Signosis
Innovative Plate Assay Solutions

SMAD/TGFβ Luciferase Reporter Stable Cell Line

HepG2 – catalog number SL-0016 NIH/3T3 – catalog number SL-0030 HEK293 – catalog number SL-0091
(For Research Use Only)

Introduction

Smad proteins are transcription factors that respond to transforming growth factor-β (TGFβ) signaling, where TGFβ induces its membrane receptors to directly activate Smad proteins. These activated Smads complex with Smad4 (co-Smad), translocate from cytoplasm into nucleus and bind to target promoter region to regulate gene transcriptions. Dysfunction in TGFβ pathway leads to immunosuppression and angiogenesis, which can make cancer more invasive. Signosis has developed the SMAD/TGFβ luciferase reporter stable cell line in HepG2 cells, which stably expresses a luciferase reporter vector containing 4 repeats of SMAD binding sites upstream of a minimal promoter of the firefly luciferase coding region. The cell line can be used as a reporter system for monitoring the activation of SMADs triggered by stimuli treatment, enforced gene expression and gene knockdown.

Product description

Signosis has developed SMAD/TGFβ luciferase reporter stable cell line by co-transfecting SMAD luciferase reporter vector and hygromycin expression vector. The hygromycin resistant clones were subsequently screened for TGFβ1-induced luciferase activity. The cell line can be used as a reporter system for monitoring the activation of SMAD triggered by stimuli treatment, such as TGFβ1, and gene overexpression and gene knockdown.

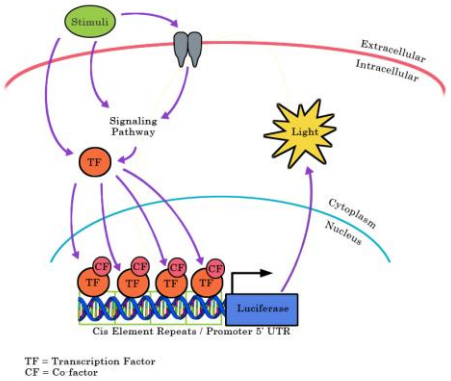
Materials provided

One vial of 2 x 10^6 cells, at passage 4, in Freezing Media.
IMPORTANT : store the frozen cells in liquid nitrogen until you are ready to thaw and propagate them.

Handling cells upon arrival

It is strongly recommended that you propagate the cells by following instructions as soon as possible upon arrival.**

IMPORTANT: It is imperative that an adequate number of frozen stocks be made from early passages as cells undergo genotypic changes. Possible genetic instability in transfected cells may result in a decreased responsiveness over time in normal cell culture conditions.



Required Cell Culture Media

- **Complete Growth Media**
  In 450mL of DMEM, add 50mL FBS (10% final) and 5mL Penicillin/Streptomycin (1% final).
  *In 450mL of EMEM, add 50mL FBS (10% final) and 5mL Penicillin/Streptomycin (1% final).
  *For HepG2 only.
- **2x Freezing Media**
  Add 10% DMSO (final) to Complete Growth Media and sterile filter. Make it fresh each time.

Materials required but not provided
(May be substituted with a comparable third-party product)

Materials	Product number
Dulbecco's Modified Eagles Medium (DMEM) Eagle's Minimum Essential Medium* (EMEM) For HepG2 only	Hyclone SH30243.01 ATCC P/N 30-2003
Fetal Bovine Serum (FBS)	Fisherbrand P/N 03-600-511
Penicillin/Streptomycin	Hyclone P/N SV30010
Trypsin	Hyclone P/N SH30236.02
Phosphate-buffered saline (PBS)	Cellgro P/N 21-040-CV
DMSO	Sigma P/N D8418
96-well white plate	Greiner Bio-One P/N 655098
Luciferase substrate	Signosis P/N LUC015
Cell lysis buffer	Signosis P/N LS-001
Hygromycin B (Optional)	Toku-E P/N H010

Initial Culture Procedure

1. Quickly thaw cells in a 37°C water bath with careful agitation. Remove from bath as soon as the vial is thawed.
2. Transfer cells to a 100mm^2 dish (or T-25cm^2 flask) containing 10ml of Complete Growth Media.
3. Gently rock the flask to ensure the cells are mixed well in the media. DO NOT PIPET.
4. Place the flask with cells in a humidified incubator at 37°C with 5% CO_2.
5. After cells adhere (wait at least 8 hours to overnight), replace media with fresh Complete Growth Media.

