Datasheet for Jurkat/NFAT-Luc cell line Cell Line

Catalog number: SL405

Product: Jurkat cell line stably expressing NFAT transcriptional response (TRE) gene .

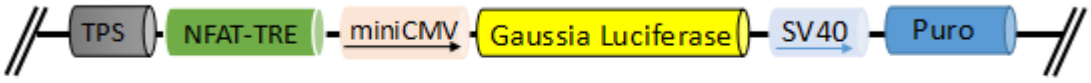
Description: The NFAT transcriptional response (TRE) cell line assays the status of the PKC/Ca++ signaling pathway through the activity of the transcription factor NFAT. The NFAT transcriptional TRE cell line contains a minimal promoter and tandem repeats of the NFAT transcriptional response element upstream of a secreted Gaussia luciferase reporter gene.

Quantity: 1 vial of 2 x 10^6 cells; frozen

Shipping conditions: Dry ice

Storage conditions: Liquid nitrogen vapor phase. Remove the item from the dry ice packaging and check all items for damage and leakage. Place immediately into storage at or below -140 ^{0}C, preferably into the liquid nitrogen vapor phase, until use.

Transgene integration:



TPS-NFAT-TRE-miniCMV-GLuc-SV40-Puro

Source of parental line:

Jurkat
Organism: *Homo sapiens*,
human Tissue: PeripheralBlood
Cell type: Lymphocyte

Quality control: >95% viability before freezing. All cells were tested and found to be free of mycoplasma, bacteria, viruses, and other toxins.

Safety instructions: To ensure safety, protective gloves, clothing, and a face mask should be worn when handling frozen vials. Some leakage may occur into the vial during storage. The liquid nitrogen will be converted to gas upon thawing. Due to the nature of nitrogen gas, pressure may build within the vial and possibly result in

the vial exploding or losing its cap. This may cause flying debris.

Thawing procedure: The vial of cells should be thawed in a 37 ^{0}C water bath with gentle agitation. For optimal performance, the vial should be thawed in under two minutes. Ensure that the cap of the vial did not loosen upon thawing, and re-tighten as needed. Spray the vial with 70% EtOH and wipe off. Repeat. Using aseptic technique, add the contents of the vial to 9 ml of complete growth medium (without selection). Centrifuge for 5 min. at 125 x g. Aspirate the medium, being careful not to disturb the pellet. Resuspend in 10 mL of complete growth medium, and place into a culture vessel of your choice. Only add selection to the medium after 24 hours in culture.

Culture conditions:

Complete Growth Medium

The base medium for this cell line is RPMI1640. For optimal growth and maintenance of selection, add the following components to the base medium: dialyzed fetal bovine serum to a final concentration of 10%.

Selection:
Puromycin to a final concentration of 1 µg/mL

Culture temperature:
37 ^{0}C with 5% CO_2

Subculture:
Cultures can be maintained by the addition or replacement of fresh medium. Start new cultures at 1 x 10^5 viable cells/mL. Subculture at 1 x 10^6 cells/mL.

Cryopreservation: Freeze slowly in complete growth medium supplemented with 5% (v/v) DMSO.

Mycoplasma: Negative
(MycoAllert Mycoplasma Detection Kit from Lonza)

Product QC:

1. Transduction

(1) Plate cells

Count the Jurkat cells before one day. Adjust the cell concentration and seed Jurkat cells in a 6-well plate ,The number of cells plated in each well should be determined so that are 50% confluent at the time of transduction.

(2) Add NFAT lentivirus

MOI=100, add NFAT lentivirus in Jurkat cells.

2. Pick single clone

Plate the Jurkat cells into dilution 96 well plate post-transduction 72 hours, add the puromycin in dish with final concentration 1 μg/mL. Culture the cells for 14 to 20 days. To avoid sibling colonies, dish should be disturbed as little as possible.

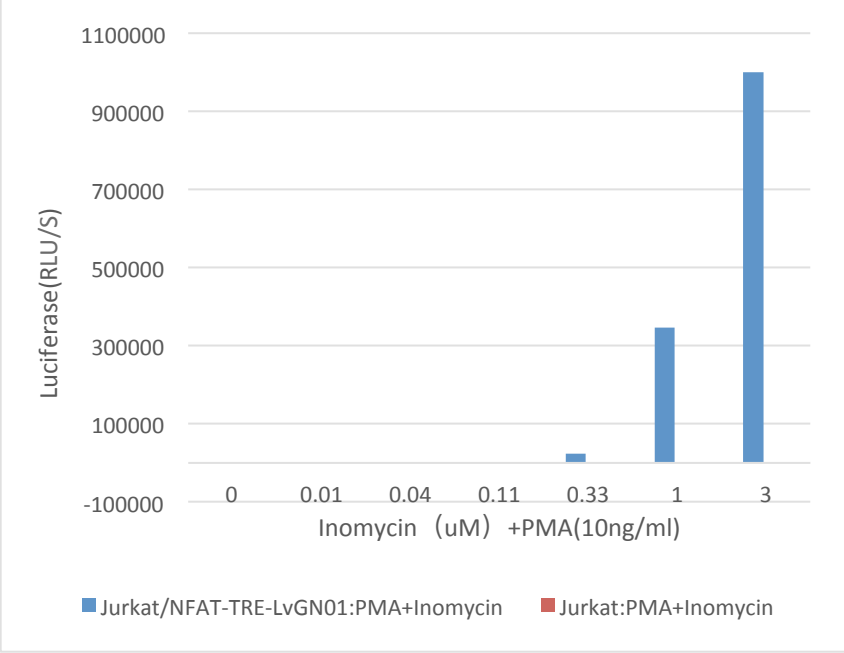
Select all the dishes with less than 20 colonies/dish, mark all the well seperated the colonies to pick. Pick the colonies by cloning cylinder. Transfer cells into 48 well plate.

3. Clone screening

Split cells from each clone into 96 well plate for stimulation assay and 24 well plate for cell culture. The cells from each clone into 96 well plate were incubated for 24 hours, followed by 18 hours of treatment with PMA and ionomycin (PMA:10ng/ml, ionomycin:1uM). A Gaussia Luciferase assay was performed, and transcriptional response activity values are expressed as luminance fold activation. Pick the highest transcriptional response activity values clone for Master Cell Bank.

4. Dose curve

Split cells from Master Cell Bank clone into 96 well plate, Jurkat cell line as negative control, cells were incubated for 24 hours, followed by 18 hours of treatment with PMA and ionomycin. PMA:10ng/ml, ionomycin(uM): 0, 0.01, 0.04, 0.11, 0.33, 1, 3. A Gaussia Luciferase Assay was performed.



Citation of product: If use of this item results in a publication, please use this information: Jurkat/NFAT-Luc Cell Line (SL405, GeneCopoeia, Inc., Rockville, MD).

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