

Product Sheet

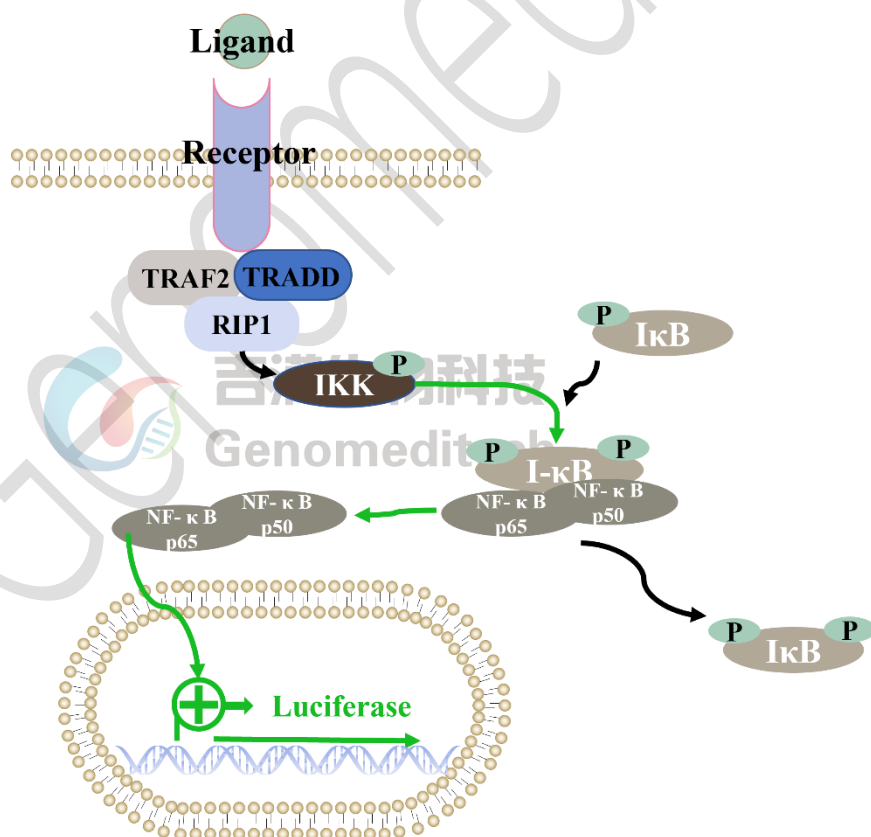
NFKB Reporter A549 Cell Line

Catalog number: GM-C40225

Version 3.3.1.250916

NF- κ B is a group of transcription factors, including p65 (RelA), RelB, c-Rel, p50/p105 (NF- κ B1), and p52/p100 (NF- κ B2). 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 (IKK β , IKK α , and NEMO), leading to the phosphorylation of I κ B proteins. Phosphorylation of I κ B results in its ubiquitination and proteasomal degradation, releasing the NF- κ B/Rel complex. The active NF- κ B/Rel complex is further activated through phosphorylation and translocates to the nucleus to induce target gene expression.

NFKB Reporter A549 Cell Line is a clonal stable A549 cell line expressing a firefly luciferase under the control of the NF- κ B response elements. Upon ligand binding (e.g., TNF- α) to its receptor, downstream signaling pathways are activated, leading to the induction of luciferase expression. The luciferase readout reflects the degree of pathway activation and can therefore be used to evaluate NF- κ B pathway activation.



