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Product Sheet

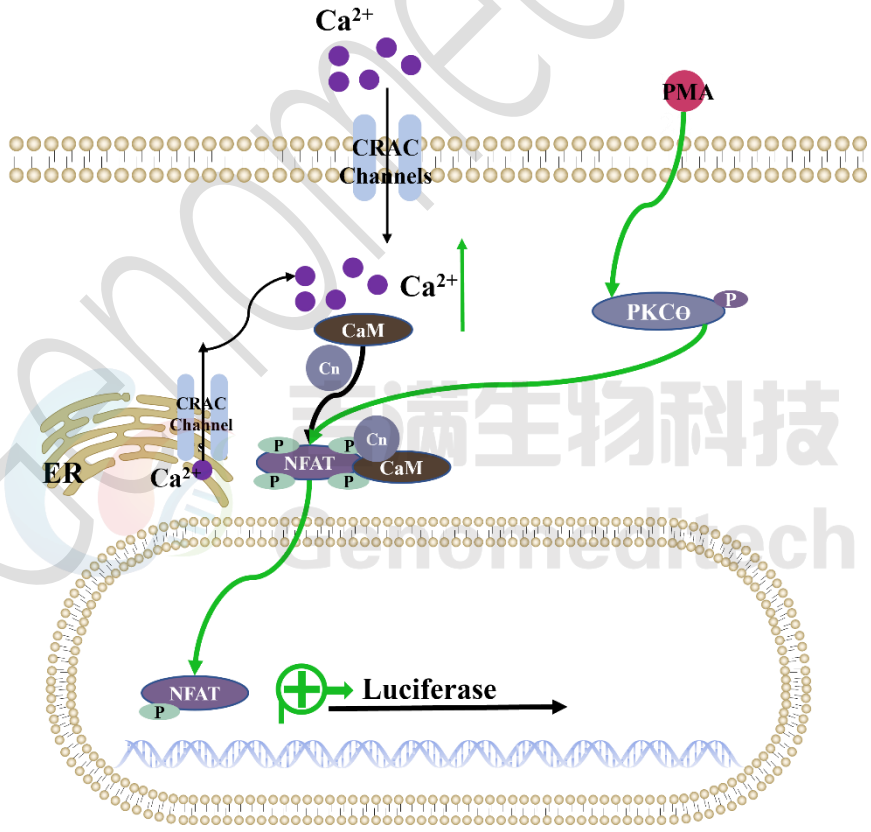
NFAT Reporter 293 Cell Line

Catalog number: GM-C39044

Version 3.3.1.250416

Nuclear factor of activated T cells (NFAT) is a family of transcription factors that play a vital role in immune regulation, being widely involved in T cell activation, differentiation, and the regulation of various cytokine genes. The protein kinase C (PKC)/Ca^{2+} response pathway is one of the key signaling mechanisms mediating NFAT activation. The activity of NFAT is jointly regulated by intracellular Ca^{2+} concentration and the Ca^{2+}/calmodulin-dependent serine phosphatase, calcineurin. In resting cells, NFAT proteins remain phosphorylated and localized in the cytoplasm; upon stimulation, calcineurin dephosphorylates NFAT, promoting its translocation into the nucleus, where it induces the expression of downstream target genes. This process is of great significance in the adaptive immune response.

NFAT Reporter 293 Cell Line is a clonal stable 293 cell line constructed using lentiviral technology that expresses a NFAT-inducible luciferase reporter gene. When the upstream signaling pathways are activated, the NFAT activates the expression of luciferase. The luciferase readout represents the activation level of the signaling pathway and can thus be used for evaluating the in vitro effects of related drugs.



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Specifications

Quantity	5E6 Cells per vial,1 mL
Product Format	1 vial of frozen cells
Shipping	Shipped on dry ice
Storage Conditions	Liquid nitrogen immediately upon receipt
Recovery Medium	DMEM+10% FBS+1% P.S
Growth medium	DMEM+10% FBS+1% P.S+4 μg/mL Blasticidin
Note	None
Freezing Medium	90% FBS+10% DMSO
Growth properties	Adherent
Growth Conditions	37°C, 5% CO_2
Mycoplasma Testing	The cell line has been screened to confirm the absence of Mycoplasma species.
Safety considerations	Biosafety Level 2
Note	It is recommended to expand the cell culture and store a minimum of 10 vials at an early passage for potential future use.

