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Product Sheet

H_CD25 CD122 CD132 Reporter Jurkat(hPD1 OE) Cell Line

Catalog number: GM-C41979

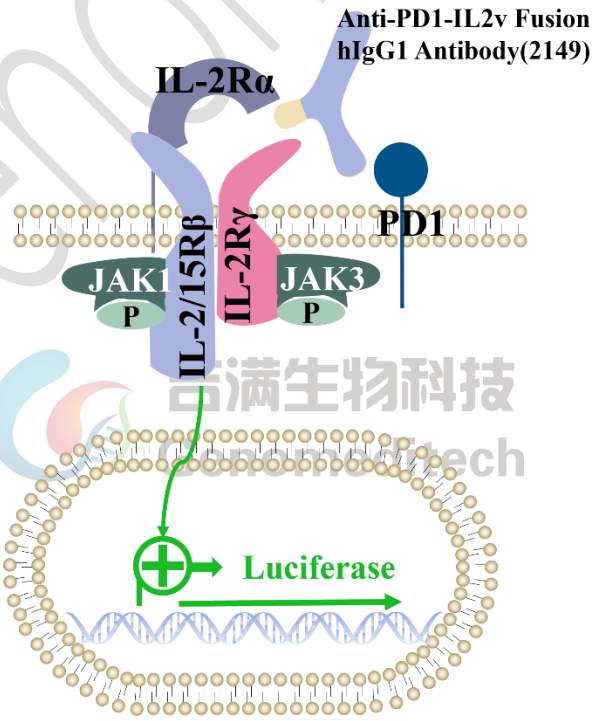
Version 3.3.1.250912

Interleukin-2 (IL-2) is produced by activated T cells after TCR recognition of antigen and is an important growth factor for tumor-infiltrating lymphocytes (TILs). Its receptor, IL-2R, is composed of CD25 (IL-2Rα), CD122 (IL-2Rβ), and CD132 (γc).

Programmed cell death 1 (PD-1, encoded by PDCD1) is an immune checkpoint receptor predominantly expressed on activated T cells, B cells, and some myeloid cells. Upon binding to its ligands PD-L1 or PD-L2, PD-1 recruits phosphatases such as SHP-2 to inhibit TCR and CD28 signaling, thereby reducing cell proliferation, cytotoxic activity, and cytokine secretion, maintaining peripheral tolerance, and preventing excessive immune activation.

Anti-PD1-IL2v represents a new class of immunotherapeutics that integrates an anti-PD-1 monoclonal antibody with an engineered IL-2 variant (IL-2v) in a fusion or conjugated format.

H_CD25 CD122 CD132 Reporter Jurkat(hPD1 OE) Cell Line is a stable clonal Jurkat cell line constructed using lentiviral technology, constitutive expression of the CD25,CD122,CD132 and PD1 genes, along with signal-dependent expression of a luciferase reporter gene. When Anti-PD-1–IL-2v binds PD-1 and delivers IL-2v to IL-2R, downstream IL-2R signaling induces luciferase expression. The luciferase readout reflects pathway activation and can be used to evaluate the in vitro efficacy of related drug candidates.



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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	PMI 1640+10% FBS+1% P.S+3.5 μg/mL Blasticidin+400 μg/mL G418+200 μg/mL Hygromycin+0.75 μg/mL Puromycin+400 μg/mL Zeocin
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
Bleomycin	Genomeditech/GM-040407
Anti-PD1-IL2v Fusion hIgG1 Antibody(2149)	Genomeditech/GM-88264AB
Anti-PD1 hIgG4 Reference Antibody (Pembio)	Genomeditech/GM-87802MAB
Anti-CD25 hIgG1 Antibody(Basiliximab)	Genomeditech/GM-52329AB
Anti-CD122 hIgG1 Antibody(HuABC-2)	Genomeditech/GM-52319AB
Anti-CD132(IL2RG) hIgG4 Antibody(REGN7257)	Genomeditech/GM-52334AB
GMOne-Step 2.0 Luciferase Reporter Gene Assay Kit	Genomeditech/GM-040513

