Genomeditech (Shanghai) Co.,Ltd.
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Product Sheet

H_CD25 CD122 CD132 Reporter 293 Cell Line

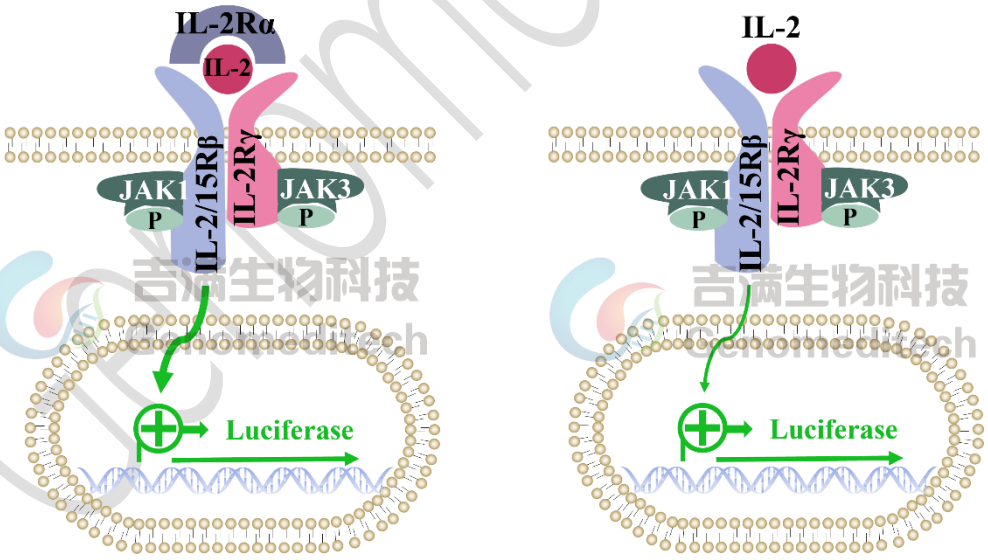
Catalog number: GM-C43769

Version 3.3.1.260827

Interleukin-2 (IL-2) is an important cytokine that mainly plays a regulatory role in the immune system. It works by binding to IL-2 receptors on the surface of lymphocytes, activating a series of signaling pathways that influence the proliferation and activity of immune cells.

The IL-2 receptor has two forms: a heterotrimer composed of IL-2Rα, IL-2Rβ, and IL-2Rγ, and a heterodimer composed of IL-2Rβ and IL-2Rγ. When IL-2 binds to its receptor, it activates the JAK signaling pathway, which in turn activates the transcription factor STAT. The phosphorylated STATs form dimers or tetramers and move into the cell nucleus, regulating the expression of specific genes to promote the immune response.

H_CD25 CD122 CD132 Reporter 293 Cell Line is a clonal stable cell line constructed using lentiviral technology, with constitutive expression of the IL-2Rα (CD25) , IL-2Rβ (CD122) , and IL-2Rγ (CD132) genes, along with signal-dependent expression of a luciferase reporter gene. When IL-2 binds to CD25, CD122, and CD132, the signaling cascade is triggered, leading to reporter gene activation. 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 IL-2.



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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+150 μg/mL Bleomycin+125 μg/mL Hygromycin+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
Bleomycin	Genomeditech/GM-040407
Hygromycin	Genomeditech/GM-040403
Recombinant Human IL-2	Novoprotein/C013
Recombinant Human IL-15	Sino Biological/10360-H07E
Anti-PD1-IL2v Fusion hIgG1 Antibody(2149)	Genomeditech/GM-88264AB
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

