TCF/LEF Reporter – HEK293 Cell Line (Wnt Signaling Pathway)

#60501

Description

The TCF/LEF Reporter – HEK293 Cell Line (Wnt Signaling Pathway) 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 and human Wnt3a and to the treatment with an inhibitor of Wnt/ß-catenin signaling pathway.

Background

The TCF/LEF Reporter – HEK293 Cell Line (Wnt Signaling Pathway) is designed for monitoring the activity of the Wnt/ß-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 ß-catenin. ß-catenin then binds to TCF/LEF transcription factors in the nucleus, leading to transcription and expression of Wnt-responsive genes.

Application

- Monitor Wnt signaling pathway activity.
- Screen for compound activity of the Wnt / ß-catenin signaling pathway.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains ~2 x 10^6 cells in 1 ml of 10% DMSO

Host Cell

HEK293

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.

Materials Required but Not Supplied

These materials are not supplied with this cell line but are necessary for cell culture and cellular assays. BPS Bioscience reagents systems are validated and optimized for use with this cell line and are highly recommended for best results. Media components are provided in the Media Formulations section.

Materials Required for Cell Culture

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187
Growth Medium 1B	BPS Bioscience #79531

Materials Required for Cellular Assay

Name	Ordering Information
LiCl	Sigma # L7026
Mouse Wnt3a or human Wnt3a	R&D Systems 1324-WN or R&D Systems 5036-WN
IWR-1-endo: inhibitor of Wnt pathway	Santa Cruz Biotechnology # sc-295215
Assay Medium: Thaw Medium 1	BPS Bioscience #60187
Growth Medium 1B	BPS Bioscience #79531

96-well tissue culture treated white clear-bottom assay plate	Corning # 3610
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
Luminometer	

Storage Conditions

Cells will arrive upon dry ice and should immediately be thawed or stored in liquid nitrogen upon receipt. Do not use a -80°C freezer for long term storage. Contact technical support at support@bpsbioscience.com if the cells are not frozen in dry ice upon arrival.

Media Formulations

For best results, it is *highly recommended* to use these validated and optimized media from BPS Bioscience. To formulate a comparable but not BPS validated media, formulation components can be found below.

Note: Thaw Media does *not* contain selective antibiotics. However, Growth Media *does* contain selective antibiotics, which are used for maintaining cell lines over many passages. Cells should be grown at 37°C with 5% CO_2. BPS cell lines are stable for at least 15 passages when grown under proper conditions.

Media Required for Cell Culture

Thaw Medium 1 (BPS Bioscience #60187):
MEM medium (Thermo Fisher, #11095098) supplemented with 10% FBS (Thermo Fisher, #26140079), 1% non-essential amino acids (Corning, #25-025-CI), 1 mM Na pyruvate (Corning, #25-000-CI), 1% Penicillin/Streptomycin (Thermo Fisher, #15140163)

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

Assay Medium: Thaw Medium 1 (BPS Bioscience #60187)

Cell Culture Protocol

Cell Thawing

1. 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.
2. 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.
3. Cells should be split before they reach complete confluence.
4. At first passage switch to Growth Medium 1B (**contains Geneticin**).

Cell Passage

1. To passage the cells, rinse cells with phosphate buffered saline (PBS), detach cells from culture vessel with 0.05% Trypsin/EDTA.

2. 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.
3. Sub cultivation ratio: 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.

Cell Freezing

1. To cryopreserve the cells, remove the medium, rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with 0.05% Trypsin/EDTA.
2. After detachment, add Growth Medium 1B and count the cells, then transfer to a tube, spin down cells, and resuspend in 4°C Freezing Medium (BPS Bioscience #79796 or 10% DMSO + 90% FBS) at ~2 x 10^6 cells/ml.
3. Dispense 1 ml of cell aliquots into cryogenic vials. Place vials in an insulated container for slow cooling and store at -80°C overnight.
4. Transfer to liquid nitrogen the next day for storage.

Note: It is recommended to expand the cells and freeze down at least 10 vials of cells at an early passage for future use.

