-catenin pathway.

Components

Catalog #	Name	Amount	Storage
Component A	TCF/LEF Luciferase Reporter Vector Mix (TCF/LEF Luciferase Reporter Vector + constitutively expressing Renilla Luciferase Vector) (60 ng DNA/ µl)	500 µl	-20°C
Component B	Negative Control Reporter Mix (Non-Inducible Luciferase Vector + constitutively expressing Renilla Luciferase Vector) (60 ng DNA/ µl)	500 µl	-20°C

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

Materials Required but Not Supplied

Materials Used in Cellular Assay

Name	Ordering Information
Mammalian cell line of interest	
Appropriate cell culture media	
Lipofectamine™ 2000 Transfection Reagent	Thermo Fisher #11668027
Opti-MEM™ I Reduced Serum Medium	Invitrogen #31985-062
Clear-bottom, white 96-well tissue culture-treated plate	Corning #3610
TWO-Step™ Luciferase (Firefly & Renilla) Assay System	BPS Bioscience #60683
LiCl	Sigma #L7026
Recombinant Mouse Wnt3-a	R&D Systems #1324-WN
IWR-1-endo	Santa Cruz Biotechnology #sc-295215
Luminometer	

Storage Conditions

This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

General Transfection and Assay Protocols

- The following procedure is designed to transfect the reporter into 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 manufacturer's transfection protocol.
- Transfection conditions should be optimized according to the cell type and study requirements. All amounts and volumes in the following setup are given on a per well basis.
- We recommend setting up at least triplicate assays for each condition and preparing transfection cocktails for multiple wells to minimize pipetting errors.

Day 1:

1. Seed cells at a density of 30,000 cells per well in 100 µl of appropriate cell growth medium.
   Note: Cells should be 90% confluent at the time of transfection.

Day 2:

1. Based on the experimental design of interest, prepare the following DNA transfection mixes, and mix gently:
    a. 1 µl of Component A in 15 µl of Opti-MEM™ I Reduced Serum Medium (no antibiotics) per well, and 1 µl of Component B in 15 µl of Opti-MEM™ I Reduced Serum Medium (no antibiotics) per well as transfection control.
    b. 1 µl of Component A + experimental vector expressing gene of interest in 15 µl of Opti-MEM™ I Reduced Serum Medium (no antibiotics) per well, and as transfection controls:
        i. 1 µl of Component A + your negative control expression vector in 15 µl of Opti-MEM™ I Reduced Serum Medium (no antibiotics) per well,
        ii. 1 µl of Component B + experimental vector expressing gene of interest, in 15 µl of Opti-MEM™ I Reduced Serum Medium (no antibiotics) per well,
        iii. 1 µl of Component B + your negative control expression vector in 15 µl of Opti-MEM™ I Reduced Serum Medium (no antibiotics) per well.
    c. 1 µl of Component A + specific siRNA in 15 µl of Opti-MEM™ I Reduced Serum Medium (no antibiotics) per well, and as transfection controls:
        i. 1 µl of Component A + Negative Control siRNA in 15 µl of Opti-MEM™ I Reduced Serum Medium (no antibiotics) per well,
        ii. 1 µl of Component B + specific siRNA in 15 µl of Opti-MEM™ I Reduced Serum Medium (no antibiotics) per well,
        iii. 1 µl of Component B + Negative Control siRNA in 15 µl of Opti-MEM™ I Reduced Serum Medium (no antibiotics) per well.

2. Mix Lipofectamine™ 2000 gently before use, then dilute 0.35 µl of Lipofectamine™ 2000 in 15 µl of Opti-MEM™ I Reduced Serum Medium (no antibiotics) per well.

    Note: Prepare this cocktail in a volume sufficient for the whole experiment.

3. Incubate for 5 minutes at Room Temperature (RT).

4. After the 5-minute incubation, combine the diluted DNA with the diluted Lipofectamine™ 2000 (you should now have 30 µl of solution per experimental well).

5. Mix gently and incubate for 25 minutes at RT.

6. Add 30 µl of DNA:Lipofectamine™ 2000 complexes to each well containing cells and medium.

7. Mix gently by tapping the plate.

8. Incubate cells at 37°C in a CO_2 incubator.

Day 3:

1. Change medium to fresh medium.

Day 4:

1. 48 hours after transfection, measure luciferase levels using the TWO-Step Luciferase Assay System following BPS Bioscience's protocol.

Validation Data

A. Effect of mouse Wnt3a on the TCF/LEF reporter activity in HEK293 cells.

- This assay should include "Cell-Free Control", "Unstimulated" and "Stimulated" conditions.

