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

Product Sheet

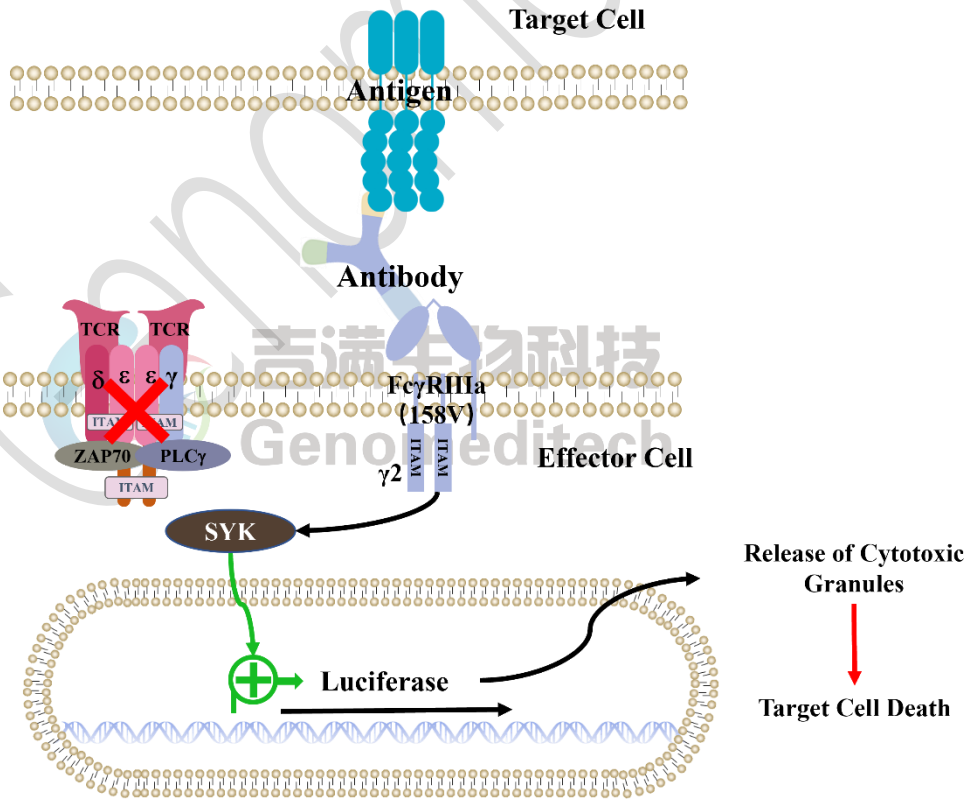
ADCC FcγRIIIa(158V) Reporter Jurkat(CD3 KO) Cell Line

Catalog number: GM-C39293

Version 3.3.1.250808

ADCC, or antibody-dependent cell-mediated cytotoxicity, refers to the process by which immune cells expressing Fc receptors directly kill target cells that specifically bind to antibodies through recognition of the Fc region of the antibodies. Nowadays, the mechanism of ADCC is used to detect and evaluate the efficacy of antibodies or target cells. Antibodies bind to target antigens on the cell surface. If the Fc region of the antibody simultaneously binds to the FcγRIIIa receptor on the surface of effector cells (primarily natural killer cells), the two types of cells undergo multiple cross-linking, leading to the activation of the ADCC signaling pathway. The 158V variant is a polymorphism where valine (V) replaces phenylalanine (F) at position 158, and the 158V mutation exhibits high affinity.

ADCC FcγRIIIa(158V) Reporter Jurkat(CD3 KO) Cell Line is a clonal stable Jurkat cell line that knockout Endogenous CD3 complex and constitutive expression of human FcγRIIIa(158V) gene, along with signal-dependent expression of a luciferase reporter gene. This design enables cells to specifically respond to receptor-mediated ADCC activation signals. By measuring the bioluminescence intensity of luciferase within the cells, the antibody activity of the ADCC pathway can be accurately quantified. This system features strong signals and low background, making it particularly suitable for functional activity and mechanism validation studies of bispecific T cell engager (TCE) drugs.