Subculture Procedure

1. After Cells have recovered and growing well subculture/passage cells when density reaches 90-100% confluency, maintain and subculture the cells in Complete Growth Media.
   Note: During the time that cells are not used for experiment, ideally, they can be maintained in Complete Growth Media with 50-100µg/ml of Hygromycin B.
2. Carefully remove the culture media from cells by aspiration.
3. Rinse cells with PBS, being careful not to dislodge attached cells. Then remove PBS by aspiration.
4. Add 1-2 mL trypsin/Tris-EDTA solution.
5. Incubate with trypsin for 2-5 minutes (or until detached). Confirm detachment by observation under the microscope.
6. Add 5-10ml of pre-warmed Complete Growth Media and gently pipet up and down to break the clumps.
7. Passage cells in 1:3 to 1:5 ratio when they reach 90% confluency.

NOTE: Stable cell lines may exhibit slower proliferation rate compared to parental cells. Do not seed cells at suboptimal density as this may hinder cell growth and division.

Preparing frozen stocks

This procedure is designed for 100mm^{2}dish or T-75cm^2 flask. Scale volumes according to other vessels.

1. When cells reach 90-100% confluency, then freeze down cells.
2. Detach cells according to "Subculture Procedure."
3. Transfer cells to a 15ml conical centrifuge tube and centrifuge at 250 x g (or 2,000 RPM) for 5 minutes to collect the cells into a pellet.
4. Carefully aspirate the media and resuspend cells in 0.5mL complete growth media.
5. Add 0.5mL of **2X Freezing Media** and gently resuspend by pipetting up and down.
6. Transfer 1mL of cells into a cryogenic vial.
7. Place cryogenic vial in a freezing container (*Nalgene # 5100-0001*) and store at -80°C freezer overnight.
8. Transfer cells to liquid nitrogen for long term storage.

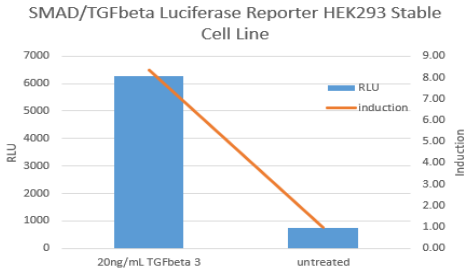
Assay procedure

The following procedure should be followed as a guideline. You will need to optimize the assay conditions based upon your experimental set up.

1. The day before performing the assay, trypsinize the cells and seed each well of a 96-well **white-wall** plate with 1-3 x 10^4 cells in 100µl.
2. Incubate the plate in a humidified incubator at 37°C with 5% CO_2 overnight.
3. Add inducing reagent directly to each well and incubate for an appropriate time to produce maximal induction.
4. Remove the media by aspiration and add 100µl of PBS to each well.
   Note: Because HEK293 cells are adherent to plate weakly, the PBS wash step can be skipped.
5. Remove PBS by aspiration and add 20µl of 1x lysis buffer to each well (To prepare 1x lysis buffer, add one volume of 5x lysis buffer to four volume of distilled water).
6. Incubate cells in lysis buffer for 15-30 minutes at room temperature with gentle agitation.
7. Add 100µl of luciferase substrate to each well and gently pipette up and down.
8. Immediately read the plate in a luminometer.

For Data, visit
http://www.signosisinc.com/data/Luciferase_Reporter_Stable_Cell_Lines

Analysis of SMAD/TGFbeta Luciferase Reporter HEK293 Stable Cell Line.