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	F12K+10% FBS+1% P.S
Growth medium	F12K+10% FBS+1% P.S+10 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.
F12K	BOSTER/PYG0036
Fetal Bovine Serum	ExCell/FSP500
Pen/Strep	Thermo/15140-122
Blasticidin	Genomeditech/ GM-040404
Recombinant Human TNF alpha	Novoprotein/C008
Human IL-1 beta / IL1B Protein	Sino Biological/10139-HNAE
GMOne-Step 2.0 Luciferase Reporter Gene Assay Kit	Genomeditech/ GM-040513

Figures

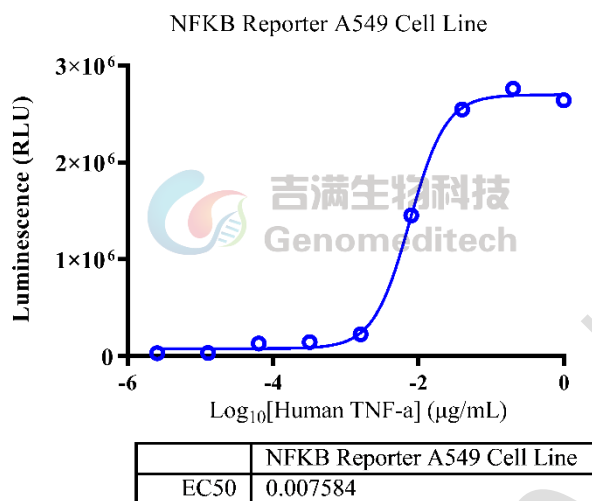


Figure 1 | Response to Human TNF alpha. The NFKB Reporter A549 Cell Line (Cat. GM-C40225) at a concentration of 1E4 cells/well (96-well format) was stimulated with serial dilutions of Recombinant Human TNF-a (Novoprotein/C008) in assay buffer (F12K + 1% FBS + 1% P.S) for 6 hours. The firefly luciferase activity was measured using the Luciferase Reporter Assay Kit (Genomeditech). The maximum induction fold was approximately [94.2]. Data are shown by drug mass concentration.

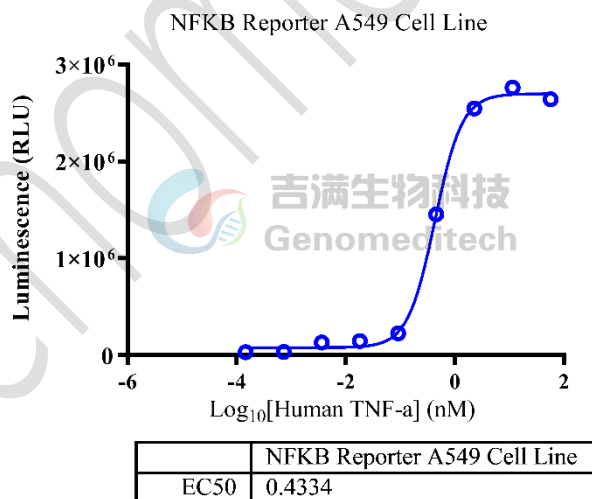


Figure 2 | Response to Human TNF alpha. The NFKB Reporter A549 Cell Line (Cat. GM-C40225) at a concentration of 1E4 cells/well (96-well format) was stimulated with serial dilutions of Recombinant Human TNF-a (Novoprotein/C008) in assay buffer (F12K + 1% FBS + 1% P.S) for 6 hours. The firefly luciferase activity was measured using the Luciferase Reporter Assay Kit (Genomeditech). The maximum induction fold was approximately [94.2]. Data are shown by drug molar concentration.

