



Estrogen Receptor Luciferase Reporter T47D Stable Cell Line

Catalog Number SL-0002 (For Research Use Only)

Introduction

Estrogen receptor (ER) belongs to the nuclear receptor family and plays a widespread role in human physiology and the development or progression of numerous diseases. In response to estrogen stimulation, estrogen bound receptor in the nucleus dimerizes and binds to specific response elements known as estrogen response elements (EREs) located in the promoters of target genes and regulates their gene expression. Signosis has established the T47D ER luciferase reporter stable cell line, in which the ERE and reporter luciferase gene are consistently expressed in the cell line to facilitate the screening and study. This stable cell line can provide a sensitive, responsive, and rapid in vitro system to detect and measure substances with potential (anti-)estrogenic activity.

Product description

The cell line was established by transfection of ER luciferase reporter vector along with neomycin expression vector followed by neomycin selection. The G418 resistant clones were subsequently screened for 17 beta-estradiol induced luciferase activity. The clone with the highest fold induction (10 fold) was selected and expanded to produce this stable cell line.

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

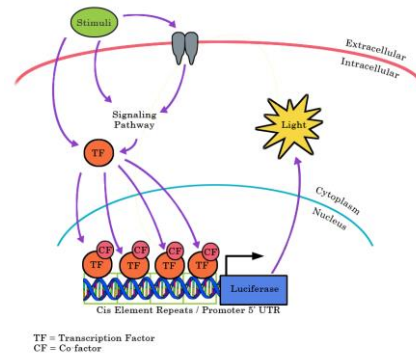


It is strongly recommended that you propagate the cells by following instructions as soon as possible upon arrival.**

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 500mL of RPMI-1640, add 50mL FBS (10% final), 5mL Penicillin/Streptomycin (1% final) and 10 ug/ml Insulin.

- **2x Freezing Media**
Add 10% DMSO (final) to Complete Growth Media and sterile filter. Make fresh each time.
- **Assay Preparation Media**
Use RPMI-1640 w/ no phenol red, no antibiotics, and with 10% dextran-charcoal stripped FBS.
- **Assay Media**
Use RPMI-1640 w/ no phenol red, no antibiotics, and with 0.5% dextran-charcoal stripped FBS.

Materials required but not provided (May be substituted)

| Materials | Product number |
|---------------------------------|--------------------------|
| RPMI-1640 Medium | Cytiva SH30027.01 |
| RPMI-1640, no phenol red | Fisher 11835030 |
| Fetal Bovine Serum (FBS) | Cytiva SH30910.03 |
| FBS, charcoal/Dextran treated | Cytiva SH30068 |
| Penicillin/Streptomycin | Cytiva SV30010 |
| Trypsin | Cytiva SH30042.02 |
| Phosphate-buffered saline (PBS) | Cellgro 21-040-CV |
| DMSO | Sigma D8418 |
| 96-well white plate | Greiner Bio-One 655098 |
| Luciferase substrate | Signosis P/N LUC015 |
| Cell lysis buffer | Signosis P/N LS-001 |
| G418 (optional) | Invitrogen P/N 10131-027 |

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 G418.

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.

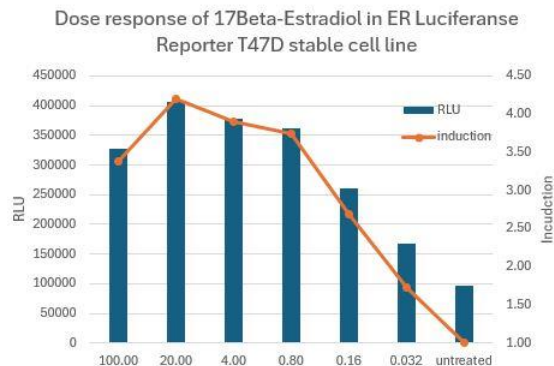
1. When cells reach 90-100% confluency, freeze down cells.
2. Detach cells according to “Subculture Procedure.”
3. Transfer cells to a 15ml conical centrifuge tube and centrifuge at 250 x g (or 2,000 RPM) for 5 minutes to collect the cells into a pellet.
4. Carefully aspirate the media and resuspend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

Note: Use Assay medium (no antibiotics, no phenol red, and replace FBS with charcoal stripped FBS), as standard growth medium has estrogens in it that will affect the assay. The assay should include “treated”, “cell-free control” and “untreated” conditions.

1. The day before performing the assay, aspirate the medium, wash the cells with PBS without Ca²⁺/Mg²⁺, and detach the cells with 0.5% Trypsin/EDTA, no phenol red.
2. Once the cells have detached, add **Assay Preparation media** and seed the cells in 100ul **Assay Preparation media** into a **clear-bottom, white 96-well plate**. Leave a few wells empty as “cell-free control” wells (as background luminescence control).
3. Incubate the plate at 37°C with 5% CO₂ incubator for 24 hours.
4. Prepare the testing compound at the concentration to be tested in **Assay Media** (100ul/well).
5. Carefully remove the **Assay Preparation media** from each well.
6. Add 100ul of the testing compound to the treated wells.
7. Add 100ul of **Assay Media** to the untreated wells and cell-free control wells.
8. Incubate the plate at 37°C with 5% CO₂ incubator for 24 hours.
9. After incubation with treatment, remove the media by aspiration and add 25µl 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).
10. Incubate cells in lysis buffer for 15 minutes at room temperature.
11. Add 50µl of luciferase substrate to each well and gently pipette up and down.
12. Immediately read the plate in a luminometer with a sensitivity of 3×10⁻²¹ moles luciferase.



T47D/ER-luc cells were treated with various concentrations in response to 17β-Estradiol.

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 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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