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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+400 μg/mL G418+200 μg/mL Hygromycin+0.75 μg/mL Puromycin
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	gibco/C11875500BT
Fetal Bovine Serum	ExCell/FSP500
Pen/Strep	Thermo/15140-122
Blasticidin	Genomeditech/GM-040404
G418	Genomeditech/GM-040402
Hygromycin	Genomeditech/GM-040403
Puromycin	Genomeditech/GM-040401
Raji Cell Line	Genomeditech/GM-C19100
ADCC FcγRIIIa(158V) Jurkat Effector Cell Line	Genomeditech/GM-C05619
Anti-CD3×CD20 hIgG1 Bispecific Antibody (Epcobio)	Genomeditech/GM-88130MAB
Anti-CD20 hIgG1 Reference Antibody (Ocrebio)	Genomeditech/GM-87986MAB
PE anti-human CD16 Antibody	BioLegend/302007
Abflo® 488 Rabbit anti-Human/Monkey CD3 mAb	Abclonal/A26283

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Figures

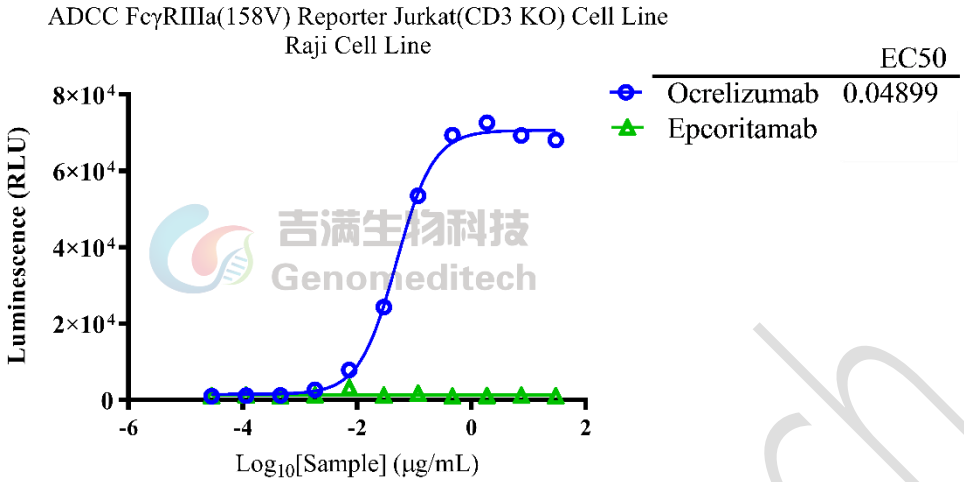


Figure 1 | Response to Ocrelizumab and Epcoritamab. Serial dilutions of Anti-CD20 hIgG1 Reference Antibody (Ocrebio)(Cat. GM-87986MAB) and Anti-CD3×CD20 hIgG1 Bispecific Antibody (Epcobio; no ADCC activity)(GM-88130AB) were prepared. For each condition, 1E5 ADCC Fc γ RIIIa(158V) Reporter Jurkat(CD3 KO) Cell Line (Cat. GM-C39293) per well were added to 2E4 Raji target cells per well, together with the antibody dilutions, followed by 6 hours of incubation. Firefly luciferase activity was then measured using the GMOne-Step 2.0 Luciferase Reporter Gene Assay Kit(Cat. GM-040513). Ocrelizumab maximum induction fold was approximately[72.9]. Data are shown by drug concentration.

