Signosis
Innovative Plate Assay Solutions

NFAT Luciferase Reporter Jurkat Stable Cell Line

Catalog number SL-0032 (For Research Use Only)

Introduction

NFATs are a family of transcriptional factors that play an important role in immune response as well as in the development of cardiac, skeletal muscle, and nervous systems. NFATs are regulated by calcium signaling. Through calcium activation of the phosphatase calcineurin, NFATc proteins translocate from the cytoplasm into the nucleus, where they cooperate with other proteins to mediate gene expression. The nuclear import of NFAT is blocked by kinases such as PKA and GSK3. NFATs are also implicated in breast cancer. Signosis has established a NFAT luciferase reporter cell line that has been stably transfected with a NFAT-luciferase reporter construct. Via the analysis of luciferase, the cell line can be employed to monitor the cellular changes of NFAT activities that are triggered by stimulation, compound treatment, enforced gene expression, or gene knockdown.

Product description

Signosis has developed NFAT luciferase reporter Jurkat stable cell line by co-transfecting NFAT luciferase reporter vector and hygromycin expression vector. The hygromycin-resistant clones were subsequently screened for PMA+ionomycin-induced luciferase activity. The cell line can be used as a reporter system for monitoring the activation of NFAT triggered by stimuli treatments, gene overexpression, and gene knockdown.

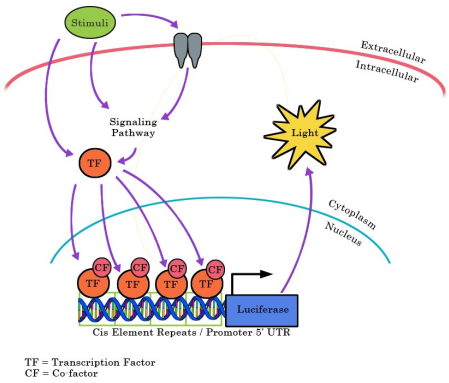
Materials provided

One vial of 5 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 RPMI-1640, add 50mL FBS (10% final) and 5mL Penicillin/Streptomycin (1% final).
- **Freezing Media**
  Add 10% DMSO (final) to Complete Growth Media and sterile filter. Make fresh each time.

Materials required but not provided

(Maybe substituted with a comparable third-party product)

Materials	Product number
RPMI-1640 Medium	Hyclone P/N SH30027.01
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
Hygromycin B	Toku-E P/N H010

Initial Culture Procedure

1. Quickly thaw cells in a 37 ºC water bath with careful agitation. Remove from the bath as soon as the vial is thawed.
2. Transfer cells to a 15ml centrifuge tube containing 7ml of pre-warmed Complete Growth Media.
3. Centrifuge tube at 1200-1500 RPM for 5 minutes
4. Remove supernatant and resuspend cells with 1ml Complete Growth Media
5. Transfer cells to a 100mm^2 dish (or T-25cm^2 flask) containing 10ml of Complete Growth Media.
6. Place the flask with cells in a humidified incubator at 37 ºC with 5% CO_2.

Cell maintenance

1. After cells recovered and are growing well (after at least one passage), maintain and subculture the cells in Complete Growth Media. **For better maintaining cells, add 100µg/ml Hygromycin B into the complete medium.**
2. Pass the cells every 3 days by inoculating 5x10^5/ml. Do not allow the cell concentration to exceed 3x10^6/ ml.

Preparing frozen stocks

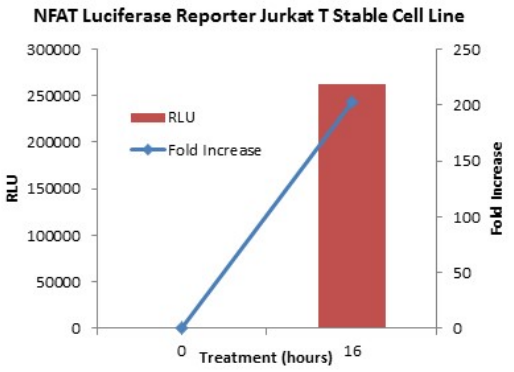
This procedure is designed for 60mm^{2}dish or T-25cm^2 flask. Scale volumes accordingly to other vessels.

1. When cells reach 2-3x10^{6}cells /ml, freeze down cells.
2. Transfer cells to a 15ml conical centrifuge tube and centrifuge at 1200-1500 RPM for 5 minutes to collect the cells into a pellet.
3. Carefully aspirate the media and resuspend cells at a density of 5-7x10^6/ml in freezing media and gently resuspend by pipetting up and down.
4. Aliquot 1ml of cells into cryogenic vials.
5. Place the cryogenic vial in a freezing container (*Nalgene # 5100-0001*) and store at -80ºC freezer overnight.
6. 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. Centrifuge cells at 1000-1500 RPM for 5 minutes
2. Remove supernatant and resuspend NFAT Jurkat cells at 1-1.2x10^6 cells/ml in pre-warmed DMEM medium+0.1%FBS.
3. Add 90µl of cell suspension (~100,000 cells) per well of a 96 well white plate.
4. Add 10µl of 10x stock of inducers per well and 10µl PBS or endotoxin-free water as a negative control.
5. Incubate the plate at 37ºC in a CO_2 incubator for the appropriate time to produce maximal induction
6. Slowly discard **80µl** of the media by using a pipette. ***Note: Do not disturb the cells on the bottom of each well***.
   Optional Step: Centrifuge the plate at 1200 RPM for 1 minute to settle the cells onto the bottom of each well, and then discard 80uL of the media.
7. Add 20µl of 2x lysis buffer to each well (To prepare 2x lysis buffer, add two volumes of 5x lysis buffer to three-volume of distilled water).
8. Incubate cells in lysis buffer for 15 minutes at room temperature with gentle agitation.
9. Transfer cell lysate solution from each well to a 1.5 ml centrifuge tubes.
10. Centrifuge the tubes at full speed for 1 minute.
11. Carefully pipet 20µl of the supernatant back to the 96 well white plate.
    Note: It is very important to centrifuge the cell lysate and test the supernatant only.
12. Add 50µl of luciferase substrate to each well and gently pipette up and down.
13. Immediately read the plate in a luminometer.



The cells were seeded on a 96-well plate in media containing 10ng/ml PMA, 1uM ionomycin, and 0.1% FBS for 16 hours. Fold induction in luciferase activity was detected when compared to untreated cells.

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

** Signosis products are warranted for 30 days from the date of shipment, and this warranty is valid only if the product is stored and handled according to this user manual or product information sheet and shipped directly by Signosis.