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Product Sheet

H_TNFRSF9(4-1BB) Reporter Jurkat Cell line

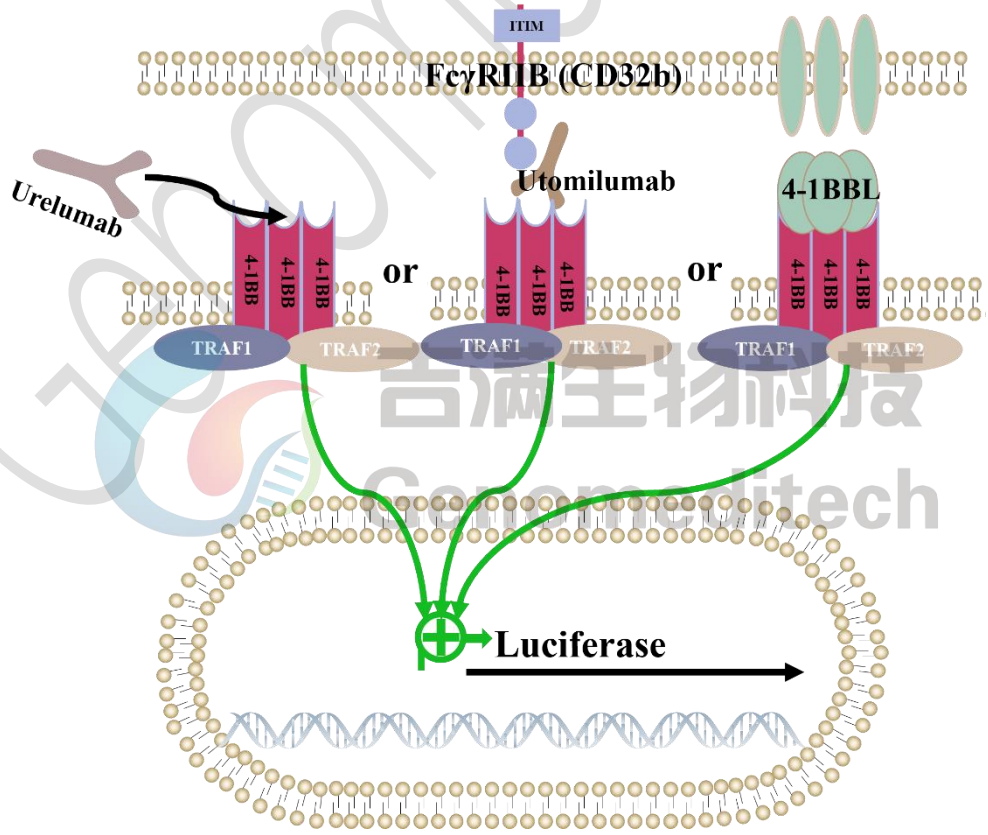
Catalog number: GM-C09468

Version 3.3.1.250718

4-1BB (CD137) is a protein in the TNF receptor superfamily, mainly found on T cells and NK cells. It is crucial for immune responses, enhancing T cell proliferation, survival, and function. Its activation is mediated by the ligand 4-1BBL (CD137L), expressed on activated dendritic cells, B cells, and some tumor cells. The signaling pathway involves TRAF and NF-κB, promoting cell survival and proliferation.

Activation of 4-1BB recruits TRAF2 and TRAF1, activating downstream pathways like NF-κB and MAPK, leading to cytokine production (e.g., IL-2 and IFN-γ). This enhances T cell immune responses, supporting anti-tumor immunity and infection clearance. Thus, 4-1BB is a key target in cancer immunotherapy, with potential as an immune checkpoint inhibitor.

H_TNFRSF9(4-1BB) Reporter Jurkat Cell line is a clonal stable cell line constructed using lentiviral technology, constitutive expression of the TNFRSF9(4-1BB) gene, along with signal-dependent expression of a luciferase reporter gene. When 4-1BBL binds to 4-1BB, it activates downstream signaling pathways, leading to the expression of luciferase. 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 TNFRSF9(4-1BB).



