Genomeditech (Shanghai) Co.,Ltd.
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Product Sheet

NFAT-Luc Reporter Jurkat (CD4-) Cell Line

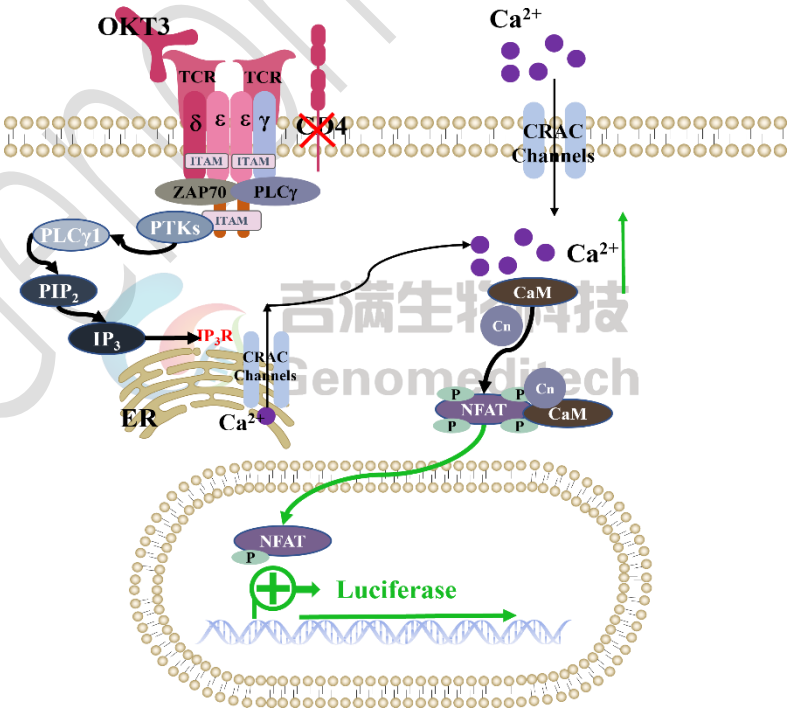
Catalog number: GM-C48178

Version 3.3.1.260805

NFAT (nuclear factor of activated T cells) is a family of transcription factors that plays a critical role in inducing gene transcription during immune responses. It is expressed not only in T cells but also in various other immune cells, including B cells, NK cells, mast cells, monocytes, and eosinophils. Its activity is regulated by calcineurin in a calcium-dependent manner. NFAT is involved in T-cell activation, differentiation, and self-tolerance, and has been implicated in the pathogenesis of multiple diseases, such as asthma, Alzheimer's disease, inflammatory bowel disease, diabetes, osteoporosis, osteoarthritis, and myocarditis.

CD4 is a coreceptor molecule expressed on the surface of T cells. Its extracellular domain binds to MHC class II molecules, while its cytoplasmic domain couples to the TCR-CD3 complex via the protein tyrosine kinase p56^lck. During antigen recognition, CD4 enhances the binding stability between TCR and the peptide–MHC class II complex and amplifies downstream TCR signaling through Lck.

NFAT-Luc Reporter Jurkat (CD4-) Cell Line is a clonal stable cell line on a CD4 knockout background and 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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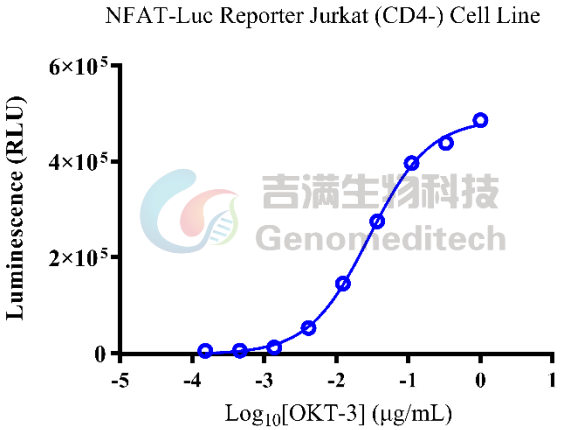
Specifications

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	RPMI 1640+10% FBS+1% P.S
Growth medium	RPMI 1640+10% FBS+1% P.S+3.5 μg/mL Blasticidin
Note	None
Freezing Medium	90% FBS+10% DMSO
Growth properties	Suspension
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

