Data Sheet

Wnt Signaling Pathway TCF/LEF Reporter (Luc) – HEK293 Cell Line
Catalog #: 60501

Background

The *Wnt Signaling Pathway TCF/LEF Reporter (Luc) – HEK293 Cell Line* is designed for monitoring the activity of the Wnt/β-catenin signaling pathway. The Wnt 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 *Wnt Signaling Pathway TCF/LEF Reporter (Luc) – HEK293 Cell Line* contains a firefly luciferase gene under the control of TCF/LEF responsive elements stably integrated into HEK293 cells. This cell line is validated for the response to the stimulation of mouse Wnt3a and to the treatment with an inhibitor of Wnt/β-catenin signaling pathway.

Application

- Monitor Wnt signaling pathway activity.
- Screen activators or inhibitors of Wnt / β-catenin signaling pathway.

Format

Each vial contains ~1.5 X 10^6 cells in 1 ml of 10% DMSO.

Functional Validation and Assay Performance

The following assays are designed for 96-well format. To perform the assay in different tissue culture formats, cell number and reagent volume should be scaled appropriately.

Materials Required but Not Supplied

- LiCl (Sigma # L7026)
- Mouse Wnt3a (R&D Systems 1324-WN)
- IWR-1-endo (Santa Cruz Biotechnology # sc-295215): inhibitor of Wnt pathway
- Assay medium: MEM medium (Hyclone #SH30024.01) + 10% FBS + 1% non-essential amino acid + 1 mM Na-pyruvate + 1% Pen/Strep
- 96-well tissue culture treated white clear-bottom assay plate (Corning # 3610)

- ONE-Glo™ luciferase assay system (Promega # E6110) or other luciferase reagents for measuring firefly luciferase activity
- Luminometer

A. Dose response of *Wnt Signaling Pathway TCF/LEF Reporter (Luc) – HEK293* cells to mouse Wnt3a

1. Harvest TCF/LEF reporter (Luc)-HEK293 cells from culture in growth medium and seed cells at a density of ~30,000 cells per well into a white clear-bottom 96-well microplate in 40µl of assay medium.

2. Prepare 50 mM LiCl solution in assay medium and add 10 µl of 50 mM LiCl solution to each well (final concentration 10 mM). Incubate cells at 37°C in a CO_2 incubator for ~ 16 hours.

3. Add 5 µl of threefold serial dilution of mouse Wnt3a in assay medium to stimulated wells.
Add 5 µl of assay medium to the unstimulated control wells.
Add 55 µl of assay medium to cell-free control wells (for determining background luminescence).
Set up each treatment in at least triplicate.

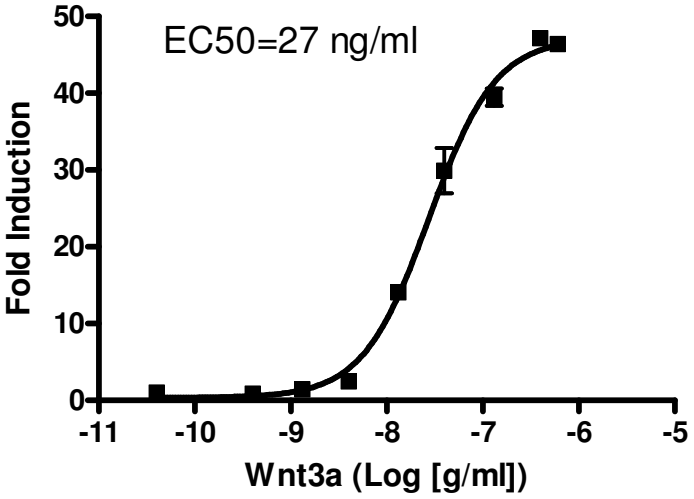
4. Incubate the plate at 37° in a CO_2 incubator for 5-6 hours.

