Genomeditech (Shanghai) Co.,Ltd.
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Product Sheet

H_IL2 Reporter DDX35™ Cell Line

Catalog number: GM-C27605

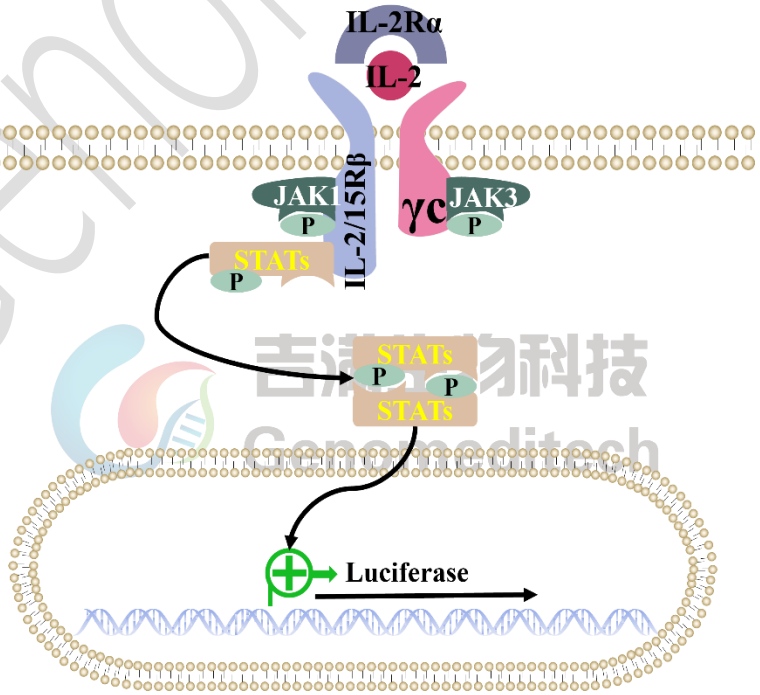
Version 3.3.1.260422

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.

The IL-2 receptor is made up of three chains: IL-2Rα, IL-2Rβ, and IL-2Rγ, which exist as a heterotrimer. 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_IL2 Reporter DDX35™ Cell Line is a clonal stable cell line constructed using lentiviral technology, constitutive expression of the IL-2Rβ gene, and it endogenously expresses IL-2Rα and IL-2Rγ, along with signal-dependent expression of a luciferase reporter gene. When IL-2 binds to IL-2R, it activates downstream signaling pathways, leading to the expression of luciferase. 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.

H_IL2 Reporter DDX35™ Cell Line was obtained through extensive monoclonal screening and multiple rounds of monoclonal selection. It possesses high stability, high sensitivity, and high amplification properties, meeting the standards for customers' batch library construction and release experiments.



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Specifications

Quantity	3E6 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+2 ng/mL GM-CSF
Growth medium	RPMI 1640+10% FBS+1% P.S+2 ng/mL GM-CSF+3 μg/mL Blasticidin+0.25 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
Recombinant Human GM-CSF	Novoprotein/C003
Blasticidin	Genomeditech/GM-040404
Puromycin	Genomeditech/GM-040401
Recombinant Human IL-2	Novoprotein/C013
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