Day 1:

1. Seed HEK293 cells at a density of 30,000 cells per well into white clear-bottom 96-well plate in 100 µl of cell culture growth medium. Leave a few empty wells as ""Cell-Free Control" (background luminescence).
2. Incubate cells overnight at 37°C in a CO_2 incubator.

Day 2:

1. Transfect cells with 1 µl of Component A per well ("Unstimulated" and "Stimulated" wells), following the procedure described above.

Day 3:

1. Prepare 10 mM LiCl in growth medium (50 µl/ well).
2. Carefully remove the media from the transfected cells.
3. Add 50 µl of LiCl to each well.
4. Incubate cells at 37°C in a CO_2 incubator for 16 hours.

Day 4:

1. Prepare a threefold serial dilution of mouse Wnt3a in growth medium (5 µl/ well).
2. ~40 hours post-transfection, add 5 µl of diluted mouse Wnt3a to the "Stimulated" wells.
3. Add 5 µl of growth medium to the "Unstimulated" wells.
4. Add 55 µl of growth medium to the "Cell-Free Control" wells.
5. Incubate cells at 37°C in a CO_2 incubator for 5-6 hours.
6. Dilute 100 x Firefly Luciferase Reagent Substrate (Component B) with Firefly Luciferase Reagent Buffer (Component A) (55 µl/ well).
7. Add 55 µl of Firefly Luciferase reagent per well and rock at RT for ~15 minutes.

8. Measure firefly luminescence using a luminometer.

9. Dilute 100x Renilla Luciferase Reagent Substrate (Component D) into Renilla Luciferase Reagent Buffer (Component C) (55 µl/ well).

10. Add 55 µl of Renilla Luciferase reagent per well, rock at RT for ~1 minute and measure Renilla luminescence.

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

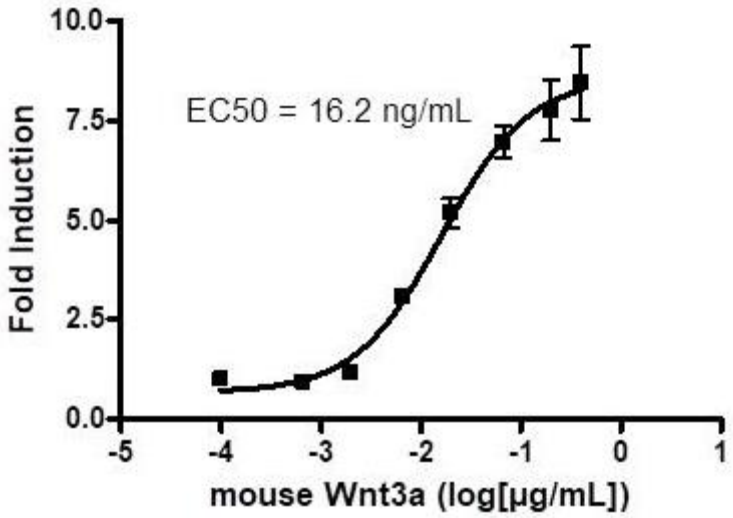

Figure 1: Dose response of TCF/LEF reporter activity to mouse Wnt3a.
HEK293 cells were transfected with and treated with increasing concentrations of mouse Wnt3a. Luciferase activity was measured with the TWO-Step Luciferase (Firefly & Renilla) Assay System. The results were shown as fold induction of normalized TCF/LEF luciferase reporter activity. Fold induction was determined by comparing values against the mean value for control cells without Wnt3a treatment.

B. Effect of Wnt antagonists on Wnt3a-induced TCF/LEF reporter activity in HEK293 cells

- This assay should include "Cell-Free Control", ""Stimulated, Antagonist", "Stimulated, No Antagonist" and "Unstimulated, No Antagonist"" conditions.

Day 1:

1. Seed HEK293 cells at a density of 30,000 cells per well into white clear-bottom 96-well plate in 100 µl of cell culture growth medium. Leave a few empty wells as "Cell-Free Control" (background luminescence).

2. Incubate cells overnight at 37°C in a CO_2 incubator.

Day 2:

1. Transfect cells with 1 µl of Component A per well following the procedure described above.
2. Incubate overnight at 37°C in a CO_2 incubator.