5. Perform luciferase assay using ONE-Glo luciferase assay system: Add 55 µl of One-Glo Luciferase reagent per well and rock at room temperature for ~10 minutes. Measure luminescence using a luminometer. *If using other luciferase reagents from other vendors follow the manufacture's assay protocol.*

6. Data Analysis: Subtract average background luminescence (cell-free control wells) from luminescence reading of all wells.
Fold induction of TCF/LEF luciferase reporter expression = background-subtracted luminescence of Wnt3a-stimulated well / average background-subtracted luminescence of unstimulated control wells

Figure 1. Dose response of TCF/LEF reporter (luc)-HEK293 cells to mouse Wnt3a. The results were shown as fold induction of TCF/LEF luciferase reporter expression.

The EC50 of mWnt3a is ~ 27 ng/ml.



B. Inhibition of Wnt3a-induced reporter activity by an inhibitor of Wnt signaling pathway in *Wnt Signaling Pathway TCF/LEF reporter (Luc)-HEK293 cells*

1. Harvest TCF/LEF reporter (Luc)-HEK293 cells from culture in Growth medium and seed cells at a density of ~30,000 cells per well into a white clear-bottom 96-well microplate in 40 µl of assay medium.

2. Add 10µl of 50 mM LiCl solution in assay medium with or without IWR-1-endo (Wnt pathway inhibitor) to each well. Incubate cells at 37°C in a CO_2 incubator for ~ 16 hours.

3. Add 5 µl of diluted mouse Wnt3a in assay medium to stimulated wells (final Wnt3a concentration = 40 ng/ml).
Add 5 µl of assay medium to the unstimulated control wells (cells treated with LiCl only for determining the basal activity).
Add 55 µl of assay medium to cell-free control wells (for determining background luminescence).
Set up each treatment in at least triplicate.

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

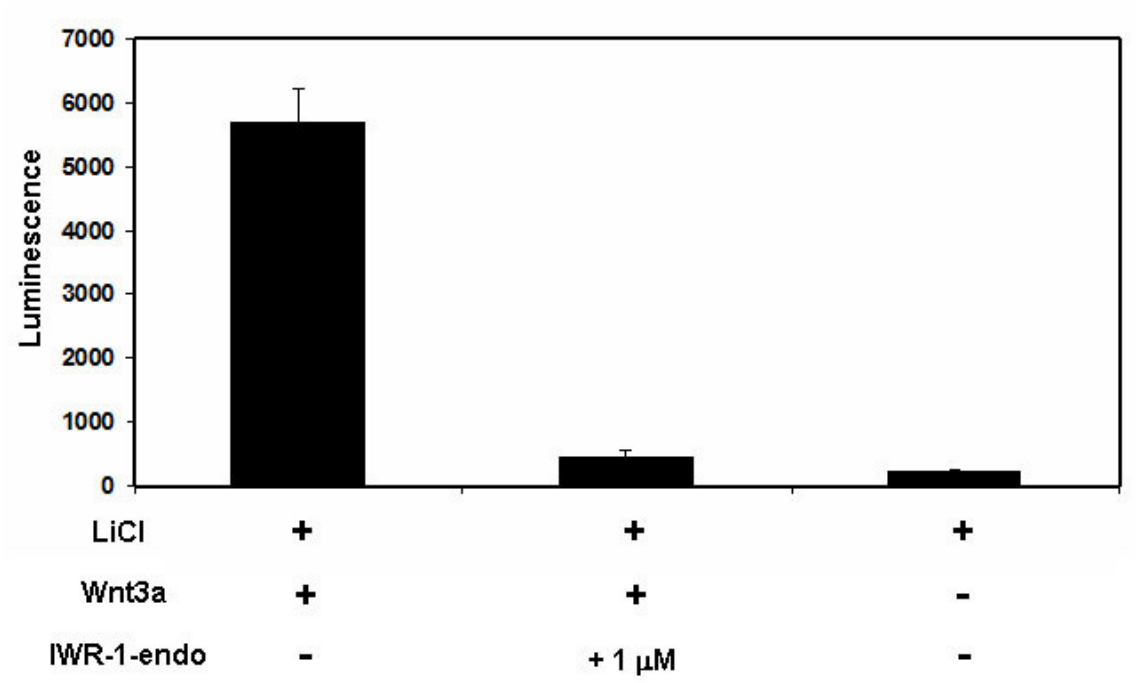
5. Perform luciferase assay using ONE-Glo luciferase assay system: Add 55 µl of One-Glo Luciferase reagent per well and rock at room temperature for ~10 minutes. Measure luminescence using a luminometer.