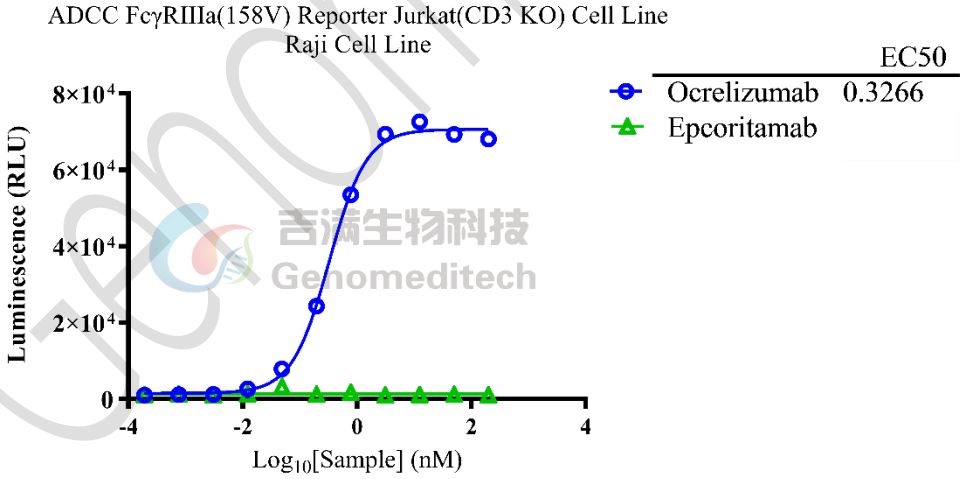
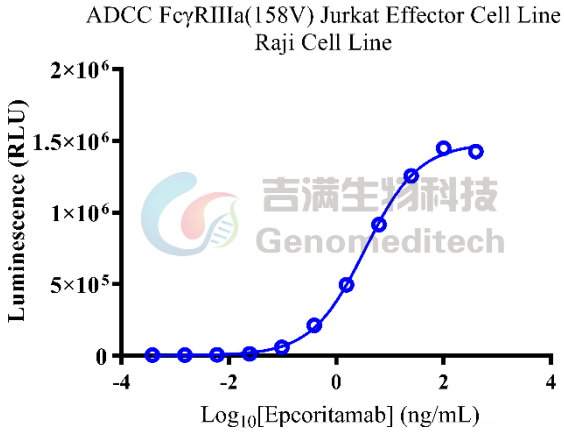
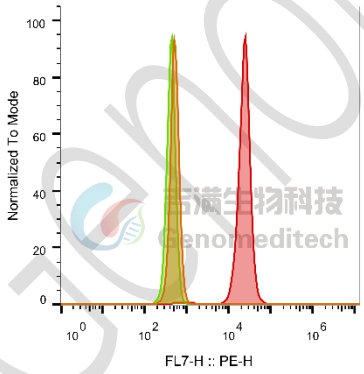


Figure 2 | Response to Ocrelizumab and Epcoritamab. Serial dilutions of Anti-CD20 hIgG1 Reference Antibody (Ocrebio)(Cat. GM-87986MAB) and Anti-CD3×CD20 hIgG1 Bispecific Antibody (Epcobio; no ADCC activity)(GM-88130AB) were prepared. For each condition, 1E5 ADCC Fc γ RIIIa(158V) Reporter Jurkat(CD3 KO) Cell Line (Cat. GM-C39293) per well were added to 2E4 Raji target cells per well, together with the antibody dilutions, followed by 6 hours of incubation. Firefly luciferase activity was then measured using the GMOne-Step 2.0 Luciferase Reporter Gene Assay Kit(Cat. GM-040513). Ocrelizumab maximum induction fold was approximately[72.9]. Data are shown by molar concentration.



	ADCC FcγRIIIa(158V) Jurkat
EC50	3.476

Figure 3 | Response to Epcoritamab. Serial dilutions of the Epcoritamab (Cat. GM-88130AB) and 1E5 cells/well of the ADCC FcγRIIIa(158V) Jurkat Effector Cell Line (Cat. GM-C05619) were added to 2E4 cells/well of the Raji cell line (Cat. GM-C19100) for 6 hours. Firefly luciferase activity was then measured using the GMOne-Step 2.0 Luciferase Reporter Gene Assay Kit (Cat. GM-040513). Epcoritamab (Epcobio) does not induce ADCC via its Fc region, as it is engineered to lack ADCC activity. The observed activation in this assay is due to the engagement of the CD3 arm of Epcoritamab with TCR on the Jurkat effector cells, together with CD20 on Raji target cells, rather than through classical Fc-mediated ADCC.