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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 Blastincidin+0.75 μg/mL Puromycin
Note	None
Freezing Medium	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	gibco/C11875500BT
Fetal Bovine Serum	ExCell/FSP500
Pen/Strep	Thermo/15140-122
Blasticidin	Genomeditech/GM-040404
Puromycin	Genomeditech/GM-040401
Anti-TNFRSF9(4-1BB) hIgG4 Reference Antibody (Urelbio)	Genomeditech/GM-87876MAB
4-1BBL	Sino Biological/15693-H01H
CD40L	Sino Biological/10239-H08E
H_FCGR2B(CD32B) CHO-K1 Cell Line	Genomeditech/GM-C16925
Anti-H_4-1BB hIgG2 Antibody(Utomilumab)	Genomeditech/GM-26840AB
GMOne-Step 2.0 Luciferase Reporter Gene Assay Kit	Genomeditech/GM-040513

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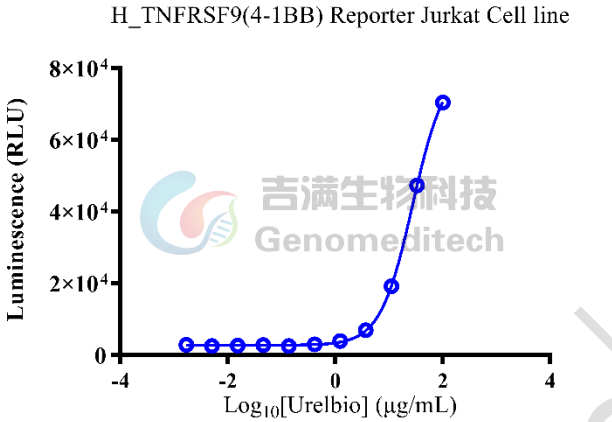
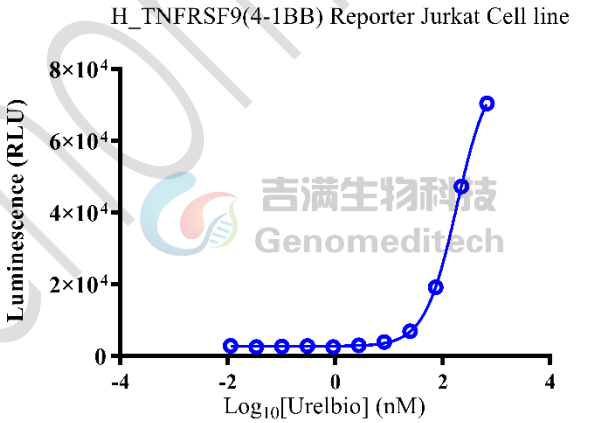
Figures



	H_TNFRSF9(4-1BB) Reporter Jurkat
EC50	27.56

Figure 1 | Response to Anti-TNFRSF9(4-1BB) hIgG4 Reference Antibody (Urelbio). The H_TNFRSF9(4-1BB) Reporter Jurkat Cell line (Cat. GM-C09468) at a concentration of 1E5 cells/well (96-well format) was stimulated with serial dilutions of Anti-TNFRSF9(4-1BB) hIgG4 Reference Antibody (Urelbio) (Cat. GM-87876MAB) in assay buffer (RPMI 1640 + 1% FBS + 1% P.S) for 7 hours. The firefly luciferase activity was measured using the GMOne-Step 2.0 Luciferase Reporter Gene Assay Kit (Cat. GM-040513). The maximum induction fold was approximately [27.1]. Data are shown by drug mass concentration.



	H_TNFRSF9(4-1BB) Reporter Jurkat
EC50	183.8

Figure 2 | Response to Anti-TNFRSF9(4-1BB) hIgG4 Reference Antibody (Urelbio). The H_TNFRSF9(4-1BB) Reporter Jurkat Cell line (Cat. GM-C09468) at a concentration of 1E5 cells/well (96-well format) was stimulated with serial dilutions of Anti-TNFRSF9(4-1BB) hIgG4 Reference Antibody (Urelbio) (Cat. GM-87876MAB) in assay buffer (RPMI 1640 + 1% FBS + 1% P.S) for 7 hours. The firefly luciferase activity was measured using the GMOne-Step 2.0 Luciferase Reporter Gene Assay Kit (Cat. GM-040513). The maximum induction fold was approximately [27.1]. Data are shown by drug molar concentration.

