Genomeditech (Shanghai) Co.,Ltd.
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Product Sheet

CD3-CD2-tsAb Reporter Jurkat(CD58 KO) Cell Line

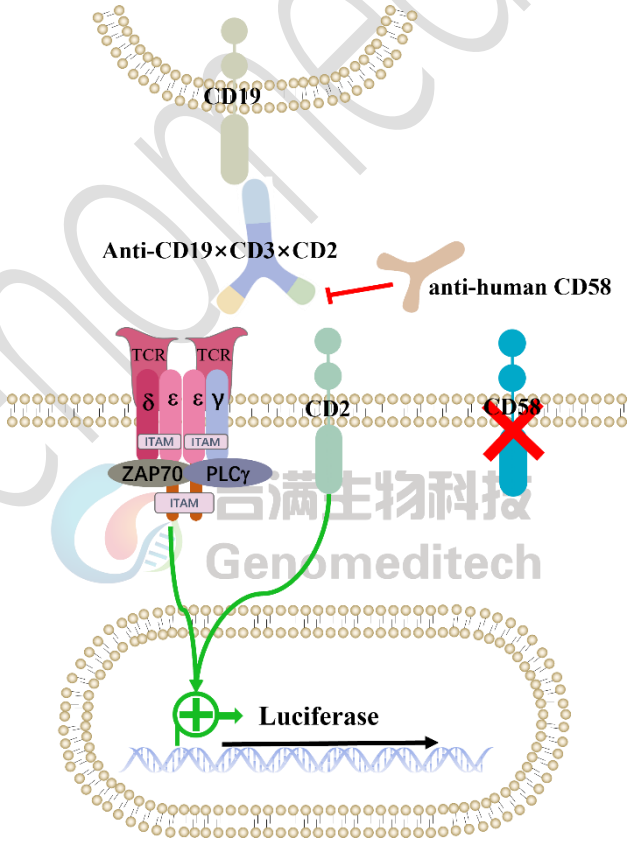
Catalog number: GM-C39956

Version 3.3.1.260701

CD3 is a core component of the T cell receptor (TCR) complex, while CD2 primarily mediates adhesion and costimulatory signaling; together, they enhance T cell responses.

PIT565, developed by Novartis, is a trispecific antibody that simultaneously binds CD3 and CD2 on T cells and CD19 on B cells; by recruiting and activating T cells to eliminate aberrant B cells, it shows promising potential in diseases characterized by B cell abnormalities, such as systemic lupus erythematosus and rheumatoid arthritis.

CD3-CD2-tsAb Reporter Jurkat(CD58 KO) Cell Line is a clonal stable Jurkat cell line constructed using non-viral vectors, knockout Endogenous CD58 gene, along with signal-dependent expression of a luciferase reporter gene. In this cell line, the reporter is expressed only when CD3 and CD2 pathways are activated, and the luciferase signal intensity directly reflects the magnitude of pathway activation. Therefore, this model provides a highly sensitive and specific platform for in vitro functional evaluation and mechanistic studies of CD3-CD2-tsAb trispecific antibodies.



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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	Jurkat cells are classified as BSL-1 by ATCC and BSL-2 by ECACC, constructed using non-viral vectors; please choose appropriate biosafety measures according to local regulations.
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
H_CD19 CHO-K1 Cell line	Genomeditech/GM-C19025
Anti-CD19×CD3×CD2 hIgG1 Reference Antibody(PIT-565)	Genomeditech/GM-87914MAB
Anti-CD19×CD3 hIgG1 Antibody[PIT-565(CD58 K34A)]	Genomeditech/GM-87921AB
Anti-CD3 hIgG1 Antibody(CH2527)	Genomeditech/GM-33037AB
Anti-CD3 hIgG1 Antibody(CH2527)	Genomeditech/GM-33037AB
Anti-CD2 hIgG1 Antibody(BTI-322)	Genomeditech/GM-79929AB
GMOne-Step 2.0 Luciferase Reporter Gene Assay Kit	Genomeditech/GM-040513

