6405 Mira Mesa Blvd Ste 100
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: support@bpsbioscience.com

Data Sheet

The Transfection Collection™ – TCF/LEF Transient Pack Wnt / β-catenin Signaling Pathway

Catalog #: 79273

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 Transient Pack* is designed to provide the tools necessary for transiently transfecting and monitoring the activity of Wnt / β-catenin signaling pathway in the cultured HEK293 cells. The TCF/LEF *Transient Pack* contains transfection-ready vectors containing firefly luciferase as a Wnt pathway-responsive reporter and constitutively expressing Renilla luciferase as a transfection control. It also includes the TWO-Step Luciferase detection reagents to detect both luciferase activities and specialized medium for growing and assaying HEK293 cells.

The key to the TCF/LEF *Transient Pack* is the 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 pack 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.

Additionally, the pack includes cell culture medium (BPS Medium 1) that has been optimized for use with HEK293 cells*. BPS Medium 1 includes MEM medium, 10% fetal bovine serum, 1% non-essential amino acids, sodium pyruvate, and 1% Pen/Strep. Finally, the pack provides the TWO-Step Luciferase (Firefly & Renilla) Assay System.

6405 Mira Mesa Blvd Ste 100
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: support@bpsbioscience.com

These luciferase reagents provide highly sensitive, stable detection of firefly luciferase activity and Renilla luciferase activity. The TWO-step luciferase reagents can be used directly in cells in growth medium, and can be detected with any luminometer; automated injectors are not required.

*Note: the kit may be used with other cell lines than HEK293, but an alternate cell culture medium may be required for optimal cell growth.

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.

Components

Component	Amount	Storage
Reporter (Component A) TCF/LEF luciferase reporter vector* + constitutively expressing Renilla luciferase vector*	500 µl (60 ng DNA/ µl)	-20°C
Negative Control Reporter (Component B) Non-inducible luciferase vector*+ constitutively expressing Renilla luciferase vector*	500 µl (60 ng DNA/ µl)	-20°C
Firefly Luciferase Reagent Buffer	10 ml	-20°C
Firefly Luciferase Reagent Substrate (100x)	100 µl	-20°C *Protect from light*
Renilla Luciferase Reagent Buffer	10 ml	Room Temp.
Renilla Luciferase Reagent substrate (100x)	100 µl	-20°C *Protect from light*
BPS Medium 1	100 ml	+4°C

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

6405 Mira Mesa Blvd Ste 100
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: support@bpsbioscience.com

Materials Required but Not Supplied

- HEK293 cells. Other mammalian cell lines may also be used, but an alternate cell culture medium may be required for optimal cell growth.
- 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 should work equally well.
- Opti-MEM I Reduced Serum Medium (Life Technologies, #31985-062)
- 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 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 provided 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 BPS Medium 1 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.

6405 Mira Mesa Blvd Ste 100
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: support@bpsbioscience.com

Note: we recommend setting up at least triplicates for each condition, and preparing a transfection cocktail for multiple wells to minimize pipetting errors.

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. Carefully remove and discard 30 µl of media from each of the wells of cell culture, taking care not to disturb the cells or touch the bottom of the well with the pipet tip. 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 CO_2 incubator. After ~24 hours of transfection, change medium to fresh growth medium. ~48 hours after transfection perform the TWO-Step Luciferase Assay System (below).

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 TWO-step luciferase assay ~48 hours after transfection (below).

TWO-Step Luciferase Assay Procedure

1. Thaw **Firefly Luciferase Reagent Buffer** by placing the reagent in a room temperature water bath. Equilibrate the buffer to room temperature and mix well before use. *Note: It is important that the **Firefly Luciferase Reagent Buffer** be at room temperature before use.*
2. Calculate the amount of Firefly Luciferase Assay Working solution needed for the experiment (**Firefly Luciferase Reagent Buffer** + **Firefly Luciferase Reagent Substrate**). Immediately prior to performing the experiment, prepare the Firefly Luciferase Assay Working Solution by diluting **Firefly Luciferase Reagent Substrate** into **Firefly Luciferase Reagent Buffer** at a 1:100 ratio and mix well. Avoid exposing to excessive light. *Only use enough of each component for the experiment, remaining* **Firefly Luciferase Reagent Buffer** *and* **Firefly Luciferase Reagent Substrate** *should be stored separately at* -20°C.
3. Remove multi-well plate containing mammalian cells from incubator. *Note: plates must be compatible with luminescence measurement by luminometer being used.*

6405 Mira Mesa Blvd Ste 100
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: support@bpsbioscience.com

4. Add equal volume of Firefly Luciferase Assay Working Solution (step 2) to the culture medium in each well. Example: 96-well plate with 100 µl of culture medium requires 100 µl of Firefly Luciferase Assay Working Solution per well.