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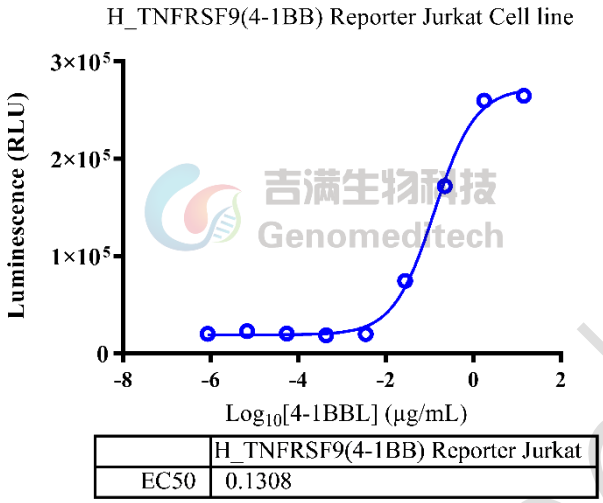
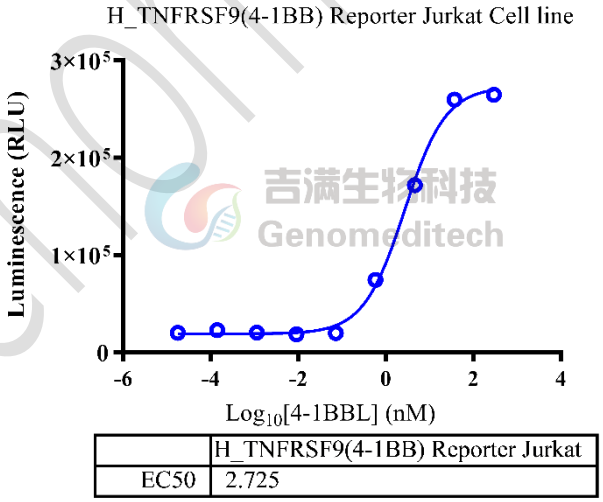


	H_TNFRSF9(4-1BB) Reporter Jurkat
EC50	0.1308

Figure 3 | Response to Recombinant Human 4-1BB Ligand/TNFSF9 Protein. The H_TNFRSF9(4-1BB) Reporter Jurkat Cell line (Cat. GM-C09468) at a concentration of 1E5 cells/well (96-well format) was stimulated with serial dilutions of Recombinant Human 4-1BB Ligand Protein (Recombinant Human 4-1BB Ligand/TNFSF9 Protein) in assay buffer (RPMI 1640 + 1% FBS + 1% P.S) for 7 hours. The firefly luciferase activity was measured using the GMOne-Step 2.0 Luciferase Reporter Gene Assay Kit (Cat. GM-040513). The maximum induction fold was approximately [10.8]. Data are shown by drug mass concentration.



	H_TNFRSF9(4-1BB) Reporter Jurkat
EC50	2.725

Figure 4 | Response to Recombinant Human 4-1BB Ligand/TNFSF9 Protein. The H_TNFRSF9(4-1BB) Reporter Jurkat Cell line (Cat. GM-C09468) at a concentration of 1E5 cells/well (96-well format) was stimulated with serial dilutions of Recombinant Human 4-1BB Ligand Protein (Recombinant Human 4-1BB Ligand/TNFSF9 Protein) in assay buffer (RPMI 1640 + 1% FBS + 1% P.S) for 7 hours. The firefly luciferase activity was measured using the GMOne-Step 2.0 Luciferase Reporter Gene Assay Kit (Cat. GM-040513). The maximum induction fold was approximately [10.8]. Data are shown by drug molar concentration.