If using other luciferase reagents from other vendors follow the manufacture's assay protocol.

6. Data Analysis: Obtain background-subtracted luminescence by subtracting average background luminescence (cell-free control wells) from luminescence reading of all wells.

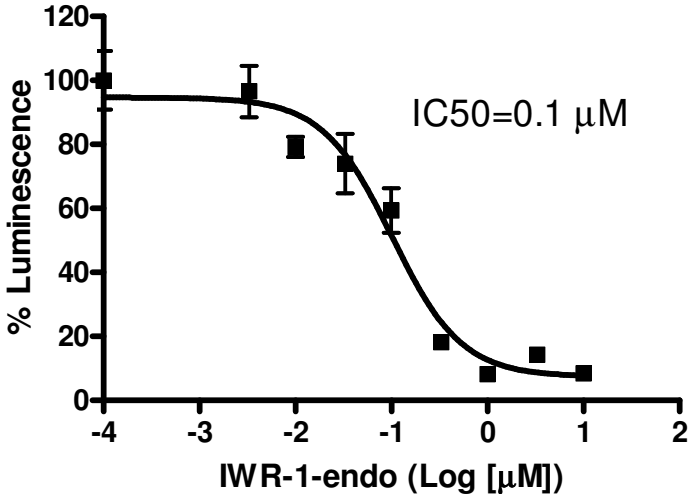
Figure 2. Inhibition of Wnt3a-induced reporter activity by IWR-1-endo in TCF/LEF reporter (Luc)-HEK293 cells

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



2b. IWR-1-endo inhibition dose response curve
The results are shown as percentage of luminescence. The background-subtracted luminescence of cells stimulated with Wnt3a in the absence of IWR-1-endo was set at 100%.

The IC50 of IWR-1-endo is ~ 0.1 µM.



Mycoplasma testing
The cell line has been screened using the PCR-based Venor™GeM Mycoplasma Detection kit (Sigma-Aldrich) to confirm the absence of Mycoplasma species.

Storage
Immediately upon receipt, store in liquid nitrogen.

Culture conditions
Cells should be grown at 37°C with 7% CO_2 using MEM medium (Hyclone #SH30024.01) supplemented with 10% FBS (Invitrogen #26140-079), 1% non-essential amino acid (Hyclone #SH30238.01), 1 mM Na pyruvate (Hyclone #SH30239.01), 1% Penicillin/Streptomycin (Hyclone SV30010.01), and 400 μg/ml of Geneticin (invitrogen #11811031). If culturing cells in medium from other vendors, it may be required to lower the percentage of CO_2 in the incubator depending on the $NaHCO_3$ level in the basal medium.

It is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, transfer to a tube containing 10 ml of growth medium without Geneticin, spin down cells, resuspend cells in pre-warmed growth medium without Geneticin, transfer resuspended cells to T25 flask and culture at 37°C in a 7% CO_2 incubator. At first passage switch to growth medium containing Geneticin. Cells should be split before they reach complete confluence.
To passage the cells, rinse cells with phosphate buffered saline (PBS), detach cells from culture vessel with Trypsin/EDTA, add complete growth medium and transfer to a tube,

spin down cells, resuspend cells and seed appropriate aliquots of cell suspension into new culture vessels.

Note: Just after thawing and at low density, the cells may grow at a slower rate. It is recommended to split the cells with ~ 1:4 ratio at the beginning of culturing. After several passages, the cell growth rate increases and the cells can be split with 1:8 -1:20 ratio weekly.

Reference

Clevers, H. (2006) Wnt/beta-catenin signaling in development and disease. Cell **127(3):**469-480.

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.

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