Genomeditech (Shanghai) Co.,Ltd.
Order: +86 021-68455258/50432826/50432825
Toll-free: +86 400 627 9288
Email: service@genomeditech.com

Product Sheet

H_CD28 Reporter Jurkat Cell Line

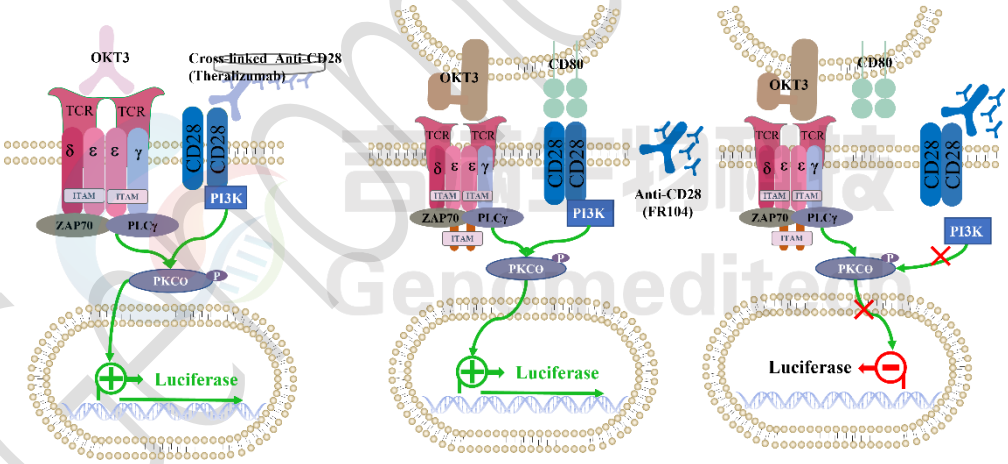
Catalog number: GM-C25147

Version 3.3.1.250108

CD28 is a key costimulatory molecule on T cells, expressed on most CD4+ and some CD8+ T cells. It binds to ligands B7-1 (CD80) or B7-2 (CD86) to provide signals that enhance T cell activation, proliferation, memory maintenance, cytokine secretion (e.g., IL-2), and anti-tumor immunity.

CD28 signaling enhances T cell activation by providing a second signal after TCR recognizes an antigen. It activates PI3K/Akt and MAPK pathways, promoting IL-2 expression, cell survival, and metabolism. CD28 also regulates NF-Kb, AP-1, and NFAT transcription factors. Without CD28 signaling, T cells may become anergic and fail to mount an immune response.

H_CD28 Reporter Jurkat Cell Line is a clonal stable Jurkat cell line constructed using lentiviral technology, endogenously expression of the TCR and CD28 gene, along with signal-dependent expression of a luciferase reporter gene. When OKT3 and CD80 binds to TCR and CD28, 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 research of drugs related to CD28.



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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
Clear Flat-Bottom Immuno Nonsterile 96-Well Plates	Thermo/442404
H_CD80 aAPC CHO-K1 Cell Line	Genomeditech/GM-C24688
Anti-H_CD28 hIgG4 Antibody(Theralizumab)	Genomeditech/GM-27197AB
Anti-CD28 hIgG4 Antibody(FR104)	Genomeditech/GM-52277AB
Anti-CD3 epsilon hIgG1 Antibody [OKT-3 (muromonab)]	Genomeditech/GM-51478AB
GMOne-Step Luciferase Reporter Gene Assay Kit	Genomeditech/GM-040503

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Figures

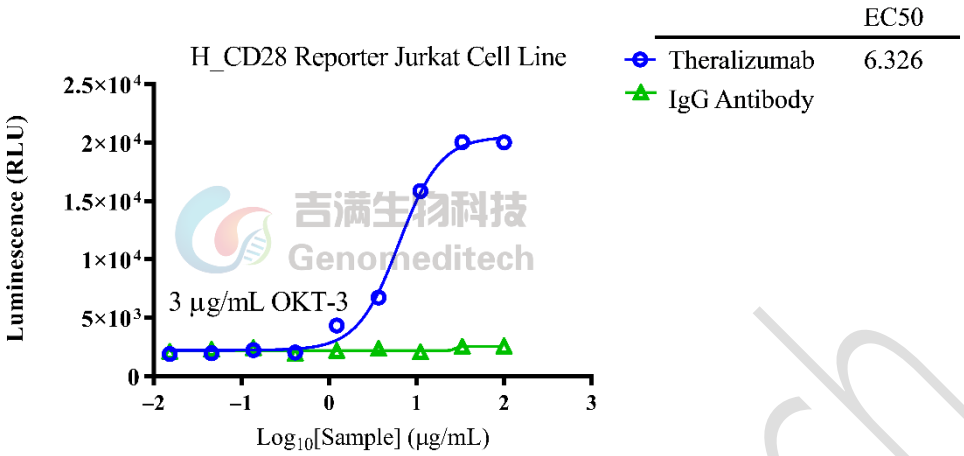


Figure 1 | Response to OKT-3 and Anti-H_CD28 hIgG4 Antibody. The wells(96-well format) were coated overnight with serial dilutions Anti-H_CD28 hIgG4 Antibody(Theralizumab) (Cat. GM-27197AB) in assay buffer (RPMI 1640 + 1% FBS + 1% P.S) . After coating, H_CD28 Reporter Jurkat Cell Line (Cat. GM-C25147) at a concentration of 1E5 cells/well and 300 ng/well Anti-CD3 epsilon Antibody [OKT-3 (muromonab)] (Cat. GM-51478AB) were added and incubated 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 [10.5]. Data are shown by drug mass concentration.