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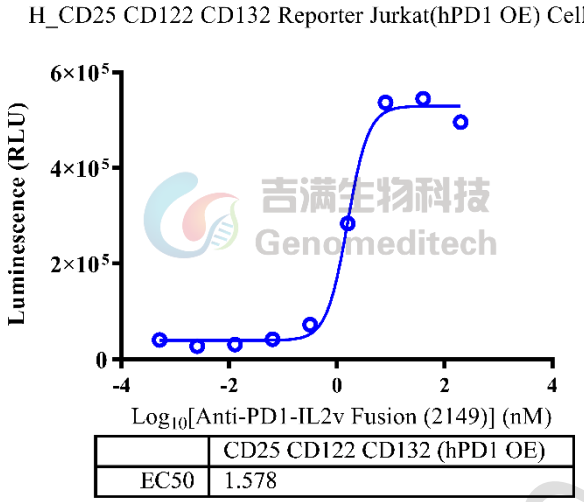
Figures



	CD25 CD122 CD132 (hPD1 OE)
EC50	1.578

Figure 1 | Response to Anti-PD1-IL2v Fusion hIgG1 Antibody(2149). The H_CD25 CD122 CD132 Reporter Jurkat(hPD1 OE) Cell Line (Cat. GM-C41979) at a concentration of 1E5 cells/well (96-well format) was stimulated with serial dilutions of Anti-PD1-IL2v (Cat. GM-88264AB) in assay buffer (RPMI 1640+1% FBS+1% P.S) for 16 hours. The firefly luciferase activity was measured using the Luciferase Reporter Assay Kit (Genomeditech). The maximum induction fold was approximately [10.5]. Data are shown by drug molar concentration.

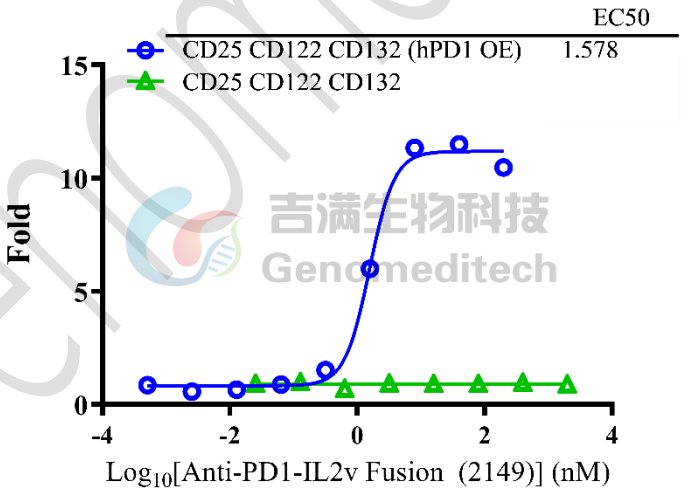


Figure 2 | Response to Recombinant Anti-PD1-IL2v Fusion hIgG1 Antibody(2149). H_CD25 CD122 CD132 Reporter Jurkat(hPD1 OE) Cell Line (Cat. GM-C41979) and H_CD25 CD122 CD132 Reporter Cell Line (Cat. GM-C29055) at a concentration of 1E5 cells/well (96-well format) were stimulated with serial dilutions of Anti-PD1-IL2v Fusion hIgG1 Antibody(2149) (Cat.GM-88264AB) in assay buffer (RPMI 1640 + 1% FBS + 1%P.S) for 16 hours. The firefly luciferase activity was measured using the Luciferase Reporter Assay Kit (Genomeditech). The maximum induction fold for the H_CD25 CD122 CD132 Reporter Jurkat(hPD1 OE) was approximately [10.5], and the H_CD25 CD122 CD132 Reporter validation was invalid/failed. Data are shown by drug molar concentration.