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Figures

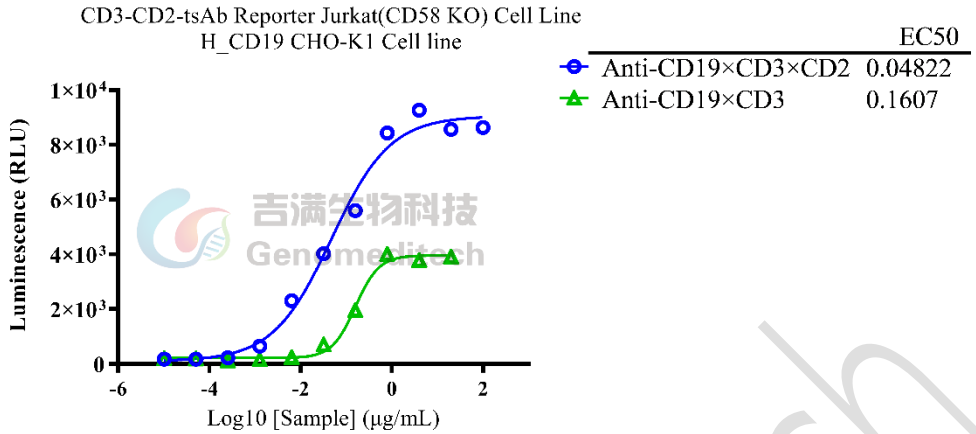


Figure 1 | Response to Anti-CD19×CD3×CD2. The CD3-CD2-tsAb Reporter Jurkat(CD58 KO) Cell Line(Cat. GM-C39956) at a concentration of 1E5 cells/well was co-cultured with H_CD19 CHO-K1 Cell line (Cat. GM-C19025) at a concentration of 1E4 cells/well, in the presence of a Serial dilutions of Anti-CD19×CD3×CD2 (Cat. GM-87914MAB) and Anti-CD19×CD3 (Cat. GM-87921AB) were applied in separate wells, respectively. The firefly luciferase activity was measured using the Luciferase Reporter Assay Kit (Genomeditech). The maximum induction folds were approximately [48.3] and [20.9], respectively. Data are shown by drug mass concentration.

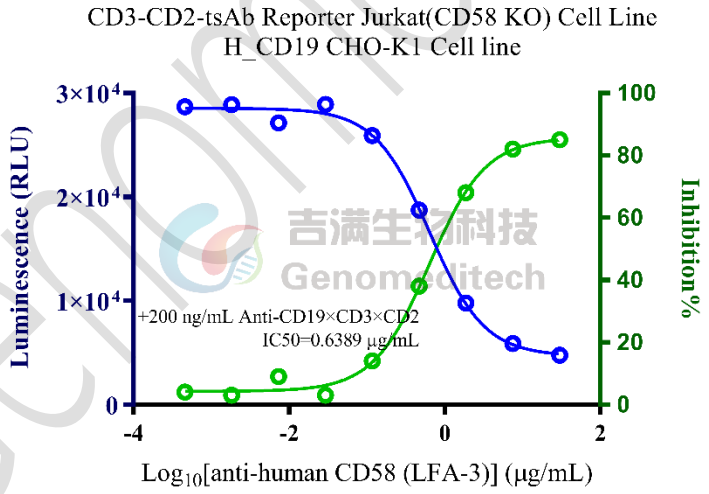
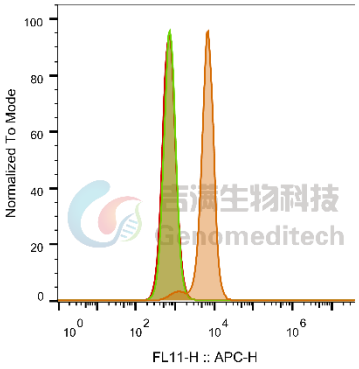
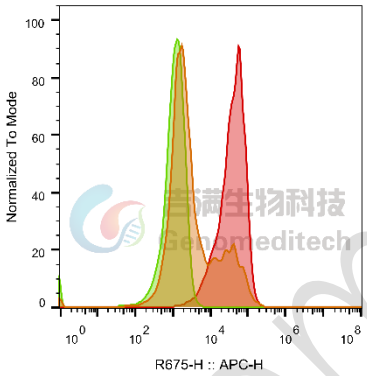


Figure 2 | Inhibition of Anti-CD19×CD3×CD2-induced reporter activity by anti-human CD58 (LFA-3) antibody. H_CD19 CHO-K1 cells (Cat. GM-C19025) were seeded at a density of 1E4 cells per well and incubated overnight. The next day, serial dilutions of the anti-human CD58 (LFA-3) antibody (BioLegend/330902) were incubated with 20 ng/well of Anti-CD19×CD3×CD2 (Cat. GM-87914MAB) for 1 hour. The mixture was then added to the pre-seeded H_CD19 CHO-K1 cells, followed by addition of CD3-CD2-tsAb Reporter Jurkat (CD58 KO) cells (Cat. GM-C39956), and incubated for 6 h. Firefly luciferase activity was measured using the Luciferase Reporter Assay Kit (Genomeditech) (left Y-axis, relative luminescence units, RLU), with inhibition percentages shown on the right Y-axis. Data are shown by drug mass concentration.