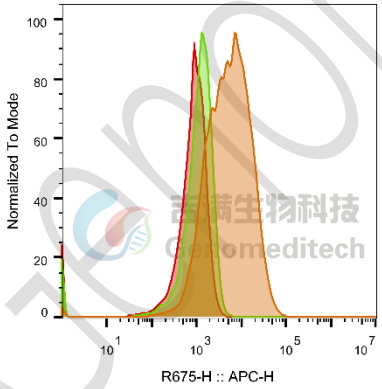
Reagent	Manufacturer/Catalogue No.
RPMI 1640	gibco/C11875500BT
Fetal Bovine Serum	ExCell/FSP500
Pen/Strep	Thermo/15140-122
Blasticidin	Genomeditech/GM-040404
Anti-H_CD4 hIgG1 Antibody(Tregalizumab)	Genomeditech/GM-28752AB
Anti-CD3 epsilon hIgG1 Antibody [OKT-3 (muromonab)]	Genomeditech/GM-51478AB
GMOne-Step 2.0 Luciferase Reporter Gene Assay Kit	Genomeditech/GM-040513

Figures



	NFAT-Luc Reporter Jurkat (CD4-)
EC50	0.02862

Figure 1 | Response to Anti-CD3 epsilon hIgG1 Antibody [OKT-3 (muromonab)]. The NFAT-Luc Reporter Jurkat (CD4-) Cell Line (Cat. GM-C48178) at a concentration of 5E4 cells/well (96-well format) was stimulated with serial dilutions of Anti-CD3 epsilon hIgG1 Antibody [OKT-3 (muromonab)] (Cat. GM-51478AB) in assay buffer (RPMI 1640+1% FBS+1% P.S) for 6 hours. The firefly luciferase activity was measured using the Luciferase Reporter Assay Kit (Genomeditech). The maximum induction fold was approximately [109.8]. Data are shown by drug mass concentration.



SampleID	Geometric Mean : R675-H
Jurkat anti-CD4+APC-2nd Ab	5005
NFAT-Luc Reporter Jurkat(CD4-) H_IgG+APC-2nd Ab	952
NFAT-Luc Reporter Jurkat(CD4-) anti-CD4+APC-2nd Ab	675

Figure 2 | NFAT-Luc Reporter Jurkat (CD4-) Cell Line (Cat. GM-C48178) was determined by flow cytometry using Anti-H_CD4 hIgG1 Antibody(Tregalizumab)(Cat. GM-28752AB).

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Figure 3 | The Sanger sequencing of the NFAT-Luc Reporter Jurkat (CD4-) Cell Line(Cat. GM-C48178) showed successful knockout of Human CD4.

Cell Recovery

Recovery Medium: RPMI 1640+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 complete medium. And dispense the suspension into 1 - 2 T-25 culture flasks.

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.

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: RPMI 1640+10% FBS+1% P.S+3.5 μg/mL Blasticidin

Approximately 48-72 hours after the initial thawing, the cells can be passaged for the first time. After this initial passage, the culture medium can be adjusted to growth medium supplemented with antibiotics. If cells are not passaged within 48 hours, it is recommended to add some fresh recovery medium and place the flask horizontally.

a) When the cell density reaches 1.5 - 2E6 cells/mL, subculture the cells. Do not allow the cell density to exceed 2E6 cells/mL.

b) It is recommended to use T-25 flasks for subculturing.

c) These cells are suspension cells, and it is recommended to use the "half-medium change" method to maintain optimal cell conditions during passaging.

d) During passaging, you can directly add fresh growth medium to the culture flask, gently pipette to resuspend the cells, and then transfer the cell suspension to a new T-25 flask for continued culture.

Subcultivation Ratio: Maintain cultures at a cell concentraion between 3E5 and 1E6 viable cells/mL.

Medium Renewal: Every 2 to 3 days

Notes

a) These cells are sensitive to density, so please ensure that the cell density is maintained within an appropriate range during culture and subculturing.

b) During the first passage, pay attention to the nutrient supply; if not subculturing, make sure to add fresh recovery medium every other day as needed.

Related Products

TCR	
H_FOXP3-Promoter Reporter Jurkat Cell Line	H_IL2-Promoter Reporter Jurkat Cell Line
NFAT-Luc Reporter Jurkat Cell Line	TCR Knockout Reporter Cell Line(CD4+)
OKT3(CD3 ScFv) CHO-K1 Cell Line	
Anti-CD3×CD19 Bispecific Antibody (Blinbio)	Anti-CD3-CD19 Bispecific Antibody(Blinatumomab)

License Agreement:

By purchasing and using this cell line product, the user voluntarily agrees to accept and abide by the following policies:

- This cell line product is restricted to research use only and shall not be used for any commercial purposes.

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