   Gently rock the plates for ≥15 minutes at room temperature. Measure firefly luminescence using a luminometer. The signal under these conditions will be stable for more than 2 hours at room temperature. For maximal light intensity, measure samples within 1 hour of reagent addition.

5. Calculate the amount of Renilla Luciferase Assay Working Solution needed for the experiment (**Renilla Luciferase Reagent Buffer** + **Renilla Luciferase Reagent Substrate**). Prepare the Renilla Luciferase Assay Working Solution by diluting **Renilla Luciferase Reagent Substrate** into **Renilla Luciferase Reagent Buffer** at a 1:100 ratio and mix well. Avoid exposing to excessive heat or light*. Only use enough of each component for the experiment,*

6. Add equal volume of Renilla Luciferase Assay Working Solution (step 5) to each well. Example: 96-well plate with 100 µl of culture medium + 100 µl Firefly Luciferase Reagent requires 100 µl of Renilla Luciferase Assay Working Solution per well.

7. Gently rock the plates for ~1 minute at room temperature. Measure Renilla luminescence using a luminometer.

8. Data analysis: subtract background (wells with medium and luciferase reagent only) from all the readings. To obtain the normalized luciferase activity for the TCF/LEF reporter, subtract the background luminescence, then calculate the ratio of firefly luminescence from TCF/LEF reporter to Renilla luminescence from the control Renilla luciferase vector. To determine the fold induction, divide the value for the treated cells by the value for the untreated cells.

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 cells
- 96-well tissue culture treated white clear-bottom assay plate (Corning, #3610)

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 BPS Medium 1. Incubate cells at 37°C in a CO_2 incubator for overnight.

6405 Mira Mesa Blvd Ste 100
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: support@bpsbioscience.com

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

3. After ~24 hours of transfection, treat transfected cells with LiCl (10 mM) in 50 µl of fresh BPS Medium 1. Incubate cells at 37°C in a CO_2 incubator for ~ 16 hours.

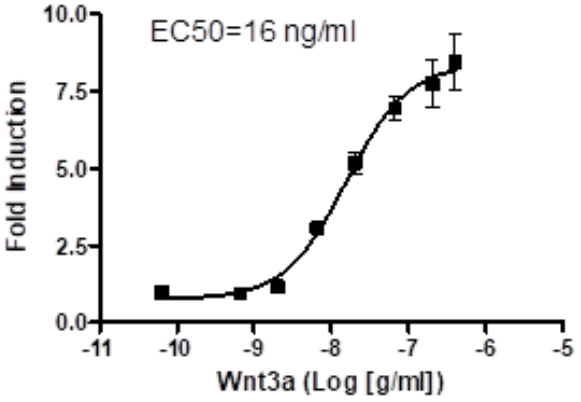
4. After ~40 hours of transfection, add threefold serial dilution of mouse Wnt3a in 5 µl of BPS Medium 1 to stimulated wells; add 5 µl of BPS Medium 1 to unstimulated control wells; add 55 µl of BPS Medium 1 to cell-free control wells (for determining background luminescence). Set up each treatment in at least triplicate.

5. Incubate at 37°C in a CO_2 incubator for 5-6 hours.

6. After ~48 hours of transfection, perform TWO-step luciferase assay as described above in **TWO-Step Luciferase Assay Procedure.** Briefly, dilute 100x Firefly Luciferase Reagent Substrate into Firefly Luciferase Reagent Buffer that has been equilibrated to room temperature. Add 55 µl of Firefly Luciferase reagent per well and rock at room temperature for ~15 minutes, then measure firefly luminescence using a luminometer. Dilute 100x Renilla Luciferase Reagent Substrate into Renilla Luciferase Reagent Buffer . Add 55 µl of Renilla Luciferase reagent per well, rock at room temperature for ~1 minute 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. To determine the fold induction, divide the value for the treated cells by the value for the untreated cells.

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.



6405 Mira Mesa Blvd Ste 100
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: support@bpsbioscience.com

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)
- 96-well tissue culture treated white clear-bottom assay plate (Corning, #3610)

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 BPS Medium 1. Incubate cells at 37°C in a CO_2 incubator for overnight.

