

# Product Sheet

## H\_IL-5 Reporter 293 Cell Line

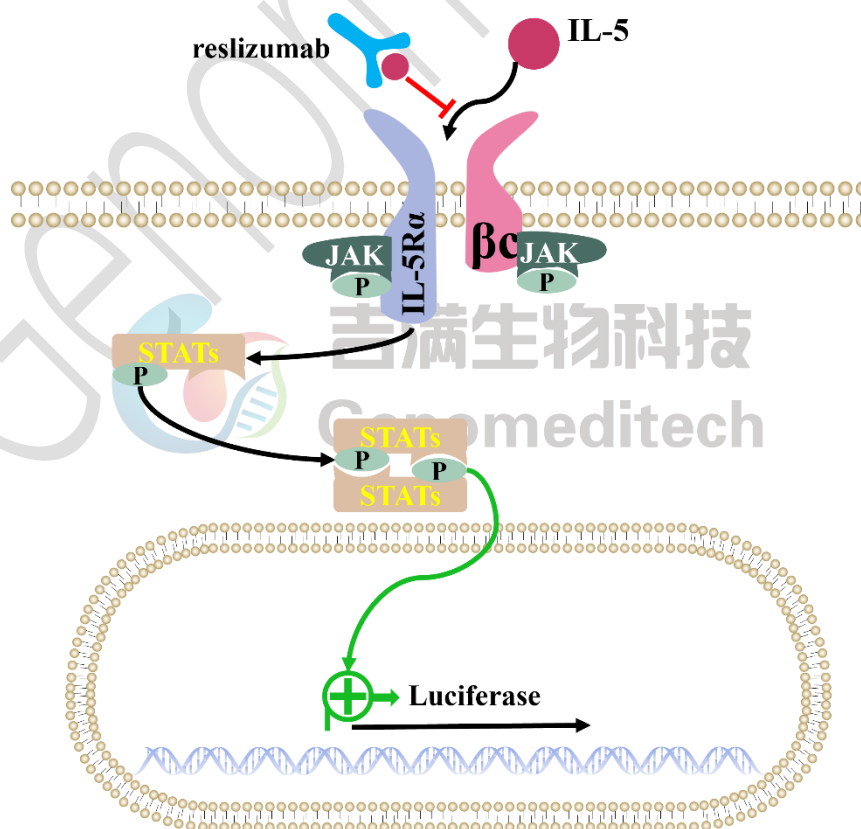
Catalog number: GM-C06723

Version 3.3.1.250916

Interleukin-5 (IL-5) is a cytokine produced by T cells, especially helper T cells, and eosinophils. It regulates the growth, differentiation, and activation of eosinophils, promoting their proliferation and survival. IL-5 is important in immune diseases like allergic reactions and asthma, enhancing eosinophil function and contributing to tissue damage and inflammation.

IL-5 activates signaling pathways by binding to its receptor IL-5R, a dimer of IL-5R $\alpha$  and  $\beta$  chains. This binding recruits signal transducer and activator of transcription 5 (STAT5), which, upon phosphorylation, moves to the nucleus to regulate genes related to eosinophil proliferation and survival. The IL-5 signaling pathway promotes eosinophil proliferation and enhances their function, playing a key role in allergic and inflammatory responses.

H\_IL-5 Reporter 293 Cell Line is a clonal stable cell line constructed using lentiviral technology, constitutive expression of the IL-5R $\alpha$  and  $\beta$ , along with signal-dependent expression of a luciferase reporter gene. When IL-5 binds to IL-5R, it activates downstream signaling pathways, leading to the expression of luciferase. Blockade antibodies can inhibit this signal transmission. 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-5.



