Genomeditech (Shanghai) Co.,Ltd.
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Product Sheet

H_TNFR2 Null Reporter Cell Line

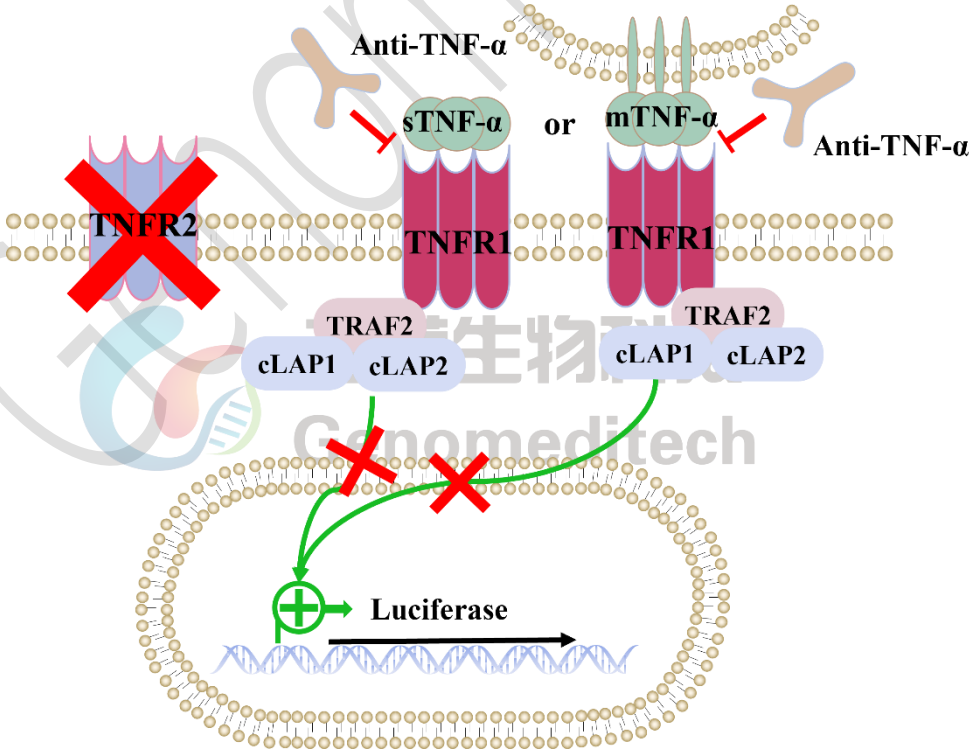
Catalog number: GM-C27615

Version 3.3.1.250107

Tumor necrosis factor-alpha (TNF-α) is a type II transmembrane protein that exists in a membrane-bound form (mTNF-α). mTNF-α can be processed by an enzyme known as TNFα-converting enzyme into a 17 kDa soluble form (sTNF-α). TNF-α functions through two type I transmembrane receptors of the TNF receptor superfamily: TNF receptor 1 (TNFR1) and TNF receptor 2 (TNFR2).

TNFR1 signal transduction occurs via its intracellular death domain binding to adaptor proteins, forming a signaling complex. Activation recruits TRADD and FADD, leading to downstream caspase activation and apoptosis. TNFR1 also recruits RIPK1 and IκB kinase (IKK) to activate the NF-κB pathway, promoting cell survival and inflammation.

H_TNFR2 Null Reporter Cell Line is a clonal stable cell line constructed using lentiviral technology, endogenously expresses the TNFR1 gene, along with signal-dependent expression of a luciferase reporter gene. It can serve as a control cell for H_TNFR2 Reporter V2 Cell Line(Cat. GM-C25776). When TNF-α binds to TNFR1, it activates downstream signaling pathways, leading to the expression of luciferase. Blockade antibodies can inhibit this signal transmission. The luciferase activity measurement indicates the activation level of the signaling pathway and can thus be used to evaluate the in vitro effects of drugs related to TNFR1 and TNF-α.



