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## **Data Sheet**

### **TCF/LEF Reporter (Luc) - HEK293 Cell line Catalog #: 60501**

#### **Background**

*TCF/LEF Reporter (Luc) – HEK293 cell line is designed for monitoring the activity of Wnt /  $\beta$ -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  $\beta$ -catenin.  $\beta$ -catenin then binds to TCF/LEF transcription factors in the nucleus, leading to transcription and expression of Wnt-responsive genes.*

#### **Description**

*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, showing Wnt pathway-responsive. This cell line is validated for the response to the stimulation of mouse Wnt3a and to the treatment of the inhibitor of Wnt /  $\beta$ -catenin signaling pathway.*

#### **Application**

- Monitor Wnt signaling pathway activity.
- Screen activators or inhibitors of Wnt /  $\beta$ -catenin signaling pathway.

#### **Format**

*Each vial contains  $\sim 1.5 \times 10^6$  cells in 1 ml of 10% DMSO.*

#### **Functional Validation and Assay Performance**

*The following assays are designed for 96-well format. To perform 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 + 1mM Na-pyruvate + 1% Pen/Strep
- 96-well tissue culture treated white clear-bottom assay plate (Corning # 3610)

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

#### **A. dose response of 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 white clear-bottom 96-well microplate in 40 $\mu$ l of assay medium.

2. Prepare 50mM LiCl solution in assay medium and add 10  $\mu$ l of 50 mM LiCl solution to each well (final concentration 10 mM). Incubate cells at 37° in a CO2 incubator for ~ 16 hours.

3. Add 5  $\mu$ l of threefold serial dilution of mouse Wnt3a in assay medium to stimulated wells.

Add 5  $\mu$ l of assay medium to the unstimulated control wells.

Add 55  $\mu$ l 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 CO2 incubator for 5-6 hours.

5. Perform luciferase assay using ONE-Glo luciferase assay system: Add 55  $\mu$ l 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.

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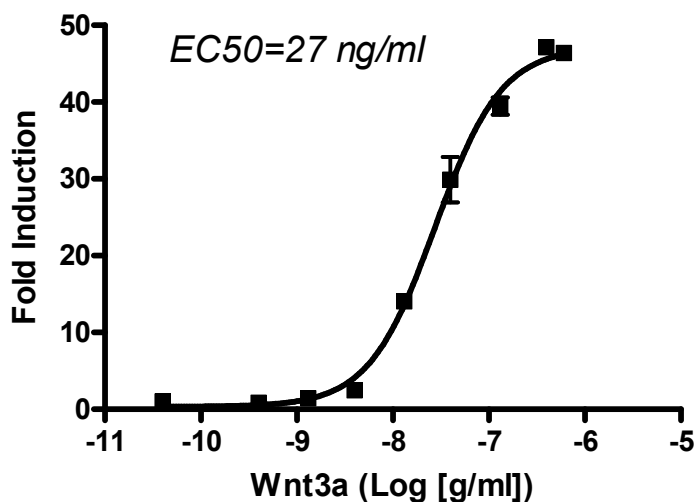
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## B. Inhibition of Wnt3a-induced reporter activity by an inhibitor of Wnt signaling pathway in TCF/LEF reporter (Luc)-HEK293 cells

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 white clear-bottom 96-well microplate in 40 $\mu$ l of assay medium.

2. Add 10 $\mu$ l of 50 mM LiCl solution in assay medium with or without IWR-1-endo (Wnt pathway inhibitor) to each well. Incubate cells at 37 $^{\circ}$  in a CO<sub>2</sub> incubator for ~ 16 hours.

3. Add 5  $\mu$ l of diluted mouse Wnt3a in assay medium to stimulated wells (final [Wnt3a] = 40 ng/ml).

Add 5  $\mu$ l of assay medium to the unstimulated control wells (cells treated with LiCl only for determining the basal activity).

Add 55  $\mu$ l 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 $^{\circ}$  in a CO<sub>2</sub> incubator for 5-6 hours.