## Specifications

<b>Quantity</b>	5E6 Cells per vial, 1 mL
<b>Product Format</b>	1 vial of frozen cells
<b>Shipping</b>	Shipped on dry ice
<b>Storage Conditions</b>	Liquid nitrogen immediately upon receipt
<b>Recovery Medium</b>	DMEM+10% FBS+1% P.S
<b>Growth medium</b>	DMEM+10% FBS+1% P.S+4 µg/mL Blasticidin+400 µg/mL G418+125 µg/mL Hygromycin+0.75 µg/mL Puromycin
<b>Note</b>	None
<b>Freezing Medium</b>	90% FBS+10% DMSO
<b>Growth properties</b>	Adherent
<b>Growth Conditions</b>	37°C, 5% CO <sub>2</sub>
<b>Mycoplasma Testing</b>	The cell line has been screened to confirm the absence of Mycoplasma species.
<b>Safety considerations</b>	Biosafety Level 2
<b>Note</b>	It is recommended to expand the cell culture and store a minimum of 10 vials at an early passage for potential future use.

## Materials

<b>Reagent</b>	<b>Manufacturer/Catalogue No.</b>
DMEM	gibco/C11995500BT
Fetal Bovine Serum	ExCell/FSP500
Pen/Strep	Thermo/15140-122
Blasticidin	Genomeditech/ <a href="#">GM-040404</a>
G418	Genomeditech/ <a href="#">GM-040402</a>
Hygromycin	Genomeditech/ <a href="#">GM-040403</a>
Puromycin	Genomeditech/ <a href="#">GM-040401</a>
Human IL-5 / Interleukin 5 Protein	Sino Biological/15673-HNCE
Anti-IL5 hIgG4 Antibody(Reslizumab)	Genomeditech/ <a href="#">GM-52408AB</a>
Anti-IL-5R hIgG1 Antibody(Benralizumab)	Genomeditech/ <a href="#">GM-46851AB</a>
Anti-IL-3R Beta/IL3RB Antibody (APC), Mouse Monoclonal	Sino Biological/10516-MM01-A
GMOne-Step 2.0 Luciferase Reporter Gene Assay Kit	Genomeditech/ <a href="#">GM-040513</a>

## Figures

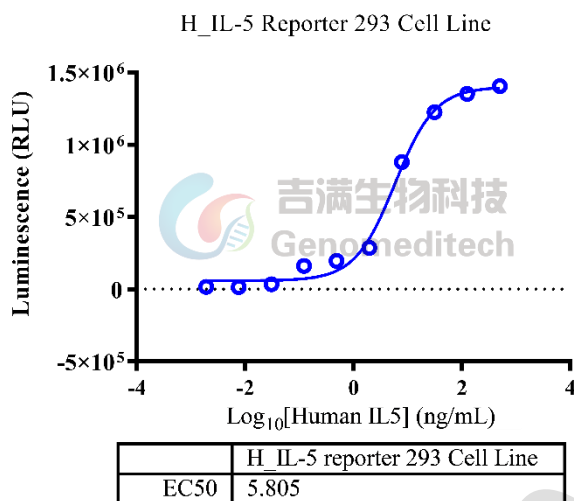


Figure 1 | Response to Human IL5 / Interleukin 5 Protein. The H\_IL-5 Reporter 293 Cell Line (Cat. GM-C06723) at a concentration of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of Human IL5 / Interleukin 5 Protein (Sino Biological/15673-HNCE) in assay buffer (DMEM + 1% FBS + 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 [90.5]. Data are shown by drug mass concentration.

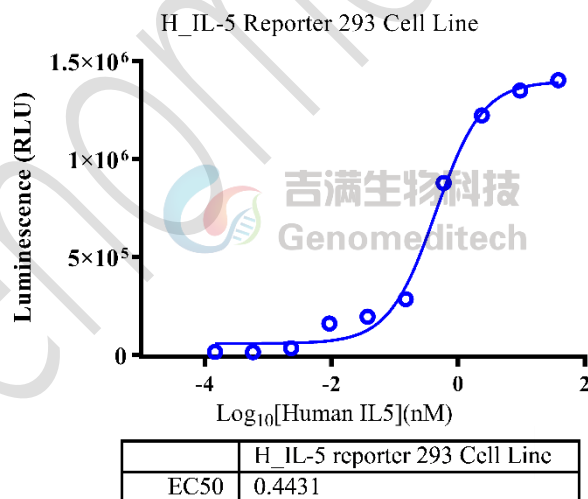


Figure 2 | Response to Human IL5 / Interleukin 5 Protein. The H\_IL-5 Reporter 293 Cell Line (Cat. GM-C06723) at a concentration of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of Human IL5 / Interleukin 5 Protein (Sino Biological/15673-HNCE) in assay buffer (DMEM + 1% FBS + 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 [90.5]. Data are shown by drug molar concentration.