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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	VivaCell/C3010-0500
Fetal Bovine Serum	Cegrogen biotech/A0500-3010
Pen/Strep	Thermo/15140-122
Blasticidin	Genomeditech/GM-040404
Recombinant Human TNF-α	PEPROTECH/300-01A
Membrane Bound H_TNFα(cleavage-resistant) CHO-K1 Cell Line	Genomeditech/GM-C33297
Anti-TNFR1 hIgG1 Antibody(Atrosab)	Genomeditech/GM-51152AB
Anti-TNF-α hIgG1 Antibody（CT-P17）	Genomeditech/GM-49267AB
GMOne-Step Luciferase Reporter Gene Assay Kit	Genomeditech/GM-040503

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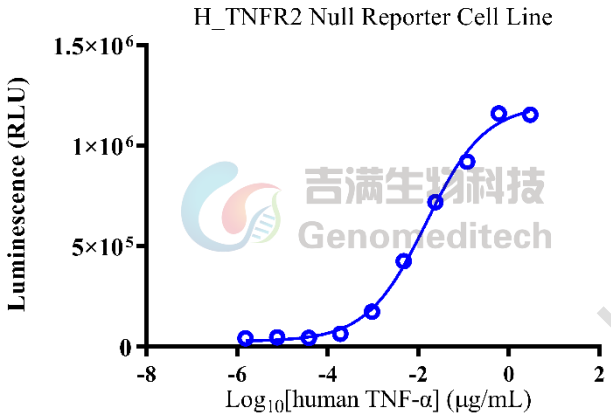
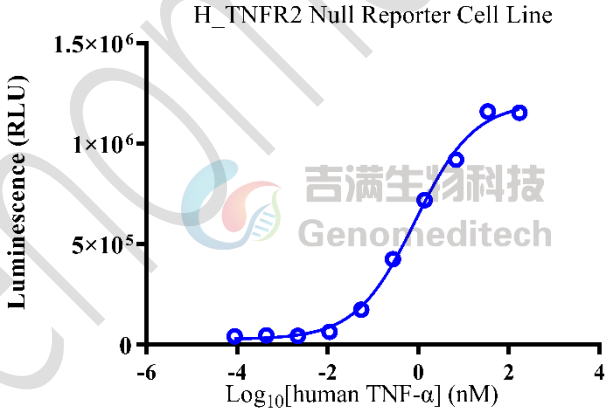
Figures



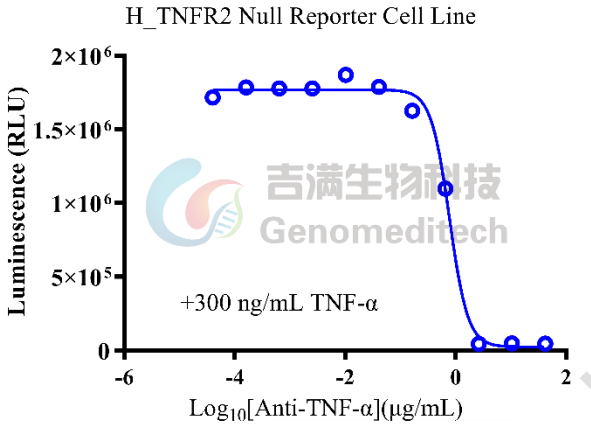
	H_TNFR2 Null Reporter Cell Line
EC50	0.01495

Figure 1 | Response to Recombinant Human TNF-α. The H_TNFR2 Null Reporter Cell Line (Cat. GM-C27615) at a concentration of 1E5 cells/well (96-well format) was stimulated with serial dilutions of Recombinant Human TNF-α (Peprotech/300-01A) 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 [27.2]. Data are shown by drug mass concentration.



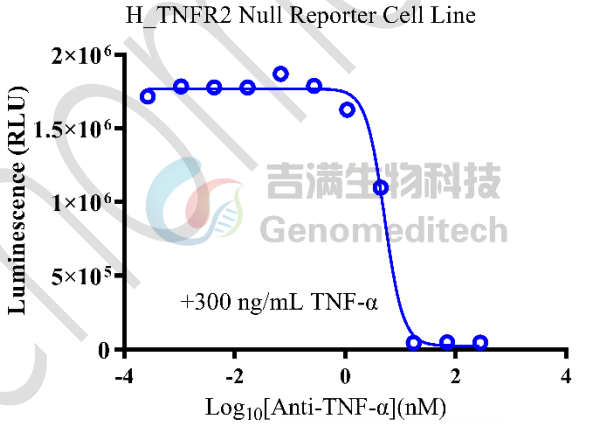
	H_TNFR2 Null Reporter Cell Line
EC50	0.8590

Figure 2 | Response to Recombinant Human TNF-α. The H_TNFR2 Null Reporter Cell Line (Cat. GM-C27615) at a concentration of 1E5 cells/well (96-well format) was stimulated with serial dilutions of Recombinant Human TNF-α (Peprotech/300-01A) 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 [27.2]. Data are shown by drug molar concentration.



