



ER Stress Luciferase Reporter Stable Cell Line (For Research Use Only)

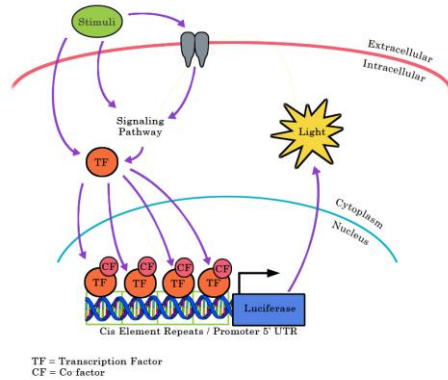
MEF - Catalog Number: SL-0067

HEK293 - Catalog number SL-0068

HepG2 – Catalog Number: SL-0069

Introduction

The endoplasmic reticulum (ER) is responsible for the proper folding and processing of secreted and transmembrane proteins. Any factors that disrupt this function may cause an accumulation of misfolded and unfolded proteins in the ER, a condition known as ER stress. Upon ER stress a signaling network called the Unfolded Protein Response (UPR) will be initiated to eliminate this stress and restore ER homeostasis. In case of failure, however, the UPR promotes apoptosis. ER stress play role in many chronic diseases like diabetes, neurodegeneration, and cancer. Researchers have to measure mRNA or protein expression to study ER stress or UPR.



Product description

Signosis has developed a luciferase reporter stable cell line with ERSE (ER stress response element) that can be easily used to monitor ER stress in the cell. Under conditions of ER stress, ER stress-responsive proteins bind to the ERSE, resulting in the transcriptional induction of the luciferase gene. The vector containing the ER stress response element has been transfected into cells along with Hygromycin vector. The hygromycin-resistant clones were subsequently screened for thapsigargin-induced luciferase activity.

Materials provided

One vial of 2×10^6 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.

Required Cell Culture Media

- **Complete Growth Media**
In 450mL of DMEM, 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 (Can be substituted with comparable third-party products):

| Materials | Product number |
|--|----------------------------|
| Dulbecco's Modified Eagles Medium (DMEM) | Cytiva SH30243.FS |
| Fetal Bovine Serum (FBS) | Cytiva SH30910.03 |
| Penicillin/Streptomycin | Cytiva SV30010 |
| Trypsin | Cytiva SH30042.02 |
| 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 100mm² dish (or T-25cm² 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% CO₂.
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 100mm² dish or T-75cm² flask. Scale volumes accordingly to other vessels.

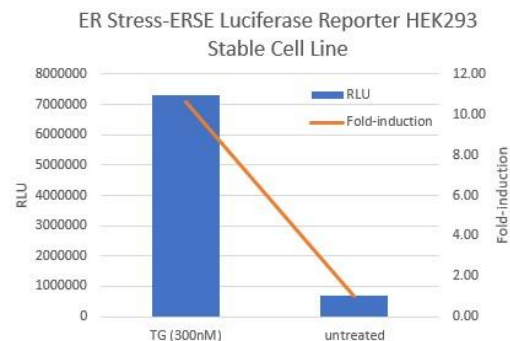
1. When cells reach 90-100% confluency, freeze them down.
2. Detach cells according to “Subculture Procedure.”
3. Transfer cells to a 15ml conical centrifuge tube and centrifuge at 250 x g (or 2,000 RPM) for 5 minutes to collect the cells into a pellet.
4. Carefully aspirate the media and resuspend cells 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 10⁴ cells in 100µl medium.
2. Incubate the plate in a humidified incubator at 37°C with 5% CO₂ 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 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 with a sensitivity of 3×10⁻²¹ moles luciferase.



ER Stress-ERSE Luciferase Reporter HEK293 Stable Cell Line response to Thapsigargin. The cells were seeded on a 96-well plate overnight in complete cell culture media. The cells were then treated with or without 300nM TG (Thapsigargin) for about 18 hours. Data was read on a luminometer with a sensitivity of 3x10⁻²¹ moles luciferase.

For more data please visit our website;

<https://www.signosisinc.com/subcategory/luciferase-reporter-stable-cell-lines>

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 |

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