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Product Sheet

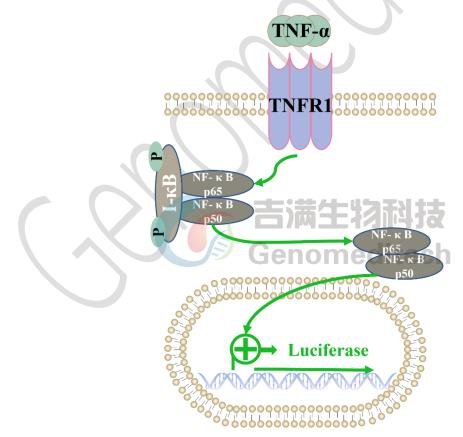
NFKB Reporter TF-1 Cell Line

Catalog number: GM-C33197

Version 3.3.1.241202

NF-κB is a group of transcription factors, including p65 (RelA), RelB, c-Rel, p50/p105 (NF-κB1), and p52/p100 (NF-κB2). These proteins function as dimeric transcription factors and play roles in controlling gene regulation across a wide range of biological processes, including innate and adaptive immunity, inflammation, stress responses, B cell development, and lymphoid organogenesis. Pro-inflammatory cytokines, LPS, growth factors, and antigen receptors activate the IKK complex (IKKβ, IKKα, and NEMO), leading to the phosphorylation of IκB proteins. Phosphorylation of IκB results in its ubiquitination and proteasomal degradation, releasing the NF-κB/Rel complex. The active NF-κB/Rel complex is further activated through phosphorylation and translocates to the nucleus to induce target gene expression.

NFKB Reporter TF-1 Cell Line is a clonal stable TF-1 cell line expressing a firefly luciferase under the control of the NF- κ B response elements, while also endogenously expressing the TNFR1. The binding of the TNF- α protein to TNFR1 activates downstream reporter genes, leading to luciferase expression. Luciferase readings reflect the activation level of signaling pathways and can be used to evaluate the activation effect of TNF- α .





Specifications

Quantity	3E6 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+2 ng/mL GM-CSF		
Growth medium	RPMI 1640+10% FBS+1% P.S+2 ng/mL GM-CSF+3 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			

Materials

Reagent	Manufacturer/Catalogue No.	
RPMI 1640	gibco/C11875500BT	
Fetal Bovine Serum	Cegrogen biotech/A0500-3010	
Pen/Strep	Thermo/15140-122	
Recombinant Human GM-CSF	Novoprotein/C003	
Blasticidin	Genomeditech/GM-040404	
GMOne-Step Luciferase Reporter Gene Assay Kit	Genomeditech/GM-040503	



Figures

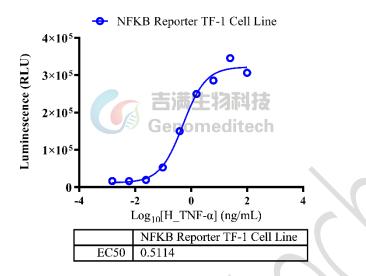


Figure 1 | Response to Human TNF- α protein. NFKB Reporter TF-1 Cell Line (Cat. GM-C33197) at a concentration of 1E5 cells/well (96-well format) was stimulated with serial dilutions of Recombinant Human TNF- α (Sino Biological/10602-HNAE) in assay buffer (RPMI 1640+1% FBS+1% P.S) for 7 hours. The firefly luciferase activity was measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). The maximum induction fold was approximately [17.0]. Data are shown by drug mass concentration.

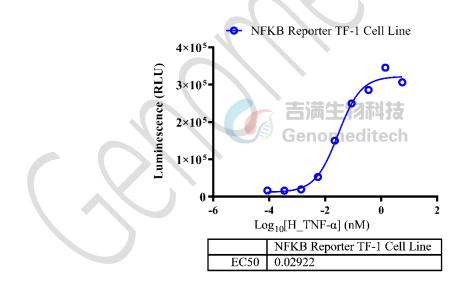


Figure 2 | Response to Human TNF- α protein. NFKB Reporter TF-1 Cell Line (Cat. GM-C33197) at a concentration of 1E5 cells/well (96-well format) was stimulated with serial dilutions of Recombinant Human TNF- α (Sino Biological/10602-HNAE) in assay buffer (RPMI 1640+1% FBS+1% P.S) for 7 hours. The firefly luciferase activity was measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). The maximum induction fold was approximately [17.0]. Data are shown by drug molar concentration.

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Cell Recovery

Recovery Medium: RPMI 1640+10% FBS+1% P.S+2 ng/mL GM-CSF

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 the cell pellet using the recommended complete medium and adjust the viable cell density to 4-6E5 cells/mL. Then dispense the suspension into an appropriate culture flask and initially place the flask in an upright position after thawing.
- e) Incubate the culture at 37°C in a suitable incubator. A 5% CO₂ 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 3E6 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+2 ng/mL GM-CSF+3 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.

- a) This cell is a human erythroid leukemia cell, lymphoblast, growing in suspension.
- b) In the suspension, they appear as large, single, round cells. Cells shed a large accumulation of cytoplasmic granules in the culture, which should not be confused with bacteria!
- c) When the cell density reaches 1-1.2E6 cells/mL, perform a 1:2 to 1:3 split, ensuring subculturing every other day. It is essential to perform a full-volume centrifugation and medium replacement during passaging. Do not let the density exceed 1.2E6 cells/mL. It is recommended to use T-25 flasks for subculturing, and you can control the cell density for subculturing by counting.

Subcultivation Ratio: Maintain cultures at a cell concentraion between 4E5 and 6E5 viable cells/mL.

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Notes

a) To minimize the presence of cytoplasmic granules, it is essential to passage the cells every other day when the cell density reaches 1-1.2E6 cells/mL. During passaging, perform a complete centrifugation and replace the culture medium to ensure appropriate cell density and cytokine concentration. Failure to do so may promote the growth of factor-independent subclones.

Related Products

NFKB			
NFKB Reporter HEK-293 Cell Line	NFKB Reporter Jurkat Cell Line		
NFKB Reporter THP1 Cell Line			

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