

Product Sheet

Membrane Bound H₂TNF α (cleavage-resistant) CHO-K1 Cell Line

Catalog number: GM-C33297

Version 3.3.1.250812

Description	Membrane Bound H ₂ TNF α (cleavage-resistant) CHO-K1 Cell Line is a clonal, stable CHO-K1 cell line that constitutively expresses a cleavage-resistant mutant form of the human TNF α (TNF) gene, constructed using lentiviral technology.
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
Target	Human_TNF α (TNF)
Gene ID/Uniprot ID	P01375(Ala76Leu & Val77Leu)
Host Cell	CHO-K1
Recovery Medium	F12K+10% FBS+1% P.S
Growth medium	F12K+10% FBS+1% P.S+4 μ g/mL Puromycin
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
Puromycin	Genomeditech/ GM-040401
ADCC FcγRIIIa(158V) Jurkat Effector Cell Line	Genomeditech/ GM-C05619
Anti-TNF- α hIgG1 Antibody (CT-P17)	Genomeditech/ GM-49267AB
GMOne-Step Luciferase Reporter Gene Assay Kit	Genomeditech/ GM-040503

Figures

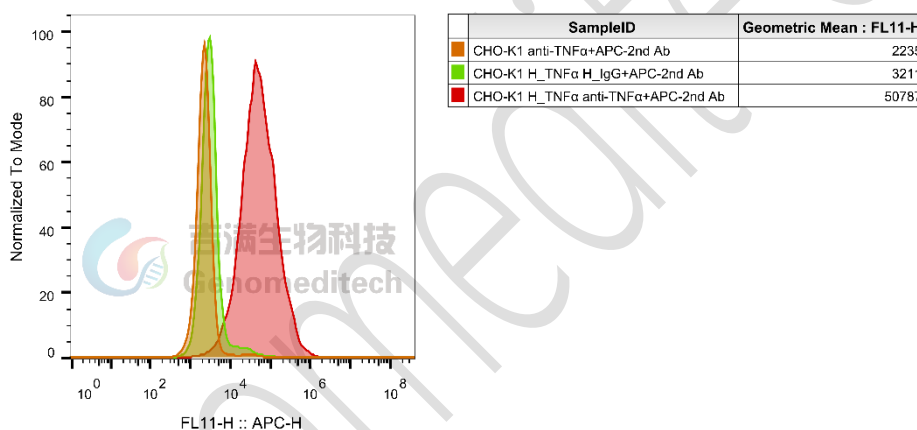


Figure 1 | Membrane Bound H_TNFα(cleavage-resistant) CHO-K1 Cell Line (Cat. GM-C33297) was determined by flow cytometry using Anti-TNF- α hIgG1 Antibody(CT-P17) (Cat. [GM-49267AB](#)).

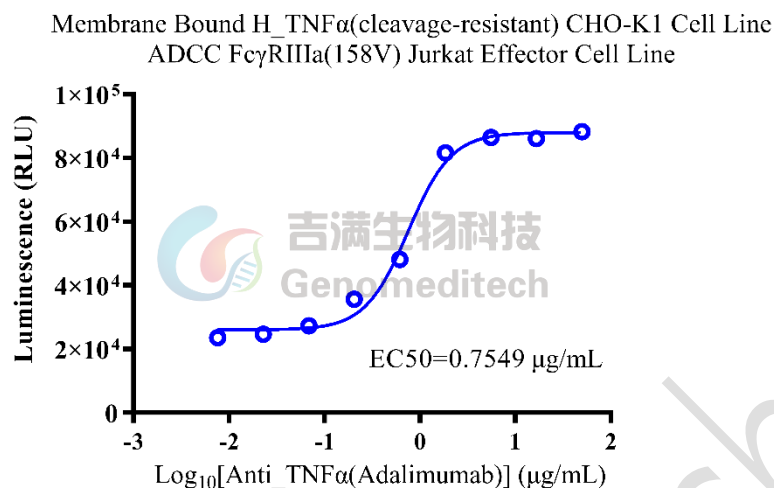


Figure 2 | Anti-TNF- α -mediated ADCC assays on CHO-K1 cells. The ADCC Fc γ RIIIa(158V) Jurkat Effector Cell Line(Cat. [GM-C05619](#)) at a concentration of 1.5E6 cells/well was co-cultured with Membrane Bound H₂ TNF α (cleavage-resistant) CHO-K1 Cell Line (Cat. GM-C33297) at a concentration of 1E4 cells/well, in the presence of serial dilutions of the Anti-TNF- α hIgG1 Antibody(CT-P17) (Cat. [GM-49267AB](#)) for 6 hours (96-well format). The firefly luciferase activity was measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. [GM-040503](#)). Data are shown by drug mass concentration.

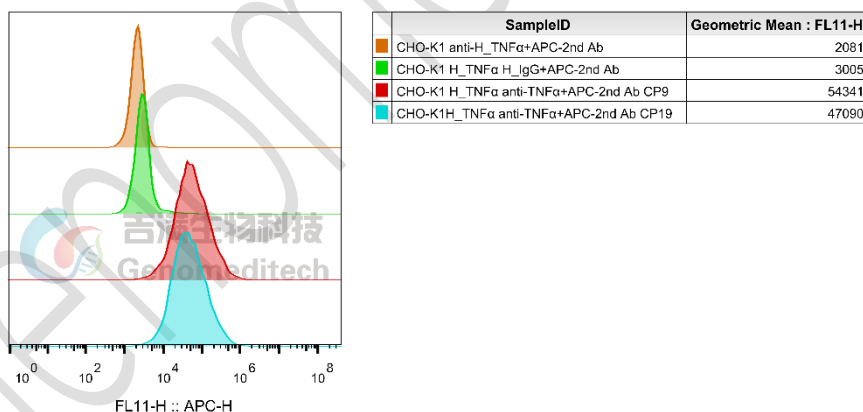


Figure 3 | The passage stability of the Membrane Bound H₂ TNF α (cleavage-resistant) CHO-K1 Cell Line (Cat. GM-C33297) was determined by flow cytometry using Anti-TNF- α hIgG1 Antibody(CT-P17) (Cat. [GM-49267AB](#)).

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 $176 \times g$ 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% 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 5×10^6 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+4 $\mu\text{g/mL}$ Puromycin

For the first 1 to 2 passages post-resuscitation, use the recovery medium. Once the cells have stabilized, switch to a growth medium.

- 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 2 to 3 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.
- Incubate cultures at 37°C .

Subcultivation Ratio: A subcultivation ratio of 1:4 - 1:5 is recommended

Medium Renewal: Every 2 to 3 days

Notes

- a) After the stabilization of the cell condition, there will be fewer dead cells post-passage, the cell growth rate will tend to stabilize, cell morphology will become uniform, and the cells will appear robust.

Sequence

TNF α (TNF) [P01375\(Ala76Leu & Val77Leu\)](#)

MSTESMIRDVELAEEALPKKTGGPQGSRRCLFLSLFSFLIVAGATTFLCLLHFGVIGPQREEFPRDLSLISPLAQ
 LLRSSRTPSDKPVAVHVVANPQAEGLQWLNRRANALLANGVELRDNQLVVPSEGLYLIYSQVLFGQGCP
 STHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRETPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLDLDF
 AESGQVYFGIIAL*

Related Products

TNF:TNFR2:TNFR1	
H_TNFR2 Null Reporter Cell Line	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	
Anti-H_TNFRSF1B(TNFR2) hIgG1 Antibody(UC2.3.8)	Anti-TNFR1 hIgG1 Antibody(Atrosab)

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