SMAD/TGFbeta Luciferase Reporter HEK293 Stable Cell Line response to TGFbeta3. The cells were seeded on a 96-well plate overnight in complete cell culture media. The cells were then treated with or without TGFbeta3 for about 18 hours. Data was read on a luminometer with a sensitivity of 3x10^{-21} moles luciferase.

HEK293 cells stably expressing SMAD luciferase reporter were treated with 20ng/mL TGF-beta1 in DMEM + 0.1% FBS for 16 hours. TGF-beta isoforms induced about 8-fold increase in SMAD-luciferase reporter activity.

Signosis Luciferase Reporter Stable Cell Lines

For a complete list of cell lines please visit our website at http://www.signosisinc.com/category/cell-based-assays

Transcription Factors	Pathway	Cell Line	Catalog Number
NFkB	NFkB	Hela; human cervical cancer	SL-0001
NFkB	NFkB	NIH/3T3; mouse fibroblast	SL-0006
NFkB	NFkB	HEK293; human embryonic kidney	SL-0012
NFkB	NFkB	MCF-7; human breast cancer	SL-0013
NFkB	NFkB	A549; human lung cancer	SL-0014
NFkB	NFkB	HepG2; human liver cancer	SL-0017
NFkB	NFkB	Neuro2a; mouse neuroblastoma	SL-0026
NFkB	NFkB	MEF; murine embryonic fibroblast	SL-0033
NFAT	Calcium Signaling	Jurkat T; human T lymphocyte	SL-0032
NFAT	Calcium Signaling	Hela; human cervical cancer	SL-0018
NFAT	Calcium Signaling	NIH/3T3; mouse fibroblast	SL-0029
p53	p53	Hela; human cervical cancer	SL-0011
p53	p53	RKO; human colon cancer	SL-0007
SMAD	TGFbeta	HepG2; human liver cancer	SL-0016
SMAD	TGFbeta	NIH/3T3; mouse fibroblast	SL-0030
NRF2	Antioxidant Response	MCF7; human breast cancer	SL-0010
STAT1	JAK-STAT	Hela; human cervical cancer	SL-0004
STAT3	JAK-STAT	Hela; human cervical cancer	SL-0003
HIF	Hypoxia Response	NIH/3T3; mouse fibroblast	SL-0005
HIF	Hypoxia Response	Hela; human cervical cancer	SL-0023
HIF	Hypoxia Response	Neuro2a; mouse neuroblastoma	SL-0027
ER	Estrogen Receptor Signaling	T47D; human breast cancer	SL-0002
AR	Androgen Receptor Signaling	MDA-MB-453; human breast cancer	SL-0008
GR	Glucocorticoid Receptor Signaling	MDA-MB-453; human breast cancer	SL-0009
GR	Glucocorticoid Receptor Signaling	Hela; human cervical cancer	SL-0021
AP-1	JNK, ERK, MAPK Signaling	Hela; human cervical cancer	SL-0019
CREB	cAMP, PKA, CaMK Signaling	HEK293; human embryonic kidney	SL-0020
CREB	cAMP, PKA, CaMK Signaling	NIH/3T3; mouse fibroblast	SL-0031
CHOP	Unfolded Protein Response, ER stress	Mia-Paca2; human pancreatic cancer	SL-0025
TCF/LEF	Wnt/b-catenin	HEK293; human embryonic kidney	SL-0015
TCF/LEF	Wnt/b-catenin	Hela; human cervical cancer	SL-0022
TCF/LEF	Wnt/b-catenin	CHO-K1;Chinese Hamster Ovary	SL-0028
ELK	MAPK Signaling	HEK293; human embyronic kidney	SL-0040
ELK	MAPK Signaling	Hela;human cervical cancer	SL-0041
IRF	Immune Response Pathway	HEK293; human embyronic kidney	SL-0035