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Figures

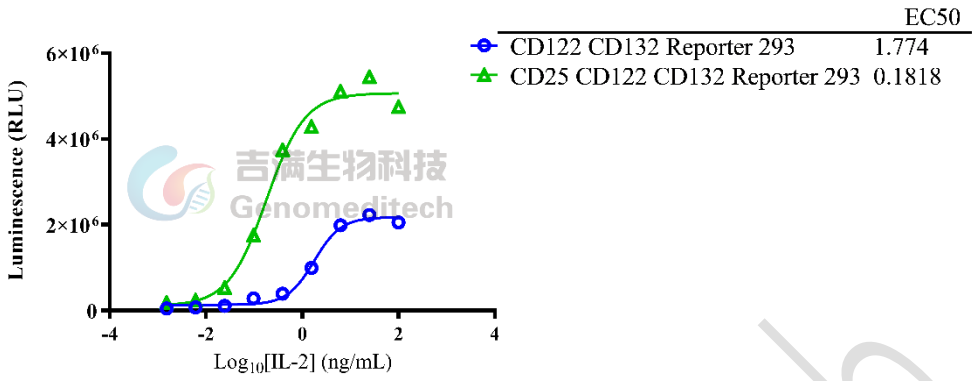


Figure 1 | Response to Recombinant Human IL-2. H_CD25 CD122 CD132 Reporter 293 Cell Line (Cat. GM-C43769) and H_CD122 CD132 Reporter 293 Cell Line (Cat. GM-C27707) were seeded at a density of 1.5E4 cells per well in a 96-well plate and incubated overnight. The next day, serial dilutions of the Human IL-2 (Novoprotein/C013) were added to the pre-seeded cells. The mixture was incubated for an additional 6 hours. The firefly luciferase activity was measured using the Luciferase Reporter Assay Kit (Genomeditech). The maximum induction fold was approximately [44.4] and [48.9]. Data are shown by drug mass concentration.

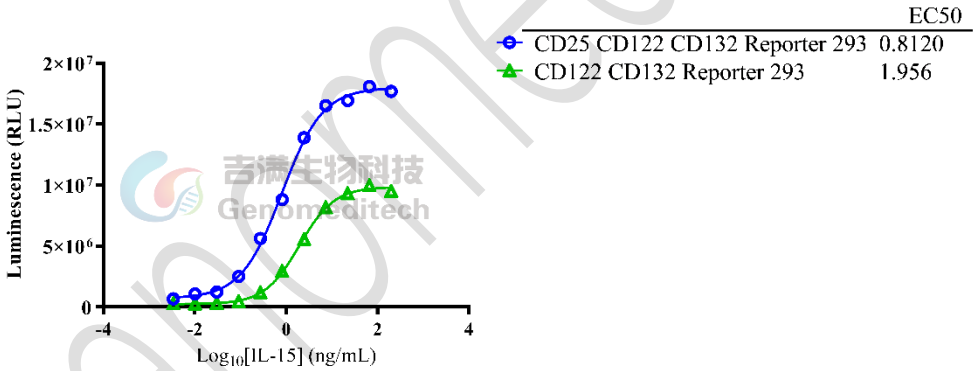
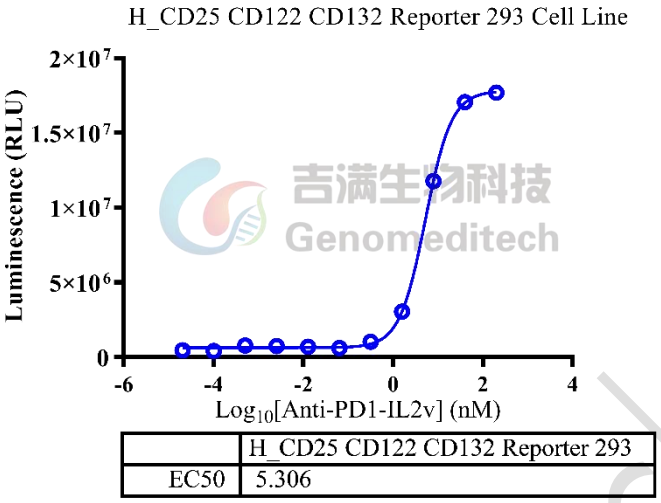
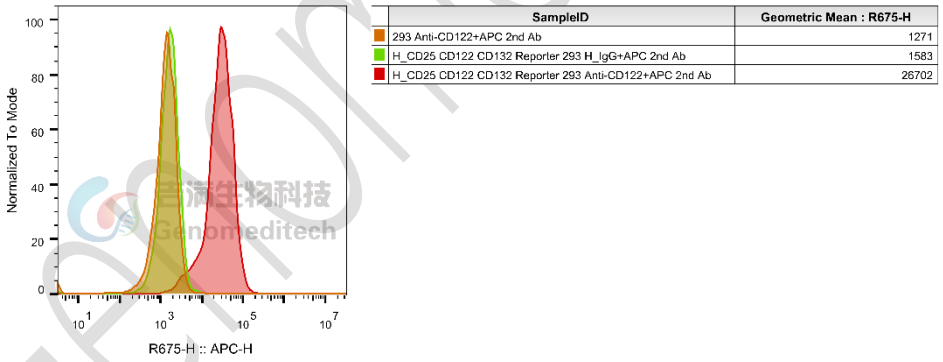


Figure 2 | Response to Recombinant Human IL-15. H_CD25 CD122 CD132 Reporter 293 Cell Line (Cat. GM-C43769) and H_CD122 CD132 Reporter 293 Cell Line (Cat. GM-C27707) were seeded at a density of 1.5E4 cells per well in a 96-well plate and incubated overnight. The next day, serial dilutions of the Human IL-15 (SinoBiological/10360-H07E) were added to the pre-seeded cells. The mixture was incubated for an additional 6 hours. The firefly luciferase activity was measured using the Luciferase Reporter Assay Kit (Genomeditech). The maximum induction fold was approximately [30.7] and [44.2]. Data are shown by drug mass concentration.