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

3. After ~24 hours of transfection, treat transfected cells with threefold serial dilution of IWR-1-endo plus LiCl (10 mM) in 50 µl of fresh BPS Medium 1. Incubate cells at 37°C in a CO_2 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 5 µl of BPS Medium 1 to stimulated wells (cells treated with Wnt3a+LiCl, and with or without IWR-1-endo); add 5 µl of BPS Medium 1 to the unstimulated control wells (cells treated with LiCl only for determining the basal activity); add 55 µl of BPS Medium 1 to cell-free control wells (for determining background luminescence). Set up each treatment in at least triplicate.

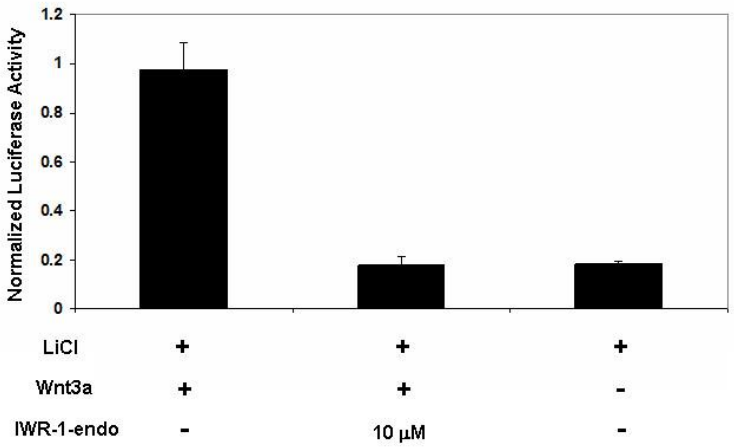
5. Incubate at 37°C in a CO_2 incubator for 5-6 hours.

6. After ~48 hours of transfection, perform TWO-step luciferase assay as described above in **TWO-Step Luciferase Assay Procedure.** Briefly, dilute 100x Firefly Luciferase Reagent Substrate into Firefly Luciferase Reagent Buffer that has been equilibrated to room temperature. Add 55 µl of Firefly Luciferase reagent per well and rock at room temperature for ~15 minutes, then measure firefly luminescence using a luminometer. Dilute 100x Renilla Luciferase Reagent Substrate into Renilla Luciferase Reagent Buffer. Add 55 µl of Renilla Luciferase reagent per well, rock at room temperature for ~1 minute and measure Renilla luminescence.

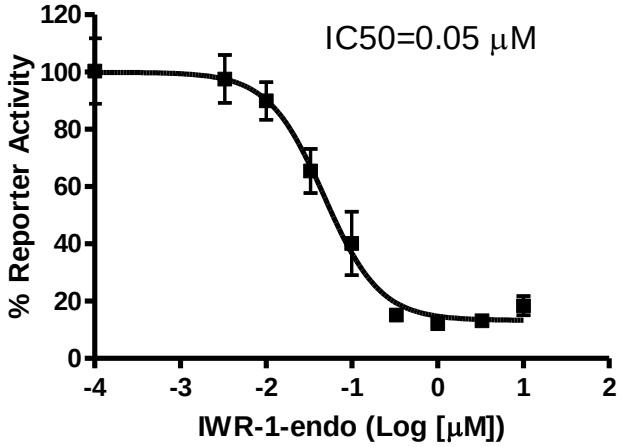
7. To obtain the normalized luciferase activity of TCF/LEF reporter, subtract background luminescence then calculate the ratio of firefly luminescence from the TCF/LEF reporter to Renilla luminescence from the control Renilla luciferase vector.

6405 Mira Mesa Blvd Ste 100
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: support@bpsbioscience.com

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



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.

6405 Mira Mesa Blvd Ste 100
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: support@bpsbioscience.com

Refills

Product	Catalog #	Size
TCF/LEF Reporter Kit	60500	500 rxns
TWO-Step Luciferase (Firefly & Renilla) Assay System	60683-1	10 ml
TWO-Step Luciferase (Firefly & Renilla) Assay System	60683-2	100 ml
TWO-Step Luciferase (Firefly & Renilla) Assay System	60683-3	1 L
BPS Medium 1	79259	100 ml

Related Products

Product	Cat. #	Size
TCF/LEF reporter cell line (Lithium-Dependent)	60501	2 vials
TCF/LEF reporter cell line (Lithium-Independent)	60531	2 vials