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Data Sheet

TCF/LEF Reporter – HEK293 Cell Line (Wnt Signaling Pathway) 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 ~2 X 10^6 cells in 1 ml of 10% DMSO.

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 Medium and Recommended Culture Conditions

Thaw Medium 1 (BPS Cat. #60187): MEM medium (Hyclone #SH30024.01) supplemented with 10% FBS (Life Technologies #26140-079), 1% non-essential amino acids (Hyclone #SH30238.01), 1 mM Na pyruvate (Hyclone #SH30239.01), 1% Penicillin/Streptomycin (Hyclone SV30010.01).

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Growth Medium 1B (BPS Cat. #79531): Thaw Medium 1 (BPS Cat. #60187) + 400 μg/ml of Geneticin (invitrogen #11811031)

Cells should be grown at 37°C with 5% CO_2 using **Growth Medium 1B (BPS Cat. #79531)** (Thaw Medium 1+ 400 μg/ml of Geneticin).

If culturing cells in medium from other vendors, it may be required to adjust the percentage of CO_2 in the incubator depending on the $NaHCO_3$ level in the basal medium

To thaw the cells, 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 Thaw Medium 1 (**no Geneticin)**, spin down cells at 1000 rpm, resuspend cells in 5 ml of pre-warmed Thaw Medium 1 (**no Geneticin)**, transfer resuspended cells to T25 flask and culture at 37°C in a 5% CO_2 incubator overnight. The next day, replace the medium with fresh warm Thaw Medium 1 (**no Geneticin)**, and continue growing culture in a CO_2 incubator at 37°C until the cells are ready to be split. Cells should be split before they reach complete confluence. At first passage switch to Growth Medium 1B (**contains Geneticin)**.

To passage the cells, rinse cells with phosphate buffered saline (PBS), detach cells from culture vessel with 0.05% Trypsin/EDTA. After detachment, add Growth Medium 1B (**contains Geneticin)** and transfer to a tube, spin down cells, resuspend cells in Growth Medium 1B (**contains Geneticin)** and seed appropriate aliquots of cell suspension into new culture vessels. Sub cultivation ration: 1:5 to 1:10 weekly or twice a week.

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 higher ratio.

To freeze down the cells, rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with 0.05% Trypsin/EDTA. After detachment, add Thaw Medium 1 (**no Geneticin)** and count the cells, then transfer to a tube, spin down cells, and resuspend in 4°C Freezing Medium (10% DMSO + 90% FBS) to ~$2x10^6$ cells/ml. Dispense 1 ml of cell aliquots into cryogenic vials. Place vials in an insulated container for slow cooling and store at -80°C overnight. Transfer to liquid nitrogen the next day for storage.
It is recommended to expand the cells and freeze down more than 10 vials of cells for future use at early passage.

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.

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Materials Required but Not Supplied

- LiCl (Sigma # L7026)
- Mouse Wnt3a (R&D Systems 1324-WN) or human Wnt3a (R&D Systems 5036-WN)
- IWR-1-endo (Santa Cruz Biotechnology # sc-295215): inhibitor of Wnt pathway
- Assay medium: Thaw Medium 1 (BPS Cat. #60187)
- Growth Medium 1B (BPS Cat. #79531)
- 96-well tissue culture treated white clear-bottom assay plate (Corning # 3610)
- ONE-Step™ Luciferase Assay System (BPS Cat. #60690)
- 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 ~35,000 cells per well into a white clear-bottom 96-well microplate in 80 µl of assay medium.

2. Prepare 50 mM LiCl solution in assay medium and add 20 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 10 µl of threefold serial dilution of mouse or human Wnt3a in assay medium to stimulated wells.
   Add 10 µl of assay medium to the unstimulated control wells.
   Add 110 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-Step™ Luciferase Assay buffer: Add 100 µl of ONE-Step™ Luciferase Assay buffer per well and rock at room temperature for ~15 to 30 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

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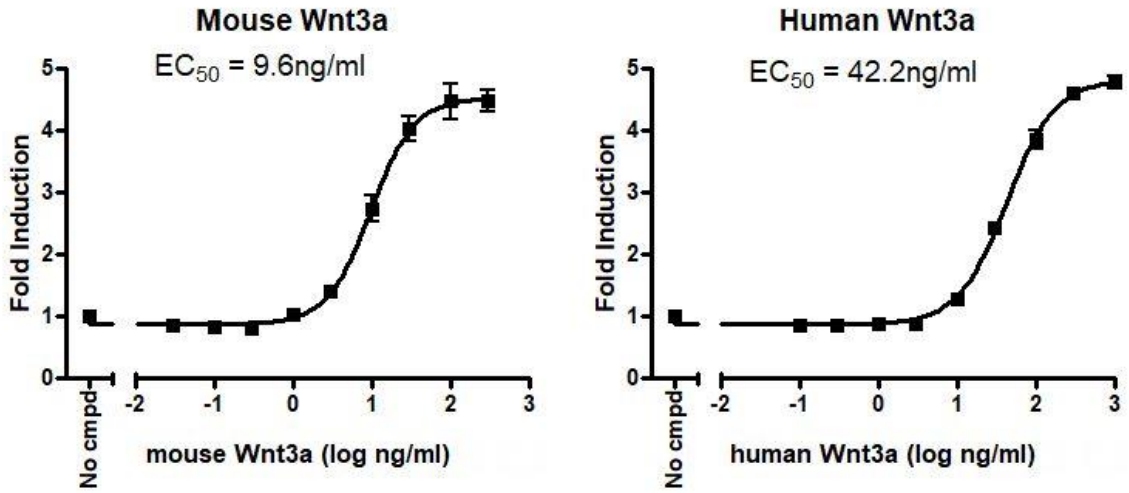
Figure 1. Dose response of TCF/LEF reporter (luc)-HEK293 cells to mouse Wnt3a.
The results are shown as fold induction of TCF/LEF luciferase reporter expression.

