Genomeditech (Shanghai) Co.,Ltd.
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Product Sheet

H_TNFRSF9(4-1BB) Reporter 293 Cell line

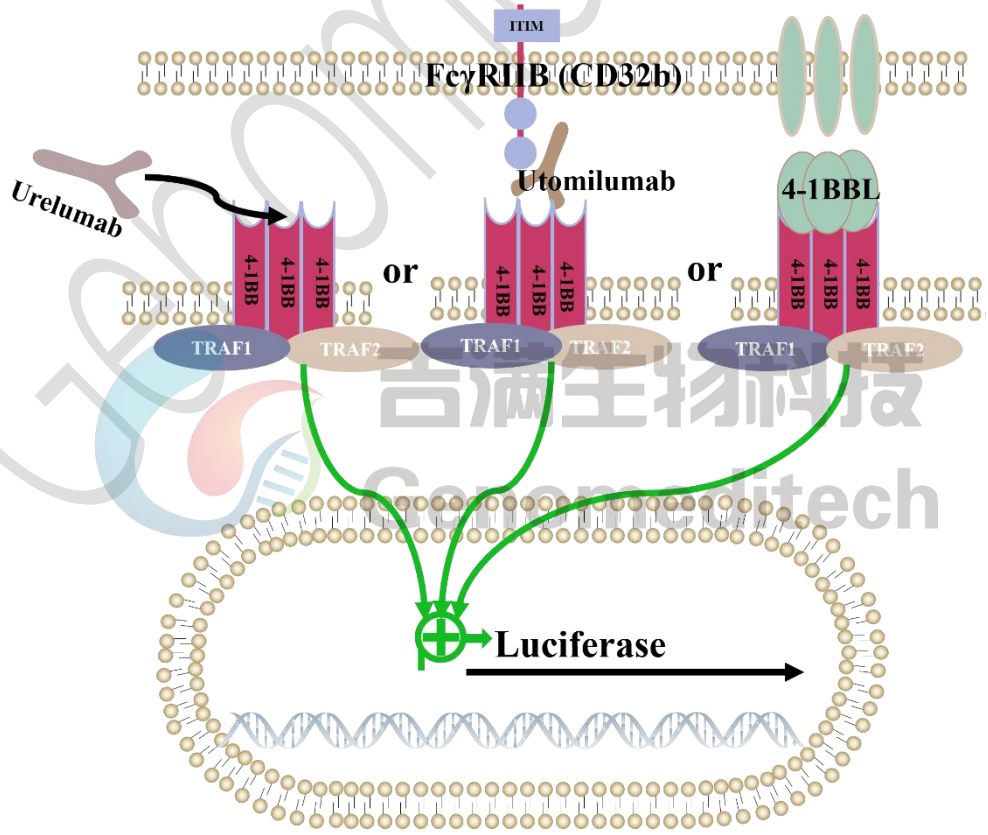
Catalog number: GM-C04832

Version 3.3.1.260817

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 293 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	DMEM+10% FBS+1% P.S
Growth medium	DMEM+10% FBS+1% P.S+4 μg/mL Blasticidin+0.75 μg/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.
DMEM	Gibco/C11995500BT
Fetal Bovine Serum	ExCell/FSP500
Pen/Strep	Thermo/15140-122
Blasticidin	Genomeditech/GM-040404
Puromycin	Genomeditech/GM-040401
Urelumab (anti-TNFRSF9)	Aladdin/Ab170654
Anti-H_4-1BB hIgG2 Antibody(Utomilumab)	Genomeditech/GM-26840AB
H_FCGR2B(CD32B) CHO-K1 Cell Line	Genomeditech/GM-C16925
GMOne-Step 2.0 Luciferase Reporter Gene Assay Kit	Genomeditech/GM-040513
Human 4-1BB Ligand/TNFSF9 Trimer Protein	kactus/BBL-HM141

