

# Product Sheet

## NFAT-Luc Reporter Jurkat Cell Line

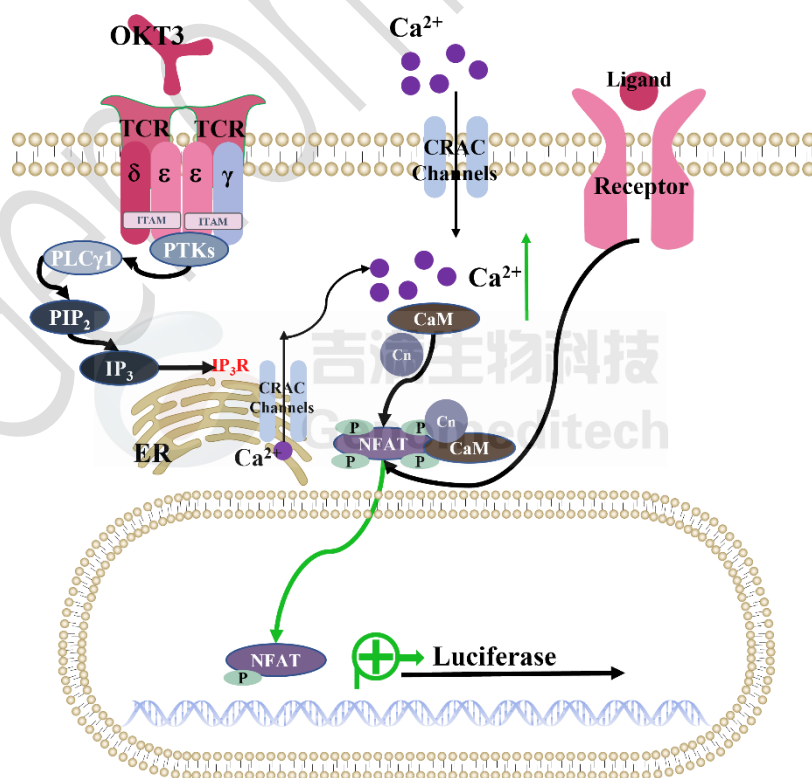
Catalog number: GM-C01459

Version 3.3.1.241226

NFAT (Nuclear Factor of Activated T-cells) is a crucial transcription factor for immune cell function, especially T cell activation. The NFAT family includes NFATc1, NFATc2, NFATc3, and NFATc4, which are involved in calcium signaling and cytokine gene expression. NFAT is activated by rising calcium levels, leading to its dephosphorylation and translocation to the nucleus, where it binds to DNA to regulate gene transcription.

The NFAT signaling pathway is activated by T cell receptors (TCR) and other surface receptors. When TCR binds to an antigen, intracellular calcium levels increase, activating phospholipase C (PLC) and producing diacylglycerol (DAG) and inositol trisphosphate (IP<sub>3</sub>). IP<sub>3</sub> triggers calcium release from the endoplasmic reticulum, activating calmodulin and calcium-dependent enzymes (like CaMK), which dephosphorylate NFAT, allowing its entry into the nucleus. NFAT interacts with other transcription factors (e.g., AP-1) to regulate immune-related gene expression, promoting T cell proliferation, differentiation, and cytokine production.

NFAT-Luc Reporter Jurkat Cell Line is a clonal stable Jurkat 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.



## Specifications

<b>Quantity</b>	5E6 Cells per vial,1 mL
<b>Product Format</b>	1 vial of frozen cells
<b>Shipping</b>	Shipped on dry ice
<b>Storage Conditions</b>	Liquid nitrogen immediately upon receipt

<b>Recovery Medium</b>	RPMI 1640+10% FBS+1% P.S
<b>Growth medium</b>	RPMI 1640+10% FBS+1% P.S+3.5 µg/mL Blasticidin
<b>Note</b>	None
<b>Freezing Medium</b>	90% FBS+10% DMSO
<b>Growth properties</b>	Suspension
<b>Growth Conditions</b>	37°C, 5% CO <sub>2</sub>

<b>Mycoplasma Testing</b>	The cell line has been screened to confirm the absence of Mycoplasma species.
<b>Safety considerations</b>	Biosafety Level 2
<b>Note</b>	It is recommended to expand the cell culture and store a minimum of 10 vials at an early passage for potential future use.

## Materials

<b>Reagent</b>	<b>Manufacturer/Catalogue No.</b>
RPMI 1640	VivaCell/C3010-0500
Fetal Bovine Serum	Cegrogen biotech/A0500-3010
Pen/Strep	Thermo/15140-122
Blasticidin	Genomeditech/ <a href="#">GM-040404</a>
Anti-CD3 epsilon hIgG1 Antibody [OKT-3 (muromonab)]	Genomeditech/ <a href="#">GM-51478AB</a>
GMOne-Step Luciferase Reporter Gene Assay Kit	Genomeditech/ <a href="#">GM-040503</a>