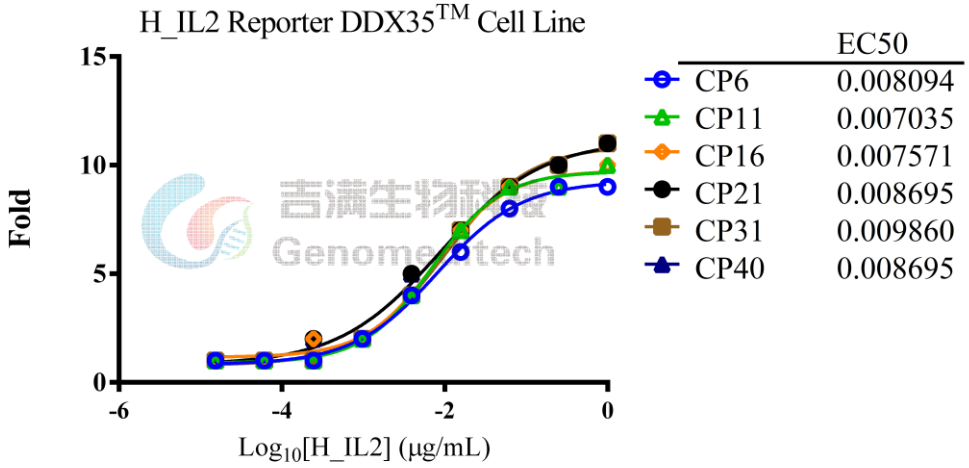


Figure 1 | The passage stability of response to Recombinant Human IL-2. The passage 6, 11, 16, 21, 31 and 40 of H_IL2 Reporter DDX35™ Cell Line (Cat. GM-C27605) at a concentration of 1E5 cells/well (96-well format) was stimulated with serial dilutions of Recombinant Human IL-2 (Novoprotein/C013) in assay buffer (RPMI 1640 + 1% FBS + 1%P.S) for 16 hours. The firefly luciferase activity was measured using the Luciferase Reporter Assay Kit (Genomeditech). Data are shown by drug mass concentration.

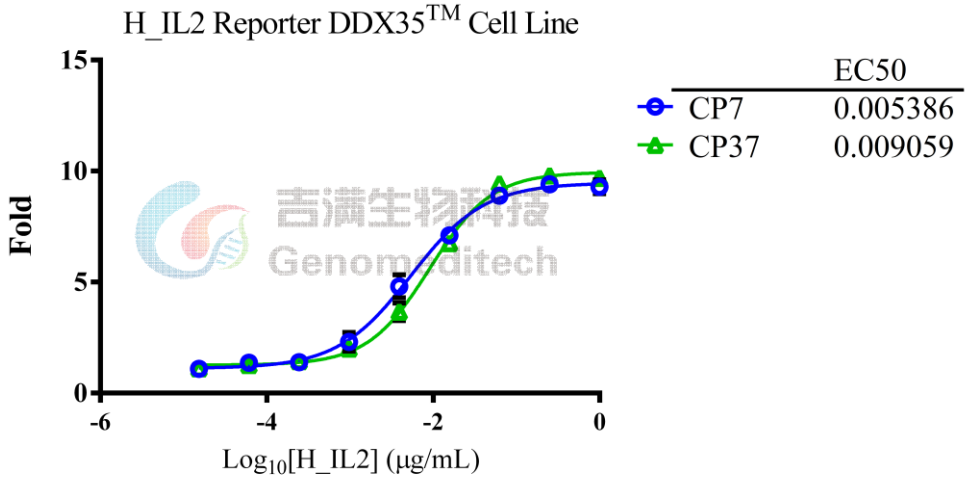
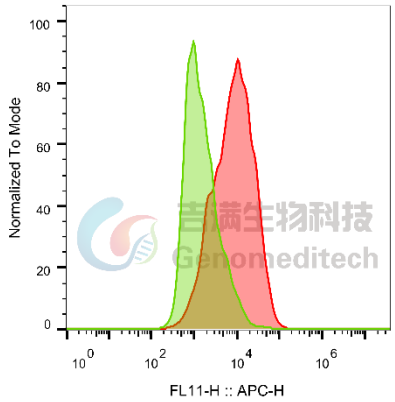
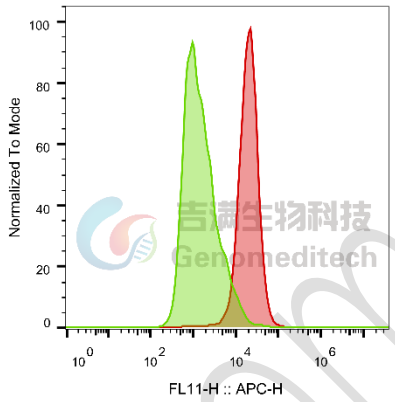


Figure 2 | The passage stability of response to Recombinant Human IL-2. The passage 7 and 37 of H_IL2 Reporter DDX35™ Cell Line (Cat. GM-C27605) at a concentration of 1E5 cells/well (96-well format) was stimulated with serial dilutions of Recombinant Human IL-2 (Novoprotein/C013) in assay buffer (RPMI 1640 + 1% FBS + 1%P.S) for 16 hours. The firefly luciferase activity was measured using the Luciferase Reporter Assay Kit (Genomeditech). Data are shown by drug mass concentration.