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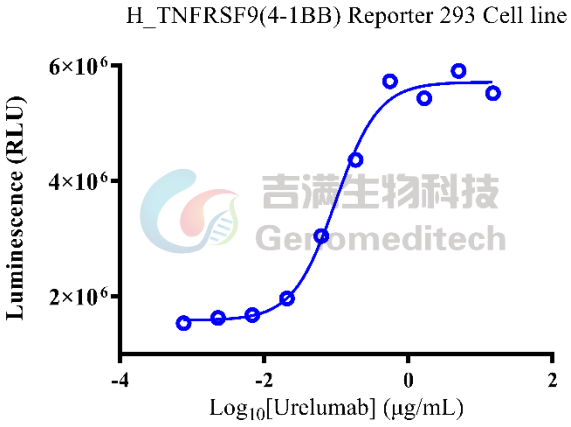
Figures



	H_TNFRSF9(4-1BB) Reporter 293
EC50	0.09817

Figure 1 | Response to Urelumab. The H_TNFRSF9(4-1BB) Reporter 293 Cell line (Cat. GM-C04832) at a concentration of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of Urelumab (anti-TNFRSF9) (Aladdin/Ab170654) in assay buffer (DMEM + 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 [3.5].

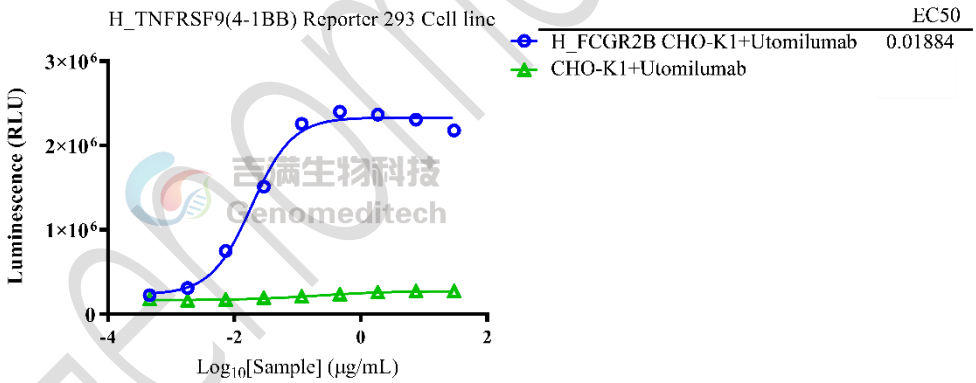
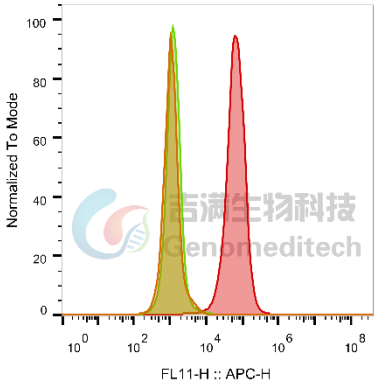
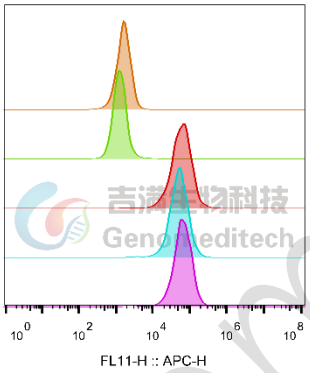


Figure 2 | Response to Anti-H_4-1BB hIgG2 Antibody(Utomilumab). H_FCGR2B(CD32B) CHO-K1 Cell Line (Cat. GM-C16925) and CHO-K1 Cell Line were seeded at a density of 1E4 cells/well in a 96-well plate and incubated overnight. The next day, serial dilutions of the Anti-H_4-1BB hIgG2 Antibody(Utomilumab) (Cat. GM-26840AB) were incubated with 1.5E4 cells/well of the H_TNFRSF9(4-1BB) Reporter 293 Cell line (Cat. GM-C04832) 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 Luciferase Reporter Assay Kit (Genomeditech). The results indicated maximum blocking folds of approximately [10.4]. Data are shown by drug mass concentration.