	H_TNFR2 Null Reporter Cell Line
IC50	0.7756

Figure 3 | Response to Anti-TNF-α hIgG1 Antibody. Serial dilutions of Anti-TNF-α hIgG1 Antibody(CT-P17) (Cat. GM-49267AB) was incubated with 30 ng/well of Recombinant Human TNF-α (Peprotech/300-01A) for 1 hour in assay buffer (RPMI 1640 + 1% FBS + 1% P.S). After pre-incubation, add the mixture to the H_TNFR2 Null Reporter Cell Line (Cat. GM-C27615) at a density of 1E5 cells/well in a 96-well format, and incubate for 6 hours. Firefly luciferase activity is then measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). The results indicated maximum blocking folds of approximately [25.5]. Data are shown by drug mass concentration.



	H_TNFR2 Null Reporter Cell Line
IC50	5.170

Figure 4 | Response to Anti-TNF-α hIgG1 Antibody. Serial dilutions of Anti-TNF-α hIgG1 Antibody(CT-P17) (Cat. GM-49267AB) was incubated with 30 ng/well of Recombinant Human TNF-α (Peprotech/300-01A) for 1 hour in assay buffer (RPMI 1640 + 1% FBS + 1% P.S). After pre-incubation, add the mixture to the H_TNFR2 Null Reporter Cell Line (Cat. GM-C27615) at a density of 1E5 cells/well in a 96-well format, and incubate for 6 hours. Firefly luciferase activity is then measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). The results indicated maximum blocking folds of approximately [25.5]. Data are shown by drug molar concentration.

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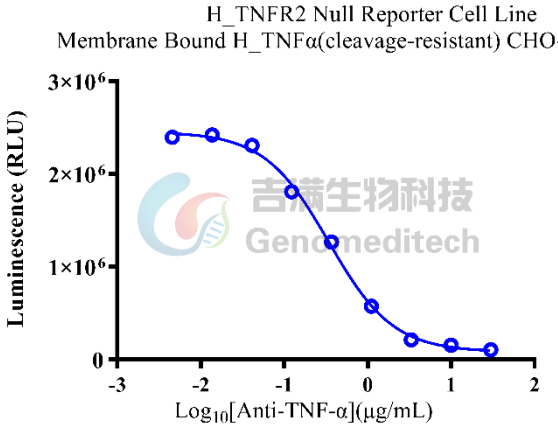
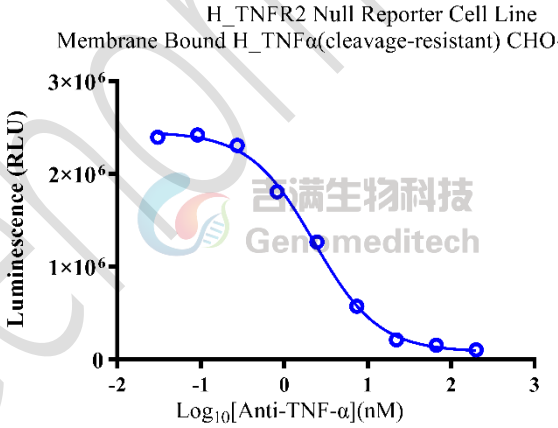


	H_TNFR2 Null Reporter Cell Line
IC50	0.3426

Figure 5 | Response to Anti-TNF-α hIgG1 Antibody. The Membrane Bound H_TNFα(cleavage-resistant) CHO-K1 Cell Line (Cat. GM-C33297) was seeded at a density of 1.5E4 cells per well in a 96-well plate and incubated overnight. The next day, serial dilutions of the Anti-TNF-α hIgG1 Antibody(CT-P17) (Cat. GM-49267AB) were incubated with 1E5 cells/well of the H_TNFR2 Null Reporter Cell Line in a 96-well plate for 1 hour, and then added to the pre-seeded cells. The mixture was incubated for an additional 6 hours. Firefly luciferase activity was measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). The maximum induction fold was approximately [23.0]. Data are presented based on drug mass concentration.



	H_TNFR2 Null Reporter Cell Line
IC50	2.284

Figure 6 | Response to Anti-TNF-α hIgG1 Antibody. The Membrane Bound H_TNFα(cleavage-resistant) CHO-K1 Cell Line (Cat. GM-C33297) was seeded at a density of 1.5E4 cells per well in a 96-well plate and incubated overnight. The next day, serial dilutions of the Anti-TNF-α hIgG1 Antibody(CT-P17) (Cat. GM-49267AB) were incubated with 1E5 cells/well of the H_TNFR2 Null Reporter Cell Line in a 96-well plate for 1 hour, and then added to the pre-seeded cells. The mixture was incubated for an additional 6 hours. Firefly luciferase activity was measured using

the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). The maximum induction fold was approximately [23.0]. Data are shown by drug molar concentration.