A. Mouse B. Human



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 ~35,000 cells per well into a white clear-bottom 96-well microplate in 80 µl of assay medium.

2. Add 20 µl 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 10 µl of diluted Wnt3a in assay medium to stimulated wells (final Wnt3a concentration = 40 ng/ml (mouse), 200 ng/ml (human)).
   Add 10 µl of assay medium to the unstimulated control wells (cells treated with LiCl only for determining the basal activity).
   Add 110 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.

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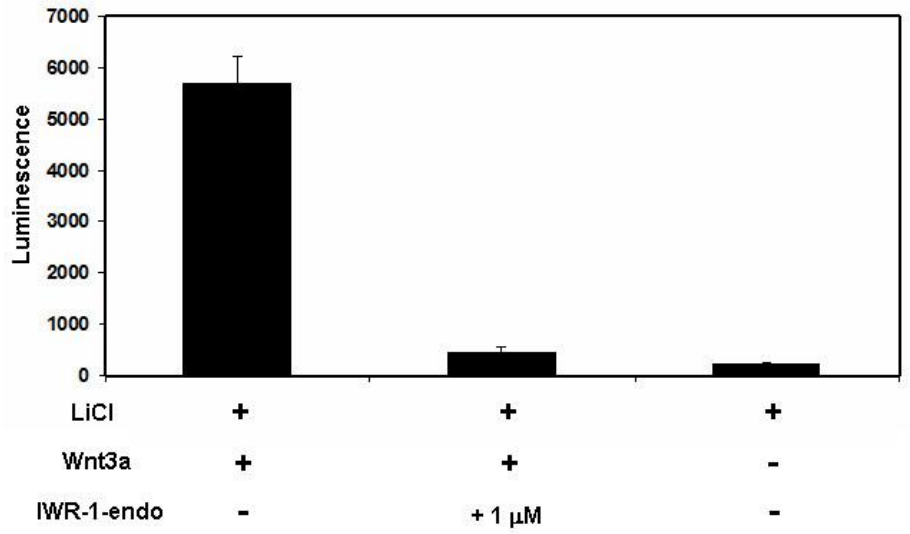
5. Perform luciferase assay using ONE-Step™ Luciferase Assay buffer: Add 100 µl of ONE-Step™ Luciferase Assay buffer per well and rock at room temperature for ~15 to 30 minutes. Measure luminescence using a luminometer.
   If using other luciferase reagents from other vendors follow the manufacture's 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 mWnt3a-induced TCF/LEF reporter activity.



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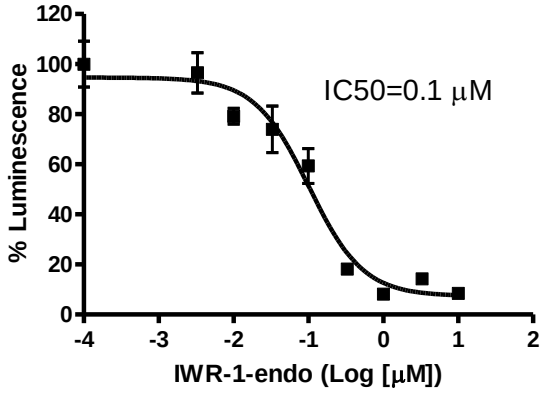
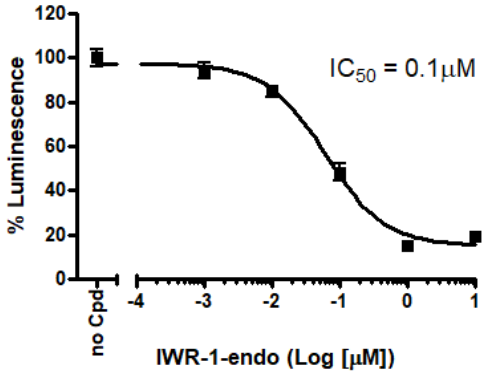
2b. IWR-1-endo inhibition dose response curves
The results are shown as percentage of luminescence. The background-subtracted luminescence of cells stimulated with Wnt3a in the absence of IWR-1-endo was set at 100%.

A. Mouse Wnt3a



B. Human Wnt3a



References

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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