Day 3:

1. Prepare a solution of 10 mM LiCl in fresh medium.
2. Prepare a mix of threefold serial dilutions of IWR-1-endo with 10 mM LiCl in fresh growth medium (50 µl/ well).
3. Carefully remove the media from the wells.
4. Add 50 µl of the mix of IWR-1-endo and LiCl to the "Stimulated, Antagonist" wells.
5. Add 50 µl of 10 mM LiCl to the "Stimulated, No Antagonist" and "Unstimulated, No Antagonist" wells.
6. Add 50 µl of growth medium to the "Cell-Free Control" wells ("Background Luminescence" control wells).
7. Incubate cells 16 hours at 37°C in a CO_2 incubator.

Day 4:

1. Prepare a solution of mouse Wnt3a in growth medium (5 µl/ well).

   Note: A final concentration of 40 ng/ ml has been used in our validation.

2. Add 5 µl of diluted Wnt3a to the "Stimulated, No Antagonist" and "Stimulated, Antagonist" wells.
3. Add 5 µl of growth medium to the "Unstimulated, No Antagonist" and "Cell-Free Control" wells.
4. Incubate for 5-6 hours at 37°C in a CO_2 incubator.
5. Dilute 100 x Firefly Luciferase Reagent Substrate (Component B) with Firefly Luciferase Reagent Buffer (Component A) (55 µl/ well).
6. Add 55 µl of Firefly Luciferase reagent per well and rock at RT for ~15 minutes.
7. Measure firefly luminescence using a luminometer.
8. Dilute 100x Renilla Luciferase Reagent Substrate (Component D) into Renilla Luciferase Reagent Buffer (Component C) (55 µl/ well).

9. Add 55 µl of Renilla Luciferase reagent per well, rock at RT for ~1 minute and measure Renilla luminescence.

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

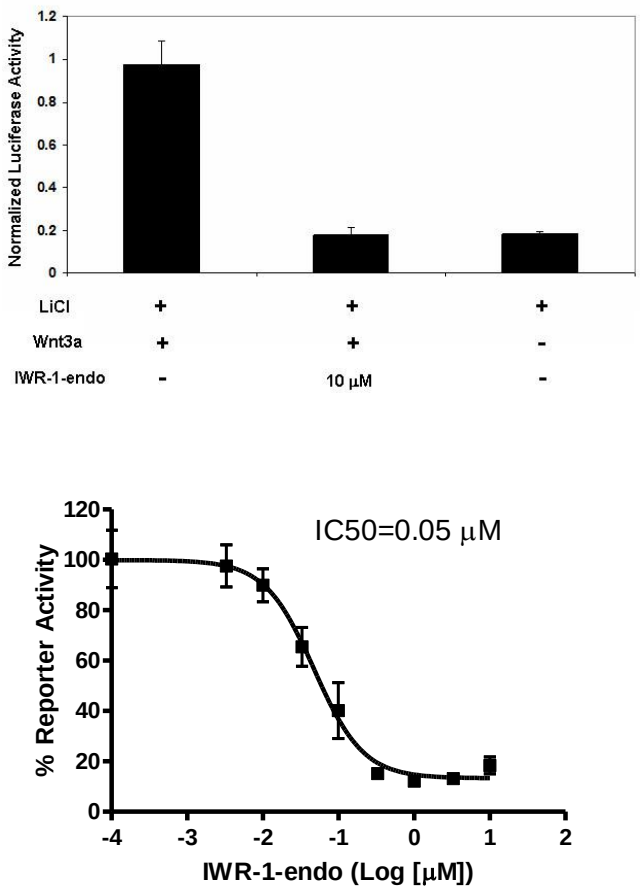

Figure 2. Inhibition of Wnt3a-induced TCF/LEF reporter activity in HEK293 cells.
Top panel: HEK293 cells were transfected and treated with different combinations of LiCl and IWR-1-endo, in the presence or absence of mouse Wnt3a. Luciferase activity was measured with the TWO-Step Luciferase (Firefly & Renilla) Assay System.
Bottom panel: HEK293 cells were transfected with and treated LiCl, with increasing concentrations of IWR-1-endo and 40 ng/ml of mouse Wnt3a. Luciferase activity was measured with the TWO-Step Luciferase (Firefly & Renilla) Assay System. 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.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

Troubleshooting Guide

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

References

Chen B., *et al*., 2009 *Nature Chemical Biology* 5(2): 100-107.

Related Products

Products	*Catalog #*	*Size*
TCF/LEF Reporter – HEK293 Cell Line (Wnt Signaling Pathway)	60501	2 vials
TCF/LEF Luciferase Reporter Lentivirus (Wnt/ β-catenin Signaling Pathway)	79787	500 µl x 2
TCF/LEF StemBright™ Luciferase iPS Cell Pool (Wnt Pathway)	78515	1 vial
CRE/CREB Reporter Assay Kit	60611	500 reactions

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