Signosis
Innovative Plate Assay Solutions

NRF2/ARE Luciferase Reporter MCF7 Cell Line

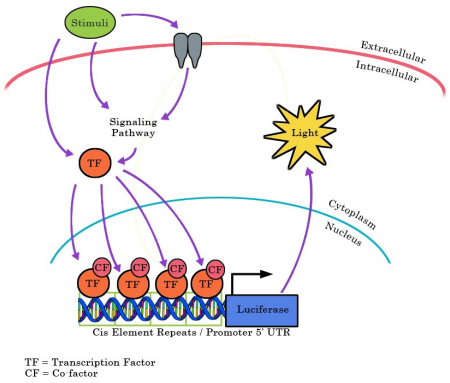
Catalog Number SL-0010 (For Research Use Only)

Introduction

NRF2 plays a crucial role in cellular antioxidant defense, making it a therapeutic target for neurodegenerative diseases and cancer. Under normal conditions, NRF2 localizes in the cytosol and is rapidly degraded by the proteasome. Under oxidative stress, NRF2 is stabilized and translocates to the nucleus where it binds to a DNA promoter and initiates gene expression. In the nucleus, NRF2 forms a heterodimer with a small Maf protein and binds to the Antioxidant Response Element in the upstream promoter region of many antioxidative genes and initiates their transcription. Signosis has established an NRF2 luciferase reporter stable cell line that has been stably transfected with pTA-NRF2-luciferase reporter vector, which contains 8 repeats of NRF2 binding sites, a minimal promoter upstream of the firefly luciferase coding region. Luciferase activity is specifically associated with the activity of NRF2. Therefore, the cell line can be used as a reporter system for monitoring the activation of NRF2 triggered by stimuli treatment, enforced gene expression, and/or gene knockdown.

Product description

The cell line was established by transfection of an NRF2 firefly luciferase reporter vector along with G418 expression vector followed by G418 selection. The G418 resistant clones were subsequently screened for tBHQ-induced luciferase activity. The clone with the highest fold induction 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 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 (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
G418	Life Technologies

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

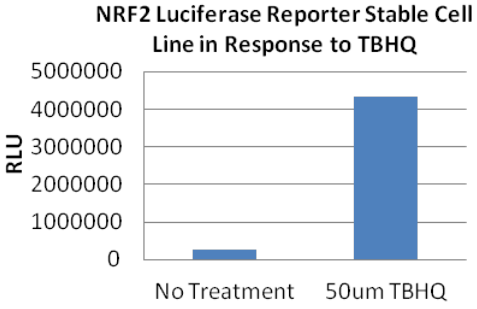
This procedure is designed for 100mm^{2}dish or T-75cm^2 flask. Scale volumes accordingly to other vessels.

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 1.0mL of **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 10^4 cells in 100µl medium.
2. Incubate the plate in a humidified incubator at 37°C with 5% CO_2 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-volume 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.
9.



The cells were seeded on a 96-well plate for overnight with DMEM including 10% FBS. The cells then were treated with or without 50nM TBHQ respectively in DMEM and 0.1% 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 river 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 river 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-Kl; 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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