



ATF6 Luciferase Reporter CHO-K1 Stable Cell Line

Catalog Number: SL-0024 (For Research Use Only)

Introduction

ATF6 is a major sensor of the endoplasmic reticulum stress (ER stress), as the accumulation of unfolded protein in the endoplasmic reticulum will lead to the proteolysis and activation of ATF6 protein. Active ATF6 translocates to the nucleus and acts as a transcription factor by binding to ER stress elements of target genes, such as ER chaperones.

Product description

Signosis has developed ATF6 luciferase reporter stable cell line by transducing cells with baculovirus containing both ATF6 luciferase reporter and hygromycin expression cassette. The hygromycin-resistant clones were subsequently screened for tunicamycin-induced luciferase activity. The cell line can be used as a reporter system for monitoring the activity of ATF6 triggered by stimuli treatment, such as tunicamycin, thapsigargin, gene overexpression, and gene knockdown. The cells contain no viral particles and require handling at biosafety level 1 protocol.

Materials provided

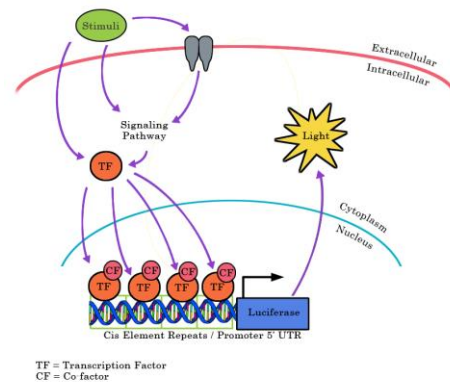
One vial of 2×10^6 cells, at passage 4, in Freezing Media. **IMPORTANT:** store the frozen cells in liquid nitrogen until you are ready to thaw and propagate them.

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. 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-12 1:1 Modified, 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 comparable third-party products):

| Materials | Product number |
|---------------------------------|----------------------------|
| DMEM-12 1:1 Modified | Cytiva SH30261.01 |
| 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 100mm²dish or T-75cm² flask. Scale volumes accordingly to other vessels.

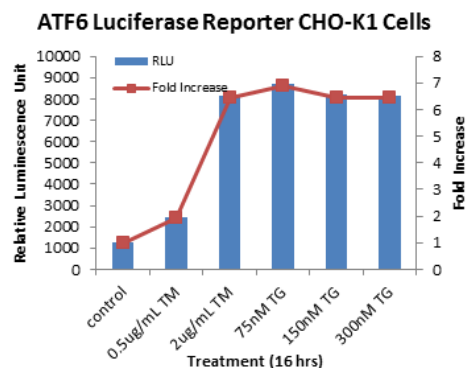
1. When cells reach 90-100% confluency, freeze them down.
2. Detach cells according to “Subculture Procedure.”
3. Transfer cells to a 15ml conical centrifuge tube and centrifuge at 250 x g (or 2,000 RPM) for 5 minutes to collect the cells into a pellet.
4. Carefully aspirate the media and resuspend cells 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.
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.



Analysis of SL-0024 ATF6 reporter activity in response to ER stress.

The CHO-K1 cells were seeded on a 96-well plate overnight with DMEM/F12 including 10% FBS. The cells then were treated with indicated concentrations of tunicamycin (TM) or thapsigargin (TG) in DMEM/F12 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 |

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