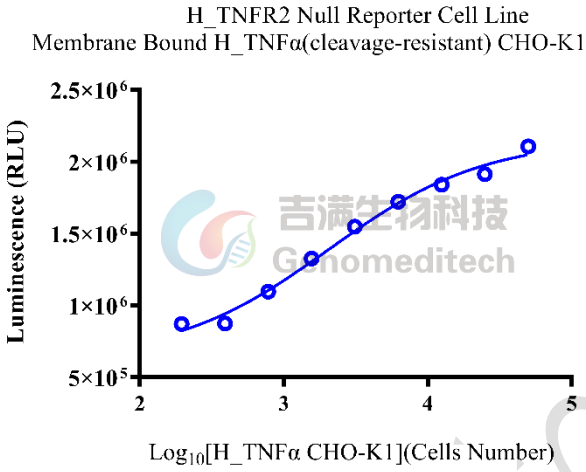
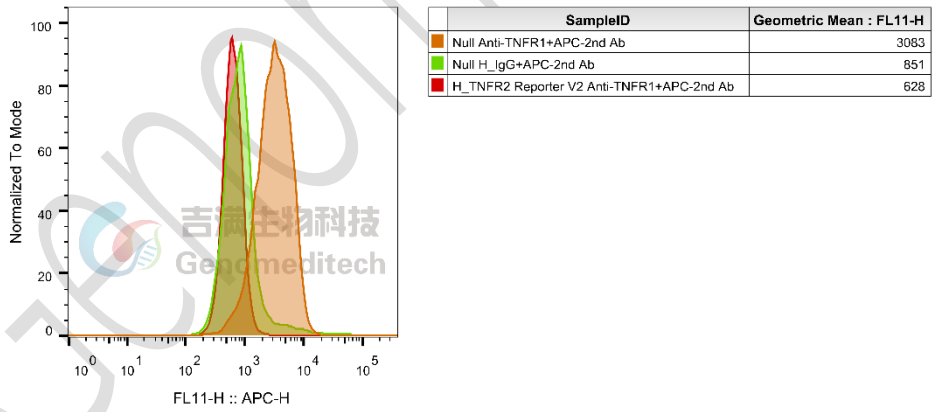


Figure 7 | Response to Membrane Bound H_TNFα(cleavage-resistant) CHO-K1 Cell Line. H_TNFR2 Null Reporter Cell Line (Cat. GM-C27615) at a concentration of 1E5 cells/well (96-well format) was stimulated with serial dilutions of Membrane Bound H_TNFα(cleavage-resistant) CHO-K1 Cell Line (Cat. GM-C33297) for 6 hours. The firefly luciferase activity was measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503).



SampleID	Geometric Mean : FL11-H
Null Anti-TNFR1+APC-2nd Ab	3083
Null H_IgG+APC-2nd Ab	851
H_TNFR2 Reporter V2 Anti-TNFR1+APC-2nd Ab	628

Figure 8 | H_TNFR2 Null Reporter Cell Line (Cat. GM-C27615) was determined by flow cytometry using Anti-TNFR1 hIgG1 Antibody(Atrosab) (Cat. GM-51152AB).

Cell Recovery

Recovery Medium: RPMI 1640+10%FBS+1%P.S

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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

TNF:TNFR2:TNFR1	
H_TNFR2 Reporter Jurkat Cell Line	H_TNFR2 Reporter V2 Cell Line
Cynomolgus_TNFRSF1B(TNFR2) CHO-K1 Cell Line	H_TNFRSF1B(TNFR2) CHO-K1 Cell Line
H_TNFRSF1B(TNFR2) HEK-293 Cell Line	Membrane Bound H_TNFα CHO-K1 Cell Line
Membrane Bound H_TNFα(cleavage-resistant) CHO-K1 Cell Line	
Anti-H_TNFR2 hIgG1 Antibody(1H10)	Anti-H_TNFRSF1B(TNFR2) hIgG1 Antibody(UC2.3.8)
Anti-TNFR1 hIgG1 Antibody(Atrosab)	Anti-TNF-α hIgG1 Antibody（CT-P17）

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