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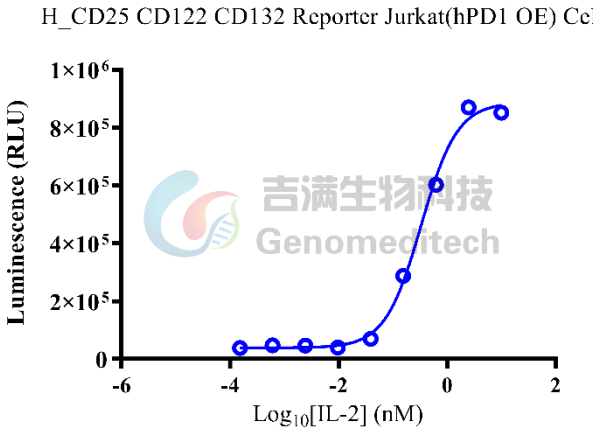
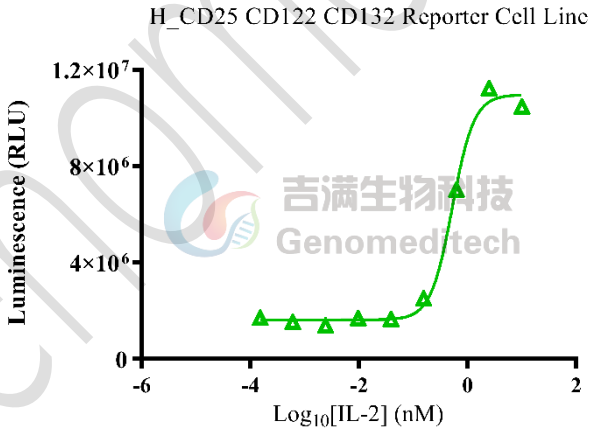


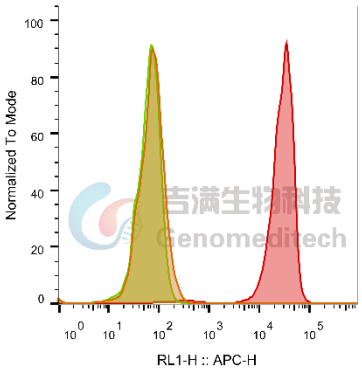
	CD25 CD122 CD132 (hPD1 OE)
EC50	0.3351

Figure 3 | Response to Recombinant Human IL-2. The H_CD25 CD122 CD132 Reporter Jurkat(hPD1 OE) Cell Line (Cat. GM-C41979) at a concentration of 1E5 cells/well (96-well format) was stimulated with serial dilutions of Recombinant Human IL-2 (Novoprotein/C013)in assay buffer (RPMI 1640+1% FBS+1% P.S) for 16 hours. The firefly luciferase activity was measured using the Luciferase Reporter Assay Kit (Genomeditech). The maximum induction fold was approximately [22.5]. Data are shown by drug molar concentration.



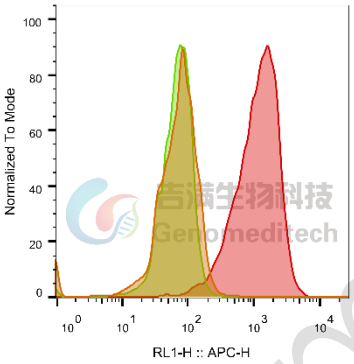
	H_CD25 CD122 CD132
EC50	0.5274

Figure 4 | Response to Recombinant Human IL-2. The H_CD25 CD122 CD132 Reporter Cell Line (Cat. GM-C29055) at a concentration of 1E5 cells/well (96-well format) was stimulated with serial dilutions of Recombinant Human IL-2 (Novoprotein/C013)in assay buffer (RPMI 1640+1% FBS+1% P.S) for 16 hours. The firefly luciferase activity was measured using the Luciferase Reporter Assay Kit (Genomeditech). The maximum induction fold was approximately [6.7]. Data are shown by drug molar concentration.



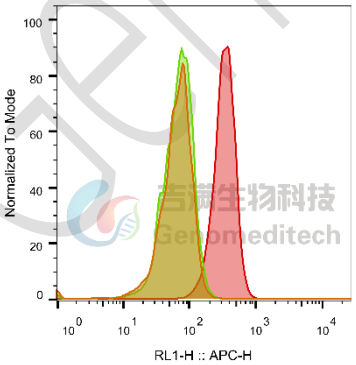
SampleID	Geometric Mean : RL1-H
Jurkat anti-PD1+APC-2nd Ab	70.4
H_CD25 CD122 CD132 Reporter (hPD1 OE) H_IgG+APC-2nd Ab	61.5
H_CD25 CD122 CD132 Reporter (hPD1 OE) anti-PD1+APC-2nd Ab	24970

Figure 5 | H_CD25 CD122 CD132 Reporter Jurkat(hPD1 OE) Cell Line (Cat. GM-C41979) was determined by flow cytometry using Anti-PD1 hIgG4 Reference Antibody (Pembio) (Cat. GM-87802MAB).