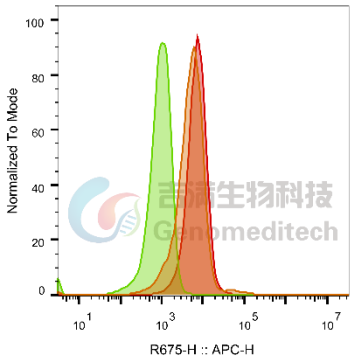
	H_CD25 CD122 CD132 Reporter 293
EC50	5.306

Figure 3 | Response to Anti-PD1-IL2v Fusion hIgG1 Antibody(2149). H_CD25 CD122 CD132 Reporter 293 Cell Line (Cat. GM-C43769) was seeded at a density of 1.5E4 cells per well in a 96-well plate and incubated overnight. The next day, serial dilutions of the Anti-PD1-IL2v Fusion hIgG1 Antibody(2149) (Cat. GM-88264AB) were added to the pre-seeded cells. The mixture was incubated for an additional 6 hours. The firefly luciferase activity was measured using the Luciferase Reporter Assay Kit (Genomeditech). The maximum induction fold was approximately [36.1]. Data are shown by drug molar concentration.



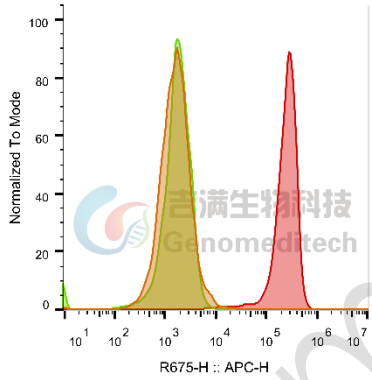
SampleID	Geometric Mean : R675-H
293 Anti-CD122+APC 2nd Ab	1271
H_CD25 CD122 CD132 Reporter 293 H_IgG+APC 2nd Ab	1583
H_CD25 CD122 CD132 Reporter 293 Anti-CD122+APC 2nd Ab	26702

Figure 4 | H_CD25 CD122 CD132 Reporter 293 Cell Line (Cat. GM-C43769) was determined by flow cytometry using Anti-CD122 hIgG1 Antibody(HuABC-2)(Cat. GM-52319AB).



SampleID	Geometric Mean : R675-H
293 Anti-CD132+APC 2nd Ab	4855
H_CD25 CD122 CD132 Reporter 293 H_IgG+APC 2nd Ab	848
H_CD25 CD122 CD132 Reporter 293 Anti-CD132+APC 2nd Ab	7109

Figure 5 | H_CD25 CD122 CD132 Reporter 293 Cell Line (Cat. GM-C43769) was determined by flow cytometry using Anti-CD132(IL2RG) hIgG4 Antibody(REGN7257)(Cat. GM-52334AB).



SampleID	Geometric Mean : R675-H
293 Anti-CD25+APC 2nd Ab	1618
H_CD25 CD122 CD132 Reporter 293 H_IgG+APC 2nd Ab	1643
H_CD25 CD122 CD132 Reporter 293 Anti-CD25+APC 2nd Ab	231494

Figure 6 | H_CD25 CD122 CD132 Reporter 293 Cell Line (Cat. GM-C43769) was determined by flow cytometry using Anti-CD25 hIgG1 Antibody(Basiliximab)(Cat. GM-52329AB).

Cell Recovery

Recovery Medium: DMEM+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 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+150 μg/mL Bleomycin+125 μg/mL Hygromycin+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.

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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 293 Cell Line	H_CD122 CD132 Reporter Cell Line
H_CD25 CD122 CD132 Reporter Cell Line	H_CD25 CD122 CD132 Reporter Jurkat(hPD1 OE) 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-IL2 hIgG1 Antibody (imneskibart)
Anti-mouse CD25 mIgG2a Antibody(PC-61.5.3)	Anti-mouse CD25 RIgG1 Antibody(PC-61.5.3)
Anti-PD1-IL2v Fusion hIgG1 Antibody(2149)	Anti-PD1-IL2v Fusion hIgG1 Antibody(KY-0118)
Cynomolgus IL-2 Protein; hFc Tag	Cynomolgus IL-2 Protein; His Tag
Cynomolgus IL-2RA Protein; hFc Tag	Cynomolgus IL-2RA Protein; His Tag
Cynomolgus IL-2RB Protein; His Tag	Human IL-2 Protein; hFc Tag
Human IL-2 Protein; His Tag	Human IL-2RA Protein; hFc Tag
Human IL-2RA Protein; His Tag	Human IL-2RB Protein; His Tag
Human IL-2RG Protein; His Tag	Mouse IL-2 Protein; His Tag

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