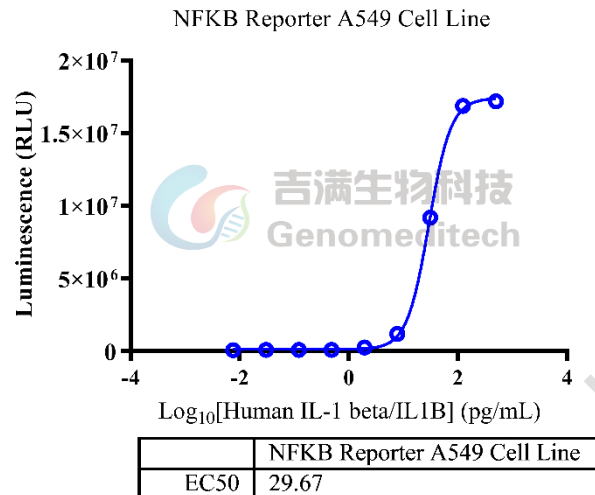


Figure 3 | Response to Human IL-1 beta Protein. The NFKB Reporter A549 Cell Line (Cat. GM-C40225) at a concentration of 1E4 cells/well (96-well format) was stimulated with serial dilutions of Recombinant Human IL-1 beta Protein (SinoBiological/10139-HNAE) in assay buffer (F12K + 1% FBS + 1% P.S) for 6 hours. The firefly luciferase activity was measured using the Luciferase Reporter Assay Kit (Genomeditech). The maximum induction fold was approximately [253.5]. Data are shown by drug mass concentration.

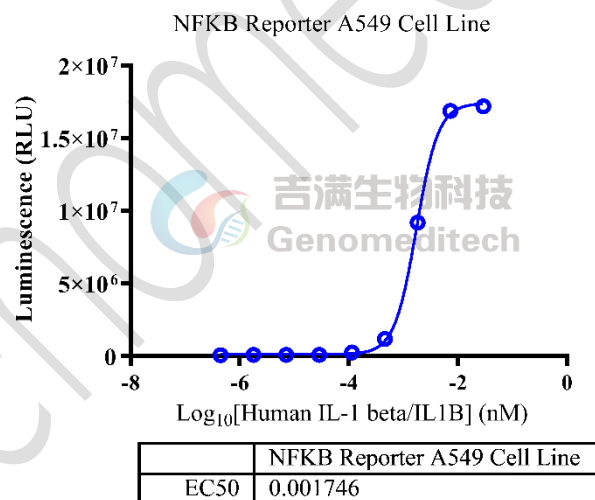


Figure 4 | Response to Human IL-1 beta Protein. The NFKB Reporter A549 Cell Line (Cat. GM-C40225) at a concentration of 1E4 cells/well (96-well format) was stimulated with serial dilutions of Recombinant Human IL-1 beta Protein (SinoBiological/10139-HNAE) in assay buffer (F12K + 1% FBS + 1% P.S) for 6 hours. The firefly luciferase activity was measured using the Luciferase Reporter Assay Kit (Genomeditech). The maximum induction fold was approximately [253.5]. Data are shown by drug molar concentration.

Cell Recovery

Recovery Medium: F12K+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.

- 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).
- 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 x g for 5 minutes. Discard supernatant.
- Resuspend cell pellet with the recommended recovery medium. And dispense into appropriate culture dishes.
- 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

- Centrifuge at 176 x g for 3 minutes to collect cells.
- Resuspend the cells in pre-cooled freezing medium and adjust the cell density to 3E6 cells/mL.
- Aliquot 1 mL into each vial.
- 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: F12K+10% FBS+1% P.S+10 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.

- Subculturing is necessary when the cell density reaches 80%. It is recommended to perform subculturing at a ratio of 1:2 to 1:3 every 2-3 days.
- Remove and discard culture medium.
- Briefly rinse the cell layer with PBS to remove all traces of serum that contains trypsin inhibitor.
- 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 3 to 5 minutes at 37°C).
- 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.
- Add 2.0 mL of growth medium to mix well and aspirate cells by gently pipetting.
- 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 1:2 - 1:3 is recommended

Medium Renewal: Every 2 to 3 days

Notes

- a) A549 cells should not be allowed to become overly confluent.
- b) FBS should be heat-inactivated at 56°C for 30 minutes, which inactivates complement and some viruses, but does not significantly affect the activity of most growth factors and cytokines.

Related Products

NFKB	
NFKB Reporter HEK-293 Cell Line	NFKB Reporter Jurkat Cell Line
NFKB Reporter TF-1 Cell Line	NFKB Reporter THP1 Cell Line

License Agreement:

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

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