Signosis
Innovative Plate Assay Solutions

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.

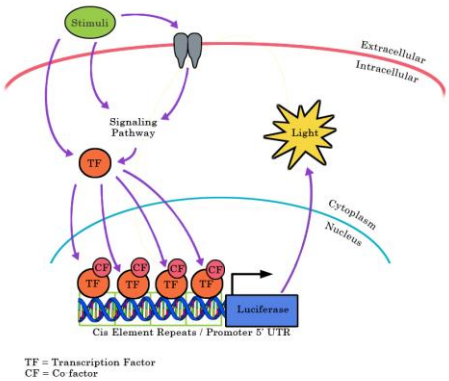
Materials provided

One vial of 2 x 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 results 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 (Signosis CC-0001)**
  Use RPMI-1640 w/ no phenol red, no antibiotics, and with 10% dextran-charcoal stripped FBS.
- **Assay Media (Signosis CC-0002)**
- 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	Hyclone P/N SH30027.01
RPMI-1640, no phenol red	Fisher P/N 11835030
Fetal Bovine Serum (FBS)	Fisherbrand P/N 03-600-511
FBS, charcoal/Dextran treated	HyClone P/N SH30068
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
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 100mm^2 dish (or T-25cm^2 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_2.
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

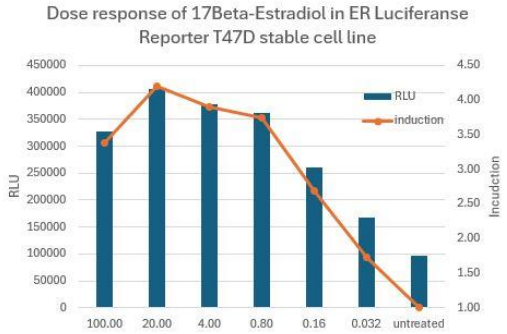
This procedure is designed for 100mm^{2}dish or T-75cm^2 flask. Scale volumes accordingly to other vessels.

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 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% CO_2 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% CO_2 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.



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 embyronic kidney	SL-0040
ELK	MAPK Signaling	Hela;human cervical cancer	SL-0041
IRF	Immune Response Pathway	HEK293; human embyronic kidney	SL-0035

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