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

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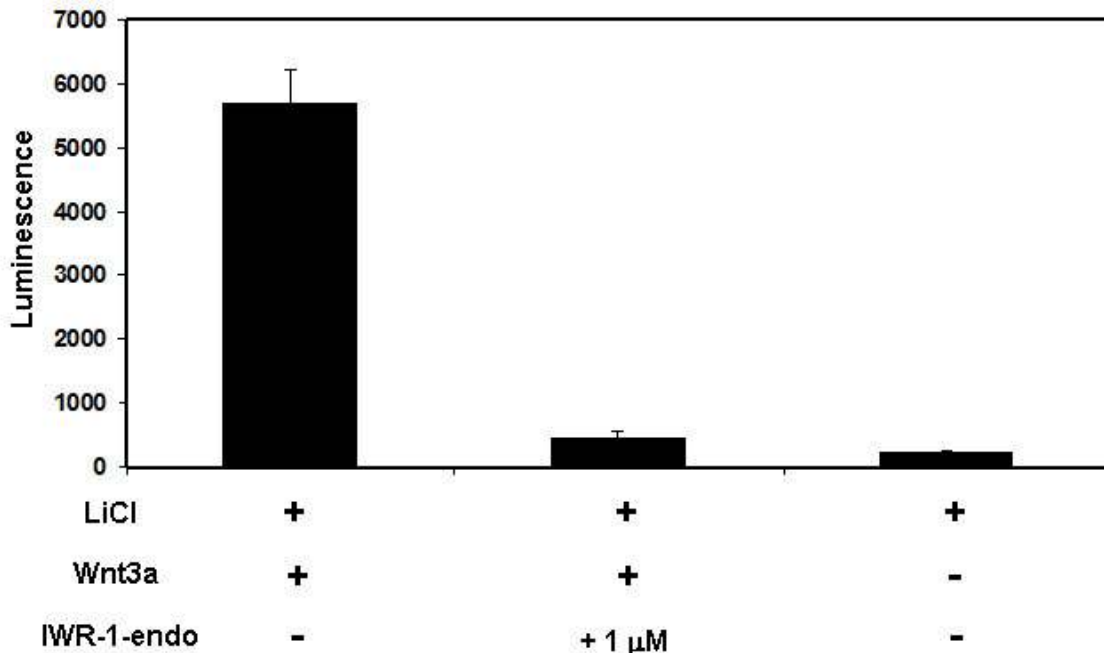
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If using other luciferase reagents from other vendors follow the manufacture's assay protocol.

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 were 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%.

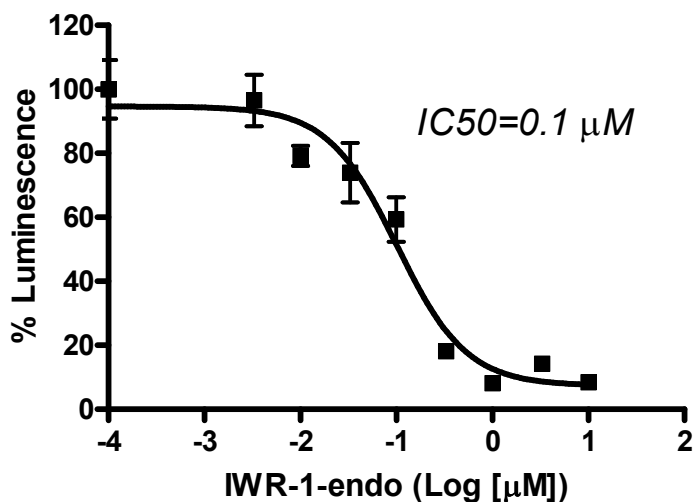
The IC<sub>50</sub> of IWR-1-endo is ~ 0.1  $\mu$ M.

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### **Mycoplasma testing**

The cell line has been screened using the PCR-based VenorGeM 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° with 7% CO<sub>2</sub> using MEM medium (Hyclone #SH30024.01) supplemented with 10% FBS (Invitrogen #26140-079), 1% non-essential amino acid (Hyclone #SH30238.01), 1mM 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<sub>2</sub> in the incubator depending on the NaHCO<sub>3</sub> 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 in 37° CO<sub>2</sub> incubator. At first passage switch to growth medium containing Geneticin. Cells should be split before they reach complete confluence.

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*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. Subcultivation ration: 1:10 to 1:20 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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