



## STAT3 Luciferase Reporter HEPG2 Stable Cell Line

Catalog Number SL-0081

(For Research Use Only)

### Introduction

STAT3 is activated in response to various cytokines and growth factors including  $IFN\gamma$ , IL-6, and OSM. The activation of STAT3 results in the formation of STAT3 homodimers and STAT3/STAT1 heterodimers that translocate to the cell nucleus and induce transcription of genes by binding to the consensus element on the promoter region of target genes associated with cell growth and apoptosis. Signosis has established Stat3 luciferase reporter HepG2 stable cell line, which can be used as a reporter system for monitoring the activation of Stat3 triggered by stimuli treatment, enforced gene expression, and gene knockdown.

### Product description

Signosis has established an IL-6 responsive luciferase reporter stable cell line that can be used as a reporter system for monitoring the activation of IL-6 responsive pathway, enforced gene expression, and gene knockdown. The cell line is established by transfection of a reporter vector, which contains 4 repeat IL-6 binding sites and a minimal promoter upstream of the firefly luciferase coding region, along with hygromycin expression vector followed by hygromycin selection. The hygromycin-resistant clones were subsequently screened for IL-6 induced luciferase activity.

### Materials provided

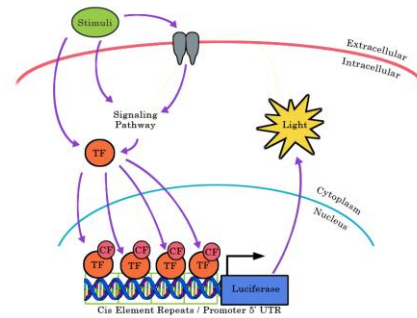
One vial of  $2 \times 10^6$  cells, at passage 4, in Freezing Media. **IMPORTANT:** store the frozen cells in liquid nitrogen until you are ready to thaw and propagate them.

### 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 may undergo genotypic changes. Possible genetic instability in transfected cells may result in a decreased responsiveness over time in normal cell culture conditions.



TF = Transcription Factor  
CF = Co factor

### Required Cell Culture Media

- **Complete Growth Media**  
In 450mL of DMEM, add 50mL FBS (10% final), 5mL Penicillin/Streptomycin (1% final), 100ug/ml hygromycin.
- **1x 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 comparable third-party products):

| Materials                                | Product number             |
|--|----------------------------|
| Dulbecco's Modified Eagles Medium (DMEM) | Hyclone SH30243.01         |
| 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 the bath as soon as the vial is thawed.
2. Transfer cells to a 100mm<sup>2</sup> dish (or T-25cm<sup>2</sup> 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<sub>2</sub>.
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 the density reaches 90-100% confluency, maintain and subculture the cells in Complete Growth Media.  
**Note: During the time that cells are not used for the 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 to not 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 a 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<sup>2</sup>dish or T-75cm<sup>2</sup> flask. Scale volumes accordingly to other vessels.*

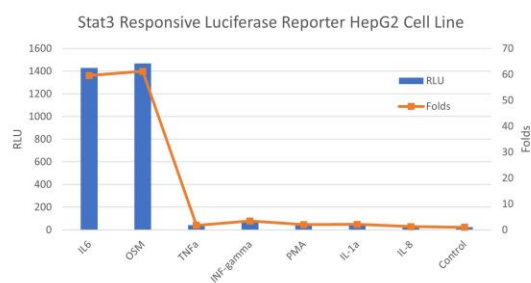
1. When cells reach 90-100% confluency, freeze them down.
2. Detach cells according to “Subculture Procedure.”
3. Transfer cells to a 15ml conical centrifuge tube and centrifuge at 250 x g (or 2,000 RPM) for 5 minutes to collect the cells into a pellet.
4. Carefully aspirate the media and resuspend cells 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 the cryogenic vial in a freezing container (*Nalgene # 5100-0001*) and store it 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 on your experimental setup.

1. The day before performing the assay, trypsinize the cells and seed each well of a **white clear-bottom** 96 well plate with 1-3 x 10<sup>4</sup> cells in 100µl medium.
2. Incubate the plate in a humidified incubator at 37°C with 5% CO<sub>2</sub> 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 1x lysis buffer to each well (To prepare 1x lysis buffer, add one volume of 5x lysis buffer to four volumes 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.  
**Note:** We recommend a luminometer with a sensitivity of at least 3x10<sup>-21</sup> moles luciferase.



Analysis of SL-0081 Stat3 activity in response to different treatments. The cells were seeded on a 96-well plate in 10% FBS DMEM medium. The cells then immediately were treated with or without 10ng/ml IL-6, OSM, TNFα, IFN $\alpha$ , PMA, IL-1 $\alpha$ , IL-8, in DMEM and 10% FBS for 16 hours.

## 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 Factor | Pathway                              | Cell Line                          | Cat #   |
|----------------------|--------------------------------------|------------------------------------|---------|
| 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                                 | MEF; murine embryonic fibroblast   | SL-0033 |
| NFAT                 | Calcium Signaling                    | Jurkat; human T lymphocytes        | SL-0032 |
| NFAT                 | Calcium Signaling                    | Hela; human cervical cancer        | SL-0018 |
| 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, PICA, CaMK Signaling           | HEK293; human embryonic kidney     | SL-0020 |
| CREB                 | cAMP, PICA, 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 embryonic kidney     | SL-0040 |
| ELK                  | MAPK Signaling                       | Hela; human cervical cancer        | SL-0041 |
| IRF                  | Immune Response Pathway              | HEK293; human embryonic kidney     | SL-0035 |

\*\* Signosis products are warranted for 30 days from the date of shipment, and this warranty is valid only if the product is stored and handled according to this user manual or product information sheet and shipped directly by Signosis.