SampleID	Geometric Mean : FL11-H
293 anti-4-1BB+APC-2nd Ab	1101
H_TNFRSF9(4-1BB) Reporter 293 H_IgG+APC-2nd Ab	1260
H_TNFRSF9(4-1BB) Reporter 293 anti-4-1BB+APC-2nd Ab	63596

Figure 3 | H_TNFRSF9(4-1BB) Reporter 293 Cell line (Cat. GM-C04832) was determined by flow cytometry using Anti-TNFRSF9(4-1BB) hIgG4 Reference Antibody.



SampleID	Geometric Mean : FL11-H
293 anti-4-1BB+APC-2nd Ab	1618
H_TNFRSF9(4-1BB) Reporter 293 H_IgG+APC-2nd Ab	1314
H_TNFRSF9(4-1BB) Reporter 293 anti-4-1BB+APC-2nd Ab CP3	57546
H_TNFRSF9(4-1BB) Reporter 293 anti-4-1BB+APC-2nd Ab CP13	49097
H_TNFRSF9(4-1BB) Reporter 293 anti-4-1BB+APC-2nd Ab CP23	61550

Figure 4 | The passage stability of the H_TNFRSF9(4-1BB) Reporter 293 Cell line (Cat. GM-C04832) was determined by flow cytometry using Anti-TNFRSF9(4-1BB) hIgG4 Reference Antibody.

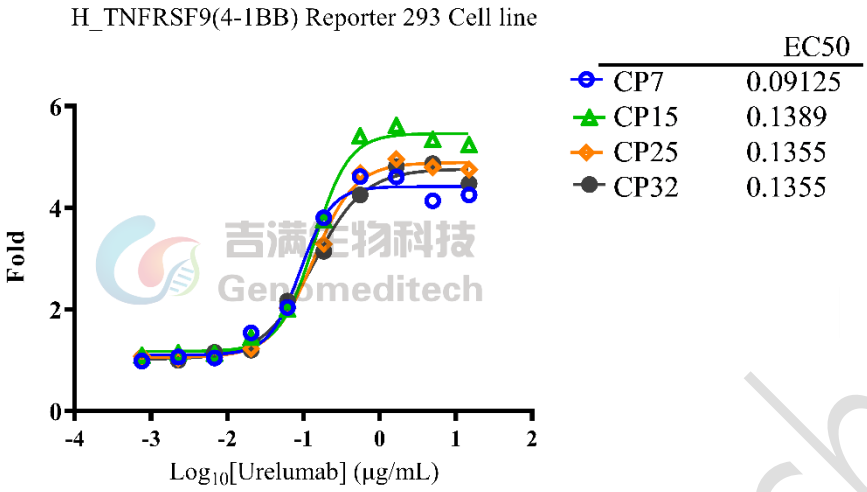
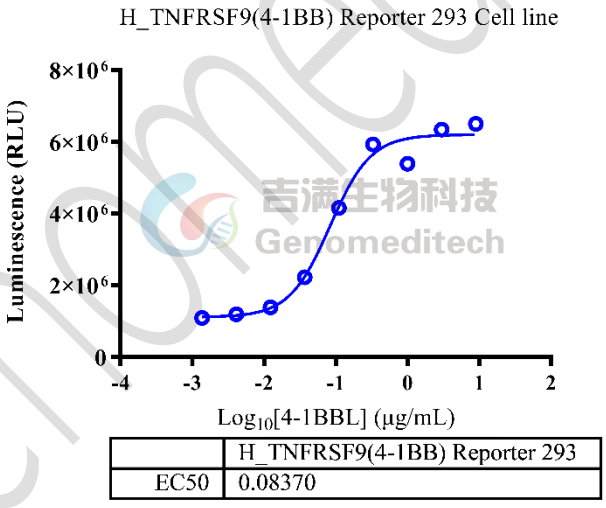


Figure 5 | The passage stability of response to Urelumab. The passages 7, 15, 25, and 32 of H_TNFRSF9(4-1BB) Reporter 293 Cell Line (Cat. GM-C04832) at a density of 1.5E4 cells/well (96-well format) were stimulated with serial dilutions of Urelumab (anti-TNFRSF9) (Aladdin/Ab170654) in assay buffer (DMEM+1% FBS+1% P.S) for 6 hours. The firefly luciferase activity was measured using the Luciferase Reporter Assay Kit (Genomeditech).