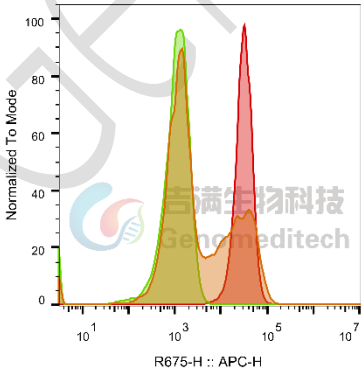
SampleID	Geometric Mean : FL11-H
Jurkat anti-CD58+APC-2nd Ab	6326
CD3 CD2 tsAb Reporter Jurkat(CD58 KO) Mouse_IgG+APC-2nd Ab	750
CD3 CD2 tsAb Reporter Jurkat(CD58 KO) anti-CD58+APC-2nd Ab	720

Figure 3 | CD3-CD2-tsAb Reporter Jurkat(CD58 KO) Cell Line(Cat. GM-C39956) was determined by flow cytometry using Purified anti-human CD58 (LFA-3) Antibody (Biolegend/330902).



SampleID	Geometric Mean : R675-H
Jurkat anti-CD3+APC-2nd Ab	3304
CD3-CD2-tsAb Reporter Jurkat(CD58 KO) H_IgG+APC-2nd Ab	1011
CD3-CD2-tsAb Reporter Jurkat(CD58 KO) anti-CD3+APC-2nd Ab	37021

Figure 4 | CD3-CD2-tsAb Reporter Jurkat(CD58 KO) Cell Line(Cat. GM-C39956) was determined by flow cytometry using Anti-CD3 hIgG1 Antibody(CH2527) (Cat. GM-33037AB).



SampleID	Geometric Mean : R675-H
Jurkat anti-CD2+APC-2nd Ab	3305
Jurkat H_IgG+APC-2nd Ab	952
CD3-CD2-tsAb Reporter Jurkat(CD58 KO) anti-CD2+APC-2nd Ab	30743

Figure 5 | CD3-CD2-tsAb Reporter Jurkat(CD58 KO) Cell Line(Cat. GM-C39956) was determined by flow cytometry using Anti-CD2 hIgG1 Antibody(BTI-322) (Cat. GM-79929AB).

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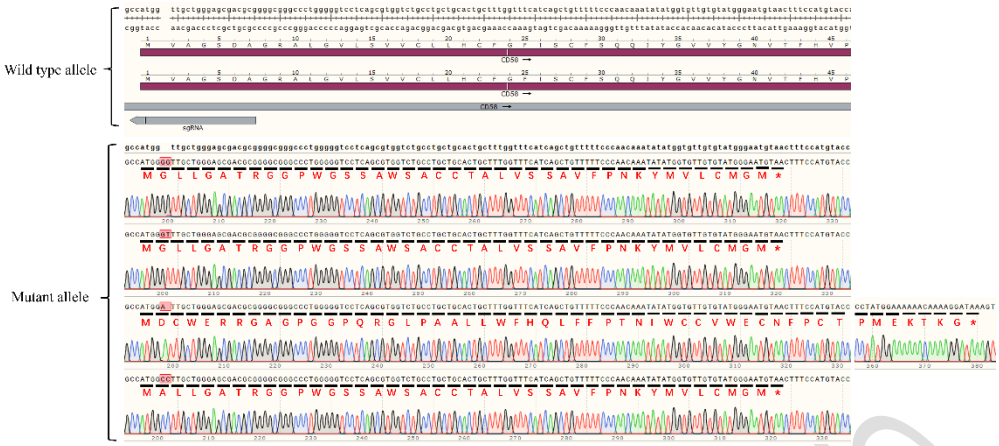

Figure 6 | The Sanger sequencing of the CD3-CD2-tsAb Reporter Jurkat(CD58 KO) Cell Line showed successful knockout of CD58.

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

CD19	
H_CD19 KO Raji Cell Line	Cynomolgus_CD19 CHO-K1 Cell Line
Cynomolgus_CD19 HEK-293 Cell Line	H_CD19 CHO-K1 Cell line
H_CD19 HEK-293 Cell Line	Mouse_CD19 CHO-K1 Cell Line
Anti-CD19 hIgG1 Reference Antibody (Loncbio)	Anti-CD19×CD3 hIgG1 Antibody[PIT-565(CD58 K34A)]
Anti-H_CD19 hIgG1/hIgG2 Antibody(Tafasitamab)	
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_CD3(TCR V2) CHO-K1 Cell Line
H_CD3(TCR V2) 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)	
CD2	
H_CD2 KO Jurkat(CD3-) Cell Line	Cynomolgus_CD2 CHO-K1 Cell Line
H_CD2 CHO-K1 Cell Line	
Anti-CD19×CD3 hIgG1 Antibody[PIT-565(CD58 K34A)]	Anti-CD2 hIgG1 Antibody(BTI-322)

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