## Figures

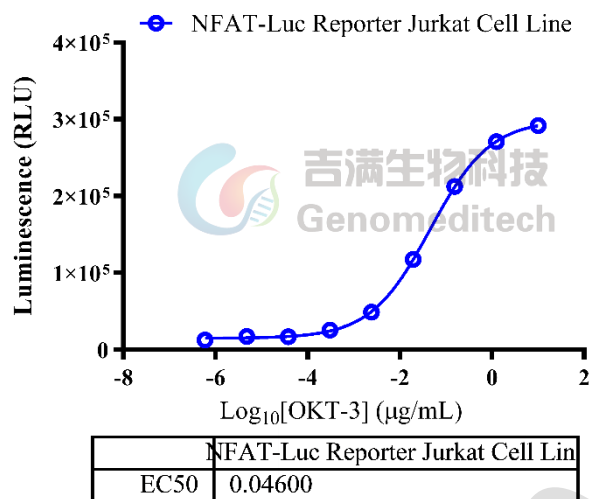


Figure 1 | Response to OKT-3 (muromonab). NFAT-Luc Reporter Jurkat Cell Line (GM-C01459) at a concentration of 1E5 cells/well (96-well format) was stimulated with serial dilutions of Anti-CD3 epsilon Antibody [OKT-3 (muromonab)] (GM-51478AB) in assay buffer (RPMI 1640+1% FBS+1% P.S) for 7 hours. The firefly luciferase activity was measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (GM-040503). The maximum induction fold was approximately [29.1]. Data are shown by drug mass concentration.

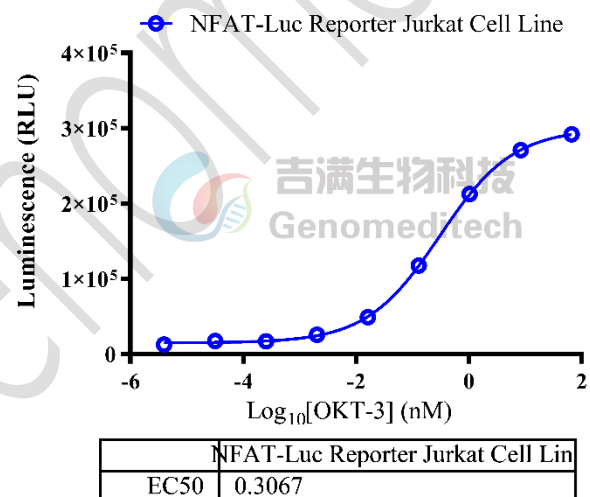


Figure 2 | Response to OKT-3 (muromonab). NFAT-Luc Reporter Jurkat Cell Line (GM-C01459) at a concentration of 1E5 cells/well (96-well format) was stimulated with serial dilutions of Anti-CD3 epsilon Antibody [OKT-3 (muromonab)] (GM-51478AB) in assay buffer (RPMI 1640+1% FBS+1% P.S) for 7 hours. The firefly luciferase activity was measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (GM-040503). The maximum induction fold was approximately [29.1]. Data are shown by drug molar concentration.

## 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  $-70^{\circ}\text{C}$ . Storage at  $-70^{\circ}\text{C}$  will result in loss of viability.

- Thaw the vial by gentle agitation in a  $37^{\circ}\text{C}$  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).
- 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.
- Transfer the vial contents to a centrifuge tube containing 5.0 mL complete culture medium. And spin at approximately  $176 \times g$  for 5 minutes. Discard supernatant.
- Resuspend cell pellet with the recommended complete medium. And dispense the suspension into 1 - 2 T-25 culture flasks.
- Incubate the culture at  $37^{\circ}\text{C}$  in a suitable incubator. A 5%  $\text{CO}_2$  in air atmosphere is recommended if using the medium described on this product sheet.

## Cell Freezing

Freezing Medium: 90% FBS+10% DMSO

- Centrifuge at  $176 \times g$  for 3 minutes to collect cells.
- Resuspend the cells in pre-cooled freezing medium and adjust the cell density to  $5 \times 10^6$  cells/mL.
- Aliquot 1 mL into each vial.
- Place the vial in a controlled-rate freezing container and store at  $-80^{\circ}\text{C}$  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  $\mu\text{g}/\text{mL}$  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.

- When the cell density reaches  $1.5 - 2 \times 10^6$  cells/mL, subculture the cells. Do not allow the cell density to exceed  $2 \times 10^6$  cells/mL.
- It is recommended to use T-25 flasks for subculturing.
- These cells are suspension cells, and it is recommended to use the "half-medium change" method to maintain optimal cell conditions during passaging.
- 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  $3 \times 10^5$  and  $1 \times 10^6$  viable cells/mL.**

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**Medium Renewal: Every 2 to 3 days**

## Notes

- These cells are sensitive to density, so please ensure that the cell density is maintained within an appropriate range during culture and subculturing.
- 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	
<a href="#">H_FOXP3-Promoter Reporter Jurkat Cell Line</a>	<a href="#">H_IL2-Promoter Reporter Jurkat Cell Line</a>
<a href="#">TCR Knockout Reporter Cell Line(CD4+)</a>	<a href="#">OKT3(CD3 ScFv) CHO-K1 Cell Line</a>
<a href="#">Anti-CD3-CD19 Bispecific Antibody(Blinatumomab)</a>	

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