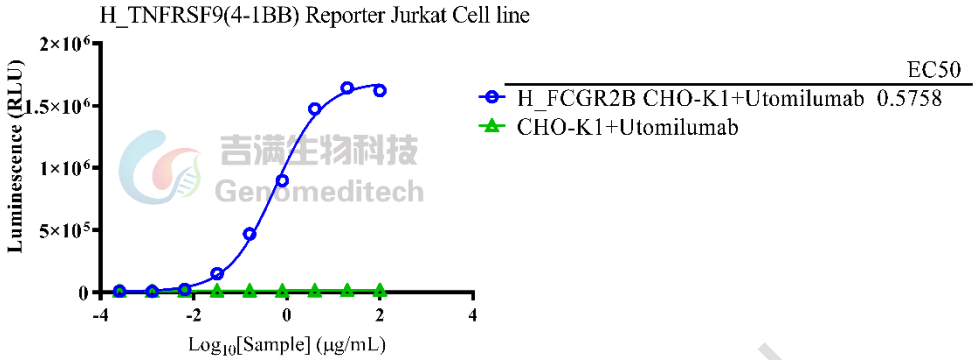


Figure 5 | Response to Anti-H_4-1BB hIgG2 Antibody(Utomilumab). H_FCGR2B CHO-K1 Cell Line (Cat. GM-C21996) and CHO-K1 Cell Line were seeded at a density of 1.5E4 cells/well in a 96-well plate and incubated overnight. The next day, serial dilutions of the Anti-H_4-1BB hIgG2 Antibody(Utomilumab) (Cat. GM-26840AB) were incubated with 1E5 cells/well of the H_TNFRSF9(4-1BB) Reporter Jurkat Cell line (Cat. GM-C09468) in a 96-well plate, and then added to the pre-seeded cells. The mixture was incubated for an additional 6 hours. Firefly luciferase activity is then measured using the GMOne-Step 2.0 Luciferase Reporter Gene Assay Kit (Cat. GM-040513). The results indicated maximum blocking folds of approximately [193.3]. Data are shown by drug mass concentration.

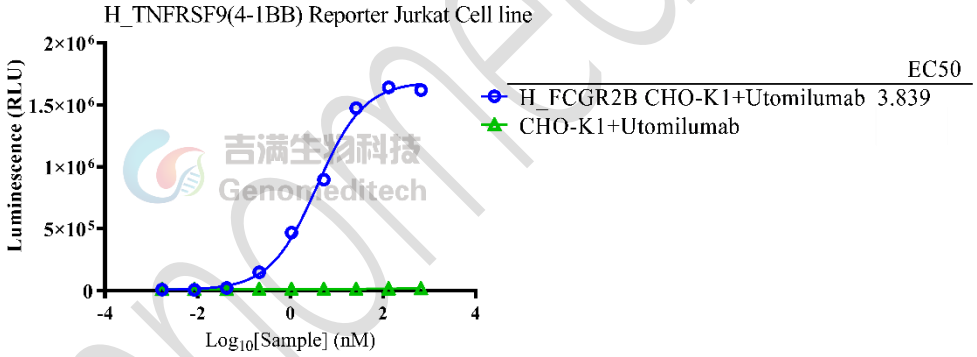


Figure 6 | Response to Anti-H_4-1BB hIgG2 Antibody(Utomilumab). H_FCGR2B CHO-K1 Cell Line (Cat. GM-C21996) and CHO-K1 Cell Line were seeded at a density of 1.5E4 cells/well in a 96-well plate and incubated overnight. The next day, serial dilutions of the Anti-H_4-1BB hIgG2 Antibody(Utomilumab) (Cat. GM-26840AB) were incubated with 1E5 cells/well of the H_TNFRSF9(4-1BB) Reporter Jurkat Cell line (Cat. GM-C09468) in a 96-well plate, and then added to the pre-seeded cells. The mixture was incubated for an additional 6 hours. Firefly luciferase activity is then measured using the GMOne-Step 2.0 Luciferase Reporter Gene Assay Kit (Cat. GM-040513). The results indicated maximum blocking folds of approximately [193.3]. Data are shown by drug molar concentration.

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: 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 Blastincidin+0.75 μg/mL Puromycin

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

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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

4-1BB	
H_TNFRSF9(4-1BB) Reporter 293 Cell line	Cynomolgus_TNFRSF9(4-1BB) CHO-K1 Cell Line
H_TNFRSF9(4-1BB) CHO-K1 Cell Line	
Anti-H_4-1BB hIgG2 Antibody(Utomilumab)	Anti-H_TNFRSF9(4-1BB) hIgG4 Antibody(Urelumab)
Anti-TNFRSF9(4-1BB) hIgG4 Reference Antibody (Urelbio)	
CD3	
H_CD3D CD3E KO Jurkat Cell Line	Jurkat CD3-BsAb Reporter Cell Line
Cynomolgus_CD3 HEK-293 Cell Line	Cynomolgus_CD3E(Membrane Bound ECD) CHO-K1 Cell Line
H_CD3 CHO-K1 Cell Line	H_CD3 HEK-293 Cell Line
H_CD3E(Membrane Bound ECD) CHO-K1 Cell Line	Mouse_CD3 HEK-293 Cell Line
Anti-CD3 epsilon hIgG1 Antibody [OKT-3 (muromonab)]	Anti-CD3 hIgG1 Antibody(CH2527)
Anti-mouse CD3ε mIgG2a Antibody(145-2C11)	

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