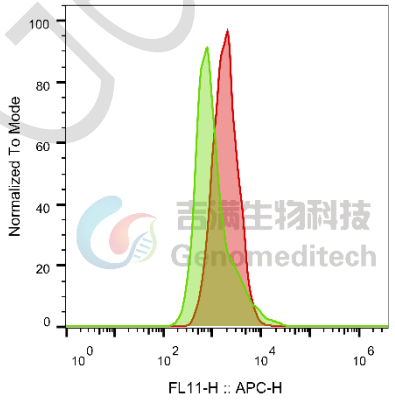
SampleID	Geometric Mean : FL11-H
H_IL2 Reporter DDX35TM H_IgG+APC-2nd Ab	1528
H_IL2 Reporter DDX35TM Anti-CD25+APC-2nd Ab	8241

Figure 3 | H_IL2 Reporter DDX35™ Cell Line (Cat. GM-C27605) was determined by flow cytometry using Anti-CD25 hIgG1 Antibody(Basiliximab) (Cat. GM-52329AB).



SampleID	Geometric Mean : FL11-H
H_IL2 Reporter DDX35TM H_IgG+APC-2nd Ab	1528
H_IL2 Reporter DDX35TM Anti-CD122+APC-2nd Ab	19825

Figure 4 | H_IL2 Reporter DDX35™ Cell Line (Cat. GM-C27605) was determined by flow cytometry using Anti-CD122 hIgG1 Antibody(HuABC-2) (Cat. GM-52319AB).



SampleID	Geometric Mean : FL11-H
H_IL2 Reporter DDX35TM H_IgG+APC-2nd Ab	1035
H_IL2 Reporter DDX35TM Anti-CD132+APC-2nd Ab	1875

Figure 5 | H_IL2 Reporter DDX35™ Cell Line (Cat. GM-C27605) 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+2 ng/mL GM-CSF

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 the cell pellet using the recommended complete medium and adjust the viable cell density to 4-6E5 cells/mL. Then dispense the suspension into an appropriate culture flask and initially place the flask in an upright position after thawing.

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 3E6 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+2 ng/mL GM-CSF+3 μg/mL Blasticidin+0.25 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.

a) This cell is a human erythroid leukemia cell, lymphoblast, growing in suspension.

b) In the suspension, they appear as large, single, round cells. Cells shed a large accumulation of cytoplasmic granules in the culture, which should not be confused with bacteria!

c) When the cell density reaches 1-1.2E6 cells/mL, perform a 1:2 to 1:3 split, ensuring subculturing every other day. It is essential to perform a full-volume centrifugation and medium replacement during passaging. Do not let the density

exceed 1.2E6 cells/mL. It is recommended to use T-25 flasks for subculturing, and you can control the cell density for subculturing by counting.

Subcultivation Ratio: Maintain cultures at a cell concentraion between 4E5 and 6E5 viable cells/mL.

Medium Renewal: Every other day

Notes

a) To minimize the presence of cytoplasmic granules, it is essential to passage the cells every other day when the cell density reaches 1-1.2E6 cells/mL. During passaging, perform a complete centrifugation and replace the culture medium to ensure appropriate cell density and cytokine concentration. Failure to do so may promote the growth of factor-independent subclones.

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_CD25 CD122 CD132 Reporter Jurkat(hPD1 OE) Cell Line	H_IL2 Reporter 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)	Anti-PD1-IL2v Fusion hIgG1 Antibody(2149)
Anti-PD1-IL2v Fusion hIgG1 Antibody(KY-0118)	
Cynomolgus IL-2RA Protein; His Tag	Cynomolgus IL-2RB Protein; His Tag
Human IL-2 Protein; His Tag	Human IL-2RA Protein; His Tag
Human IL-2RB Protein; His Tag	Human IL-2RG Protein; His Tag

License Agreement:

By purchasing and using this cell line product, the user voluntarily agrees to accept and abide by the following policies:

- This cell line product is restricted to research use only and shall not be used for any commercial purposes.
- This product is strictly prohibited from being used in the diagnosis or treatment of human or animal diseases, and shall not be directly used in experiments involving humans.

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