Materials

Reagent	Manufacturer/Catalogue No.
DMEM	Gibco/C11995500BT
Fetal Bovine Serum	Cegrogen biotech/A0500-3010
Pen/Strep	Thermo/15140-122
Blasticidin	Genomeditech/GM-040404
Ionomycin	MCE/HY-13434
PMA/TPA	Beyotime/S1819
GMOne-Step Luciferase Reporter Gene Assay Kit	Genomeditech/GM-040503

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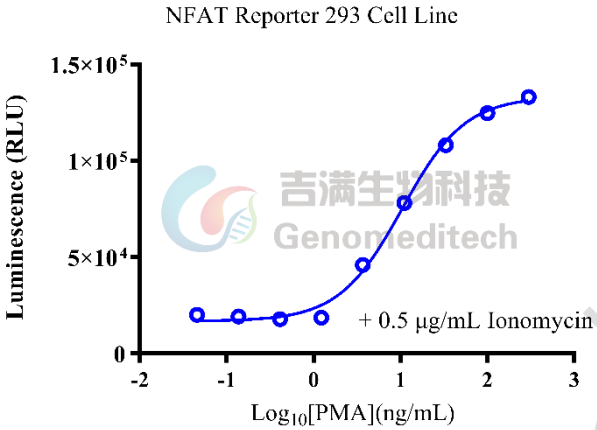
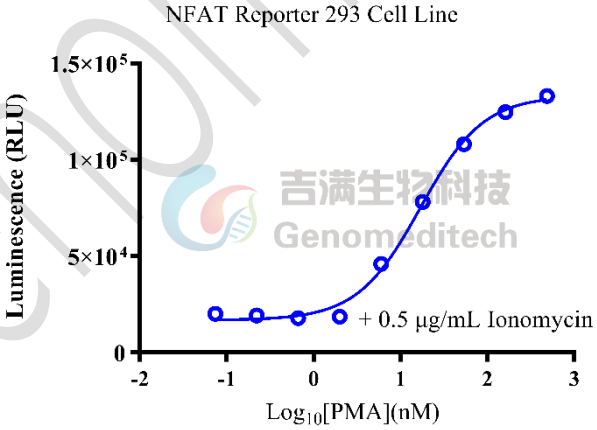
Figures



	NFAT Reporter 293 Cell Line
EC50	10.46

Figure 1 | Response to Response to PMA/TPA and Ionomycin. NFAT Reporter 293 Cell Line (Cat. GM-C39044) was seeded at a density of 1.5E4 cells per well in a 96-well plate and incubated overnight. The next day, stimulated with 0.05 μg/wells Ionomycin (MCE/HY-13434) and serial dilutions of PMA/TPA (Beyotime/S1819) in assay buffer (DMEM+1% FBS+1% P.S) for 16 hours. The firefly luciferase activity was measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). The maximum induction fold was approximately [7.1]. Data are shown by drug mass concentration.



	NFAT Reporter 293 Cell Line
EC50	16.96

Figure 2 | Response to Response to PMA/TPA and Ionomycin. NFAT Reporter 293 Cell Line (Cat. GM-C39044) was seeded at a density of 1.5E4 cells per well in a 96-well plate and incubated overnight. The next day, stimulated with 0.05 ug/wells Ionomycin (MCE/HY-13434) and serial dilutions of PMA/TPA (Beyotime/S1819) in assay buffer (DMEM+1% FBS+1% P.S) for 16 hours. The firefly luciferase activity was measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). The maximum induction fold was approximately [7.1]. Data are shown by drug molar concentration.