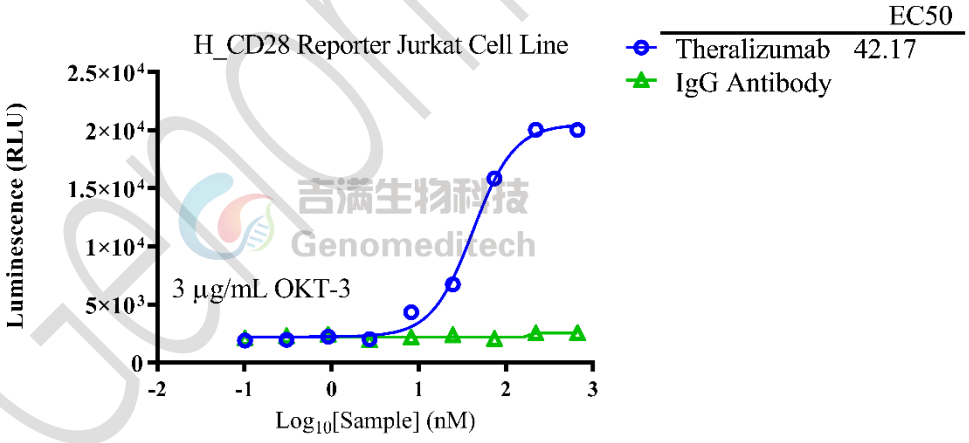
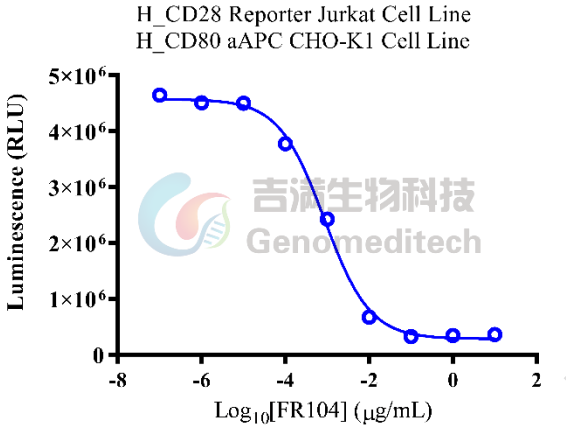
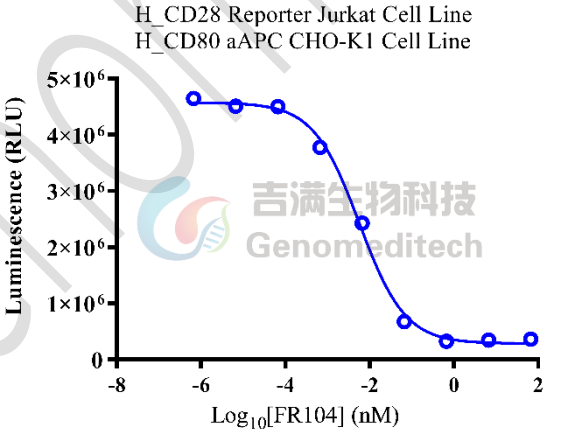


Figure 2 | Response to OKT-3 and Anti-H_CD28 hIgG4 Antibody. The wells(96-well format) were coated overnight with serial dilutions Anti-H_CD28 hIgG4 Antibody(Theralizumab) (Cat. GM-27197AB) in assay buffer (RPMI 1640 + 1% FBS + 1% P.S) . After coating, H_CD28 Reporter Jurkat Cell Line (Cat. GM-C25147) at a concentration of 1E5 cells/well and 300 ng/well Anti-CD3 epsilon Antibody [OKT-3 (muromonab)] (Cat. GM-51478AB) were added and incubated 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 [10.5]. Data are shown by drug molar concentration.



	H_CD28 Reporter Jurkat Cell Line
IC50	0.0008563

Figure 3 | Response to Anti-CD28 hIgG4 Antibody(FR104). H_CD80 aAPC CHO-K1 Cell Line (Cat. GM-C24688) was seeded at a density of 2E4 cells per well in a 96-well plate and incubated overnight. The next day, serial dilutions of the Anti-CD28 hIgG4 Antibody(FR104) (Cat. GM-52277AB) were incubated with 1E5 cells/well of the H_CD28 Reporter Jurkat Cell Line (Cat. GM-C25147) 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 is then measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). The results indicated a maximum blocking fold of approximately [12.9]. Data are shown by drug mass concentration.