	H_TNFRSF9(4-1BB) Reporter 293
EC50	0.08370

Figure 6 | Response to Human 4-1BB Ligand/TNFSF9 Trimer Protein. The H_TNFRSF9(4-1BB) Reporter 293 Cell line (Cat. GM-C04832) at a density of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of Human 4-1BB Ligand/TNFSF9 Trimer Protein (kactus/BBL-HM141) in assay buffer (DMEM + 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 [6.0]. Data are shown by drug mass concentration.

Cell Recovery

Recovery Medium: DMEM+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 recovery medium. And dispense into appropriate culture dishes.
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: DMEM+10% FBS+1% P.S+4 μg/mL Blasticidin+0.75 μg/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.

a) Subculturing is necessary when the cell density reaches 80%. It is recommended to perform subculturing at a ratio of 1:3 to 1:4 every 2-3 days. Ensure that the density does not exceed 80%, as overcrowding can lead to reduced viability due to compression.
b) Remove and discard culture medium.
c) Briefly rinse the cell layer with PBS to remove all traces of serum that contains trypsin inhibitor.
d) 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 30 to 60 seconds at 37°C).
e) 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.
f) Add 2.0 mL of growth medium to mix well and aspirate cells by gently pipetting.
g) 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 1:3 - 1:4 is recommended

Medium Renewal: Every 2 to 3 days

Notes

a) Upon initial thawing, a higher number of dead cells is observed, which is a normal phenomenon. Significant improvement is seen after adaptation. Once the cells reach a stable state, the number of dead cells decreases after subculturing and the cell growth rate becomes stable.

b) Ensure that the cell density does not exceed 80%, as overcrowding may lead to reduced viability due to compression.

Related Products

4-1BB	
H_TNFRSF9(4-1BB) Reporter Jurkat Cell Line	H_TNFRSF9(4-1BB) Reporter Jurkat Cell Line (Low Expression)
Cynomolgus_TNFRSF9(4-1BB) CHO-K1 Cell Line	H_TNFRSF9(4-1BB) CHO-K1 Cell Line
Anti-H_4-1BB hIgG2 Antibody(Utomilumab)	
Cynomolgus TNFRSF9(4-1BB) Protein; His Tag	Human TNFRSF9(4-1BB) Protein; His Tag
Human TNFSF9(4-1BBL) Protein; hFc Tag	
CD3	
Cynomolgus_CD3D CD3E Reporter Jurkat(CD3DE KO) 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_CD3(TCR V2) CHO-K1 Cell Line	H_CD3(TCR V2) HEK-293 Cell Line
H_CD3(TCRαβ delta V) HEK-293 Cell Line	H_CD3D CD3E KO Jurkat Cell Line
H_CD3E KO Jurkat Cell Line	H_CD3E(Membrane Bound ECD) CHO-K1 Cell Line
Mouse_CD3 HEK-293 Cell Line	
Anti-CD19×CD3 hIgG1 Antibody[PIT-565(CD58 K34A)]	Anti-CD3 epsilon hIgG1 Antibody [OKT-3 (muromonab)]
Anti-CD3 hIgG1 Antibody(CH2527)	Anti-CD3×CD20 hIgG1 Bispecific Antibody (Epcobio)
Anti-CD3×FCRL5 hIgG1 Bispecific Antibody(cevostamab)	Anti-CD3E×BCMA hIgG4 Reference Antibody (Tecbio)
Anti-CD3E×DLL3 hIgG1 Bispecific Antibody(Tarlabio)	Anti-CD3E×MUC17 hIgG1 Bispecific Antibody(Vepsitbio)
Anti-mouse CD3ε mIgG2a Antibody(145-2C11)	

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