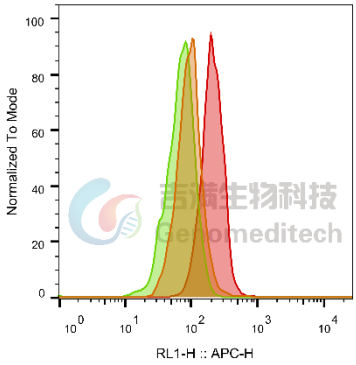
SampleID	Geometric Mean : RL1-H
Jurkat anti-CD25+APC-2nd Ab	66.1
H_CD25 CD122 CD132 Reporter (hPD1 OE) H_IgG+APC-2nd Ab	66.1
H_CD25 CD122 CD132 Reporter (hPD1 OE) anti-CD25+APC-2nd Ab	1070

Figure 6 | H_CD25 CD122 CD132 Reporter Jurkat(hPD1 OE) Cell Line (Cat. GM-C41979) was determined by flow cytometry using Anti-CD25 hIgG1 Antibody(Basiliximab) (Cat. GM-52329AB).



SampleID	Geometric Mean : RL1-H
Jurkat anti-CD122+APC-2nd Ab	61.0
H_CD25 CD122 CD132 Reporter (hPD1 OE) H_IgG+APC-2nd Ab	65.5
H_CD25 CD122 CD132 Reporter (hPD1 OE) anti-CD122+APC-2nd Ab	329

Figure 7 | H_CD25 CD122 CD132 Reporter Jurkat(hPD1 OE) Cell Line (Cat. GM-C41979) was determined by flow cytometry using Anti-CD122 hIgG1 Antibody(HuABC-2) (Cat. GM-52319AB).



SampleID	Geometric Mean : RL1-H
Jurkat anti-CD132+APC-2nd Ab	93.8
H_CD25 CD122 CD132 Reporter (hPD1 OE) H_IgG+APC-2nd Ab	67.4
H_CD25 CD122 CD132 Reporter (hPD1 OE) anti-CD132+APC-2nd Ab	206

Figure 8 | H_CD25 CD122 CD132 Reporter Jurkat(hPD1 OE) Cell Line (Cat. GM-C41979) was determined by flow cytometry using Anti-CD132(IL2RG) hIgG4 Antibody(REGN7257) (Cat. GM-52334AB).

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.

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Cell passage

Growth medium: PMI 1640+10% FBS+1% P.S+3.5 μg/mL Blasticidin+400 μg/mL G418+200 μg/mL Hygromycin+0.75 μg/mL Puromycin+400 μg/mL Zeocin

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

IL-15	
H_IL15 Reporter Cell Line	Cynomolgus_CD122 HEK-293 Cell Line
H_CD122 CD132 CHO-K1 Cell Line	H_CD122 CHO-K1 Cell Line
H_CD122 HEK-293 Cell Line	H_CD215(IL15RA) HEK-293 Cell Line
IL-2	
H_CD122 CD132 Reporter Cell Line	H_CD25 CD122 CD132 Reporter Cell Line
H_IL2 Reporter Cell Line	H_IL2 Reporter DDX35TM Cell Line
Cynomolgus_CD25 HEK-293 Cell Line	H_CD25 CHO-K1 Cell Line
H_CD25 HEK-293 Cell Line	
Anti-CD122 hIgG1 Antibody(HuABC-2)	Anti-CD132(IL2RG) hIgG4 Antibody(REGN7257)
Anti-CD25 hIgG1 Antibody(Basiliximab)	Anti-mouse CD25 mIgG2a Antibody(PC-61.5.3)
Anti-mouse CD25 RIgG1 Antibody(PC-61.5.3)	

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