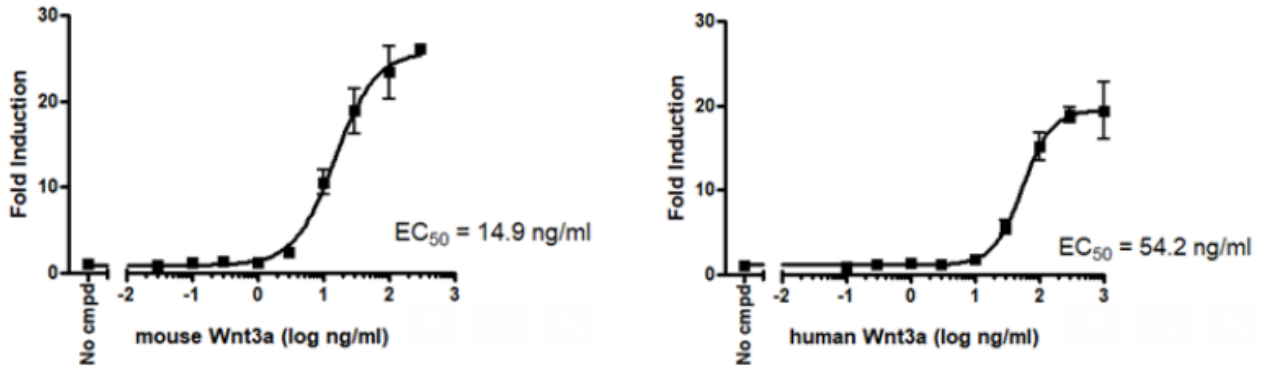
Validation Data

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

A. Dose response of Wnt Signaling Pathway TCF/LEF Reporter (Luc) – HEK293 cells to mouse or human 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 LiCl concentration = 10 mM). Incubate cells at 37°C in a CO2 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°C in a CO_2 incubator for 5-6 hours.
5. Perform luciferase assay using ONE-Step™ Luciferase Assay buffer: Add 110 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

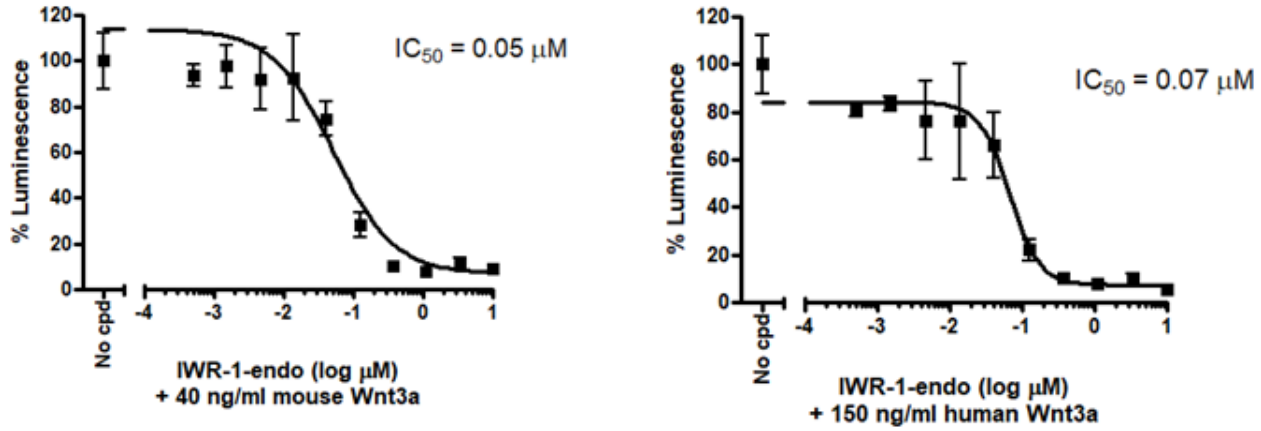


A. Mouse B. Human

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

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 ~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 (final LiCl concentration = 10 mM). 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), 150 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.
5. Perform luciferase assay using ONE-Step™ Luciferase Assay buffer: Add 110 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 the background-subtracted luminescence by subtracting the average background luminescence (cell-free control wells) from the luminescence reading of all wells. The percent luminescence of TCF/LEF luciferase reporter expression = background- subtracted luminescence of stimulated well / average background-subtracted luminescence of unstimulated control wells x 100%



A. Mouse Wnt3a B. Human Wnt3a

Figure 2. 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%.

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Troubleshooting Guide

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

References

1. Clevers, H. (2006) Wnt/beta-catenin signaling in development and disease. Cell **127(3):**469-480.
2. 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.