Signosis
Innovative Plate Assay Solutions

SMAD/TGFβ Luciferase Reporter Stable Cell Line

(For Research Use Only)

HepG2 – catalog number SL-0016

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 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 will undergo genotypic changes. Genetic instability in transfected cells will results in a decreased responsiveness over time in normal cell culture conditions.

Required Cell Culture Media

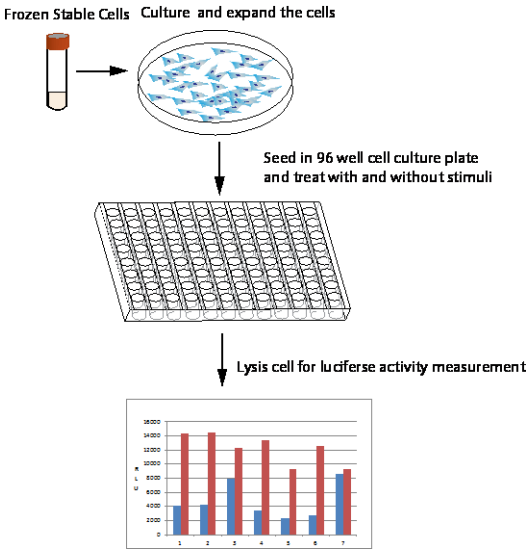
- **Complete Growth Media**
  In 450mL of EMEM, add 50mL FBS (10% final) and 5mL Penicillin/Streptomycin (1% final).

- **2x Freezing Media**
  Add 10% DMSO (final) to Complete Growth Media and sterile-filter. Make fresh each time.

Materials required but not provided

(May be substituted with a comparable third-party product)

- Eagle's Minimum Essential Medium (EMEM) -- *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 assay -- *Signosis P/N LUC100*
- Passive lysis buffer -- *Promega P/N E-1941*
- Hygromycin B – *Toku-E P/N H010* **(OPTIONAL)**



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 T25cm^2 flask (or 60mm^2 dish) containing 5-10ml 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 4 hours to overnight), **replace media** with fresh Complete Growth Medium.

Subculture Procedure

1. Subculture/passage cells when density reaches 90-100% confluency.
2. Carefully remove the culture media from cells by aspiration.
3. Rinse cells with PBS, being careful to not dislodge attached cells. Then remove PBS by aspiration.
4. Add 1-2 mL trypsin/Tris-EDTA solution. DO NOT add directly to cells.
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 line 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 T75cm^2 flask. Scale volumes accordingly to other vessels.

1. When cells reach 90-100% confluency, 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 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.
5. Remove PBS by aspiration and add 20µl of passive lysis buffer to each well.
6. Incubate cells in lysis buffer for 15 minutes at room temperature.
7. Add 100µl of luciferase substrate to each well and gently pipette up and down.
8. Immediately read the plate in a luminometer.