	H_CD28 Reporter Jurkat Cell Line
IC50	0.005709

Figure 4 | Response to Anti-CD28 hIgG4 Antibody(FR104). H_CD80 aAPC CHO-K1 Cell Line (Cat. GM-C24688) was seeded at a density of 2E4 cells per well in a 96-well plate and incubated overnight. The next day, serial dilutions of the Anti-CD28 hIgG4 Antibody(FR104) (Cat. GM-52277AB) were incubated with 1E5 cells/well of the H_CD28 Reporter Jurkat Cell Line (Cat. GM-C25147) 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 is then measured using the GMOne-

Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). The results indicated a maximum blocking fold of approximately [12.9]. Data are shown by drug molar concentration.

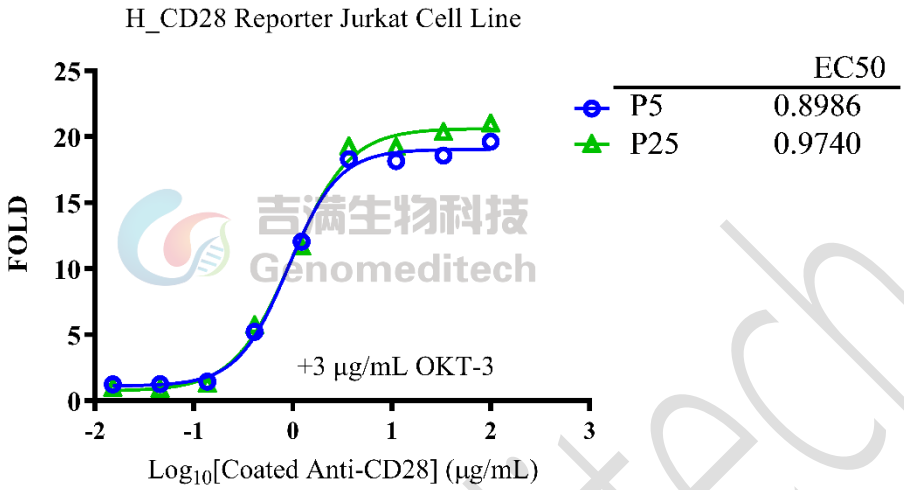
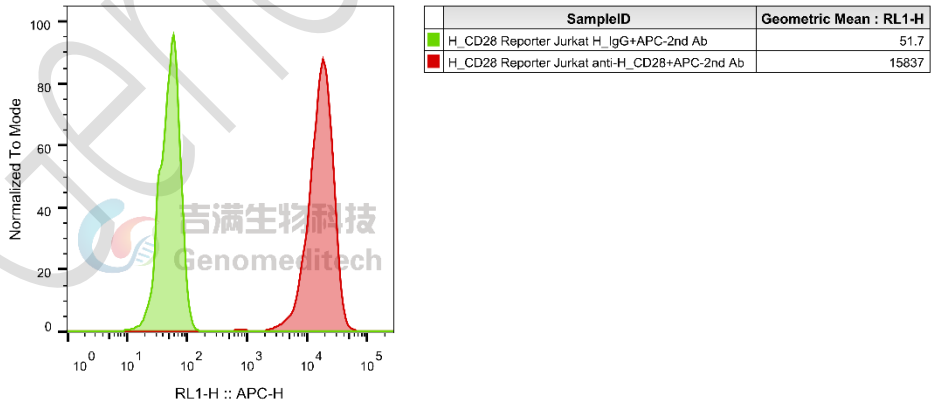


Figure 5 | The passage stability of response to OKT-3 and Anti-H_CD28 hIgG4 Antibody. The wells(96-well format) were coated overnight with serial dilutions Anti-H_CD28 hIgG4 Antibody(Theralizumab) (Cat. GM-27197AB) in assay buffer (RPMI 1640 + 1% FBS + 1% P.S) . After coating, the passage 5 and 25 of H_CD28 Reporter Jurkat Cell Line (Cat. GM-C25147) at a concentration of 1E5 cells/well and 300 ng/well Anti-CD3 epsilon Antibody [OKT-3 (muromonab)] (Cat. GM-51478AB) were added and incubated for 7 hours. 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.



SampleID	Geometric Mean : RL1-H
H_CD28 Reporter Jurkat H_IgG+APC-2nd Ab	51.7
H_CD28 Reporter Jurkat anti-H_CD28+APC-2nd Ab	15837

Figure 6 | H_CD28 Reporter Jurkat Cell Line (Cat. GM-C25147) was determined by flow cytometry using Anti-H_CD28 hIgG4 Antibody(Theralizumab) (Cat. GM-27197AB).

Cell Recovery

Recovery Medium: RPMI 1640+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.

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

CD28	
Cynomolgus_CD28 CHO-K1 Cell Line	H_CD28 CHO-K1 Cell Line
H_CD28 HEK-293 Cell Line	
Anti-CD28 hIgG4 Antibody(FR104)	Anti-H_CD28 hIgG4 Antibody(Theralizumab)
Anti-mouse CD28 Syrian Hamster IgG2 Antibody(37. 51)	
CD3	
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)

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