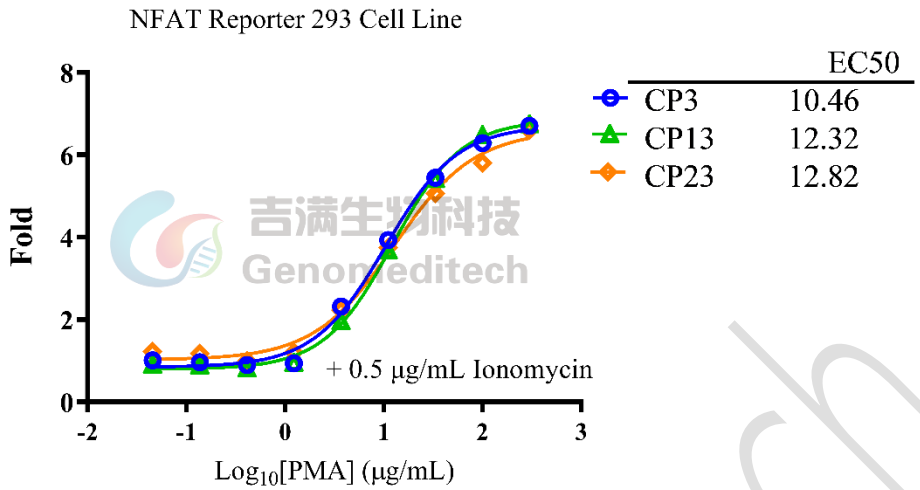


Figure 3 | The passage stability of response to PMA/TPA and Ionomycin. The passage 3, 13, and 23 of NFAT Reporter 293 Cell Line (Cat. GM-C39044) was seeded at a density of 1.5E4 cells per well in a 96-well plate and incubated overnight. The next day, stimulated with 0.05 μg/wells Ionomycin (MCE/HY-13434) and serial dilutions of PMA/TPA (Beyotime/S1819) in assay buffer (DMEM+1% FBS+1% P.S) for 16 hours. The firefly luciferase activity was measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). Data are shown by drug mass concentration.

Cell Recovery

Recovery Medium: DMEM+10% FBS+1% P.S

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70°C. Storage at -70°C will result in loss of viability.

a) Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 - 3 minutes).

b) Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.

c) Transfer the vial contents to a centrifuge tube containing 5.0 mL complete culture medium and spin at approximately 176 x g for 5 minutes. Discard supernatant.

d) Resuspend cell pellet with the recommended recovery medium. And dispense into appropriate culture dishes.

e) Incubate the culture at 37°C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended if using the medium described on this product sheet.

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Cell Freezing

Freezing Medium: 90% FBS+10% DMSO

a) Centrifuge at 176 x g for 3 minutes to collect cells.

b) Resuspend the cells in pre-cooled freezing medium and adjust the cell density to 5E6 cells/mL.

c) Aliquot 1 mL into each vial.

d) Place the vial in a controlled-rate freezing container and store at -80°C for at least 1 day, then transfer to liquid nitrogen as soon as possible.

Cell passage

Growth medium: DMEM+10% FBS+1% P.S+4 μg/mL Blasticidin

For the first 1 to 2 passages post-resuscitation, use the recovery medium. Once the cells have stabilized, switch to a growth medium.

a) Subculturing is necessary when the cell density reaches 80%. It is recommended to perform subculturing at a ratio of 1:3 to 1:4 every 2-3 days. Ensure that the density does not exceed 80%, as overcrowding can lead to reduced viability due to compression.

b) Remove and discard culture medium.

c) Briefly rinse the cell layer with PBS to remove all traces of serum that contains trypsin inhibitor.

d) Add 1.0 mL of 0.25% (w/v) Trypsin-EDTA solution to dish and observe cells under an inverted microscope until cell layer is dispersed (usually within 30 to 60 seconds at 37°C).

e) Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.

f) Add 2.0 mL of growth medium to mix well and aspirate cells by gently pipetting.

g) After centrifugation, resuspend the pellet and add appropriate aliquots of the cell suspension to new culture vessels.

h) Incubate cultures at 37°C.

Subcultivation Ratio: A subcultivation ratio of 1:3 - 1:4 is recommended

Medium Renewal: Every 2 to 3 days

Notes

a) Upon initial thawing, a higher number of dead cells is observed, which is a normal phenomenon. Significant improvement is seen after adaptation. Once the cells reach a stable state, the number of dead cells decreases after subculturing and the cell growth rate becomes stable.

b) Ensure that the cell density does not exceed 80%, as overcrowding may lead to reduced viability due to compression.

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