SampleID	Geometric Mean : FL7-H
Jurkat(CD3 KO) PE-anti-CD16 Ab	526
ADCC FcγRIIIa(158V) Reporter Jurkat(CD3 KO) PE-Mouse_IgG Ab	433
ADCC FcγRIIIa(158V) Reporter Jurkat(CD3 KO) PE-anti-CD16 Ab	22285

Figure 4 | ADCC FcγRIIIa(158V) Reporter Jurkat(CD3 KO) Cell Line (Cat. GM-C39293) was determined by flow cytometry using PE anti-human CD16 Antibody (Biolegend/302007).

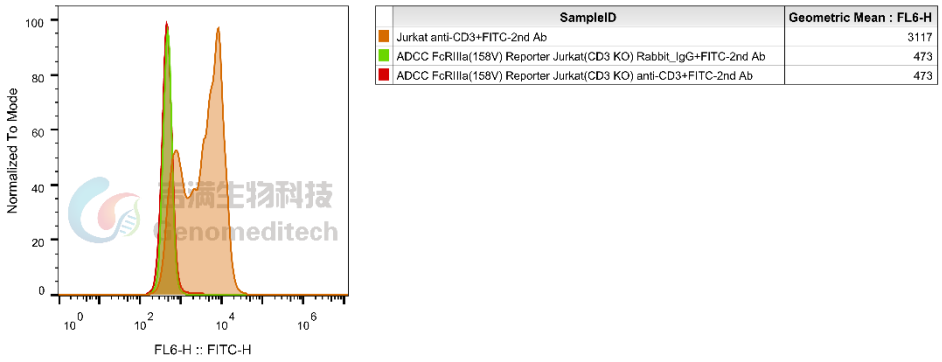


Figure 5 | ADCC FcγRIIIa(158V) Reporter Jurkat(CD3 KO) Cell Line (Cat. GM-C39293) was determined by flow cytometry using Abflo® 488 Rabbit anti-Human/Monkey CD3 mAb (Abclonal/A26283).

Figure 6 | The Sanger sequencing of the HADCC FcγRIIIa(158V) Reporter Jurkat(CD3 KO) Cell Line showed successful knockout of CD3E.

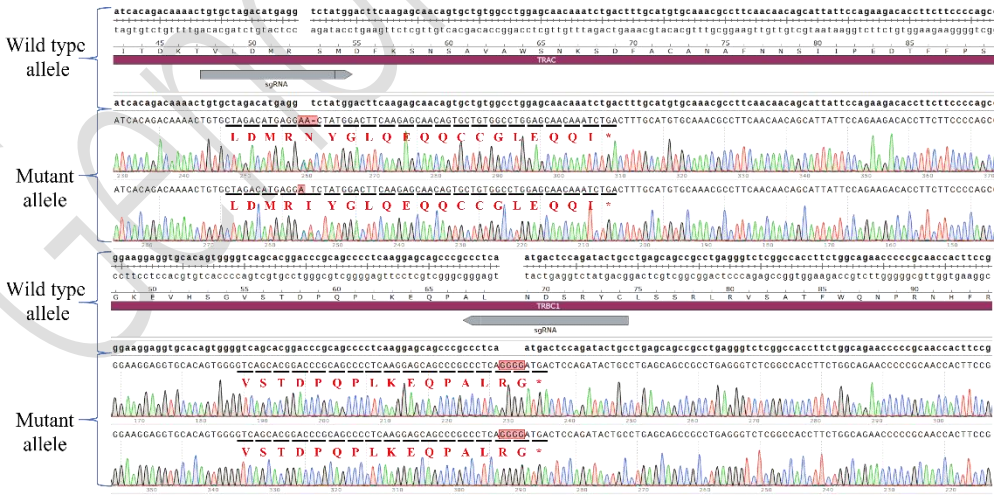


Figure 7 | The Sanger sequencing of the parental cells of the ADCC FcγRIIIa(158V) Reporter Jurkat(CD3 KO) Cell Line showed successful knockout of TCR.

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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+400 μg/mL G418+200 μg/mL Hygromycin+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

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	
H_CD28 Reporter Jurkat Cell Line	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)	
CD19	
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-H_CD19 hIgG1/hIgG2 Antibody(Tafasitamab)
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)	
CD2	
Cynomolgus_CD2 CHO-K1 Cell Line	H_CD2 CHO-K1 Cell Line
Anti-CD2 hIgG1 Antibody(BTI-322)	

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