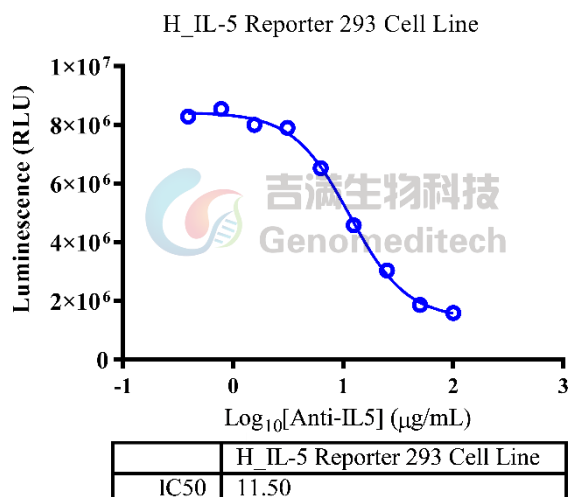


Figure 3 | Response to Anti-IL5 hIgG4 Antibody(Reslizumab). Serial dilutions of Anti-IL5 hIgG4 Antibody(Reslizumab) (Cat. [GM-52408AB](#)) was incubated with 0.581 ng/well of Human IL5 / Interleukin 5 Protein (Sino Biological/15673-HNCE) for 1 hour in assay buffer (DMEM + 1% FBS + 1% P.S). After pre-incubation, add the mixture to the H\_IL-5 Reporter 293 Cell Line (Cat. GM-C06723) at a density of 1.5E4 cells/well in a 96-well format, and incubate for 16 hours. Firefly luciferase activity is then measured using the Luciferase Reporter Assay Kit (Genomeditech). The results indicated maximum blocking folds of approximately [5.2]. Data are shown by drug mass concentration.

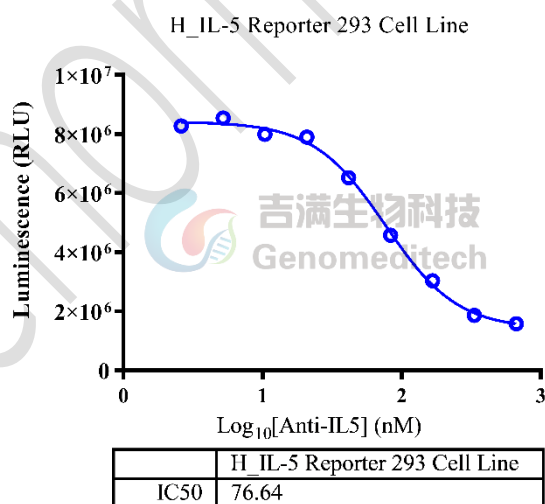


Figure 4 | Response to Anti-IL5 hIgG4 Antibody(Reslizumab). Serial dilutions of Anti-IL5 hIgG4 Antibody(Reslizumab) (Cat. [GM-52408AB](#)) was incubated with 0.581 ng/well of Human IL5 / Interleukin 5 Protein (Sino Biological/15673-HNCE) for 1 hour in assay buffer (DMEM + 1% FBS + 1% P.S). After pre-incubation, add the mixture to the H\_IL-5 Reporter 293 Cell Line (Cat. GM-C06723) at a density of 1.5E4 cells/well in a 96-well format, and incubate for 16 hours. Firefly luciferase activity is then measured using the Luciferase Reporter Assay Kit

(Genomeditech). The results indicated maximum blocking folds of approximately [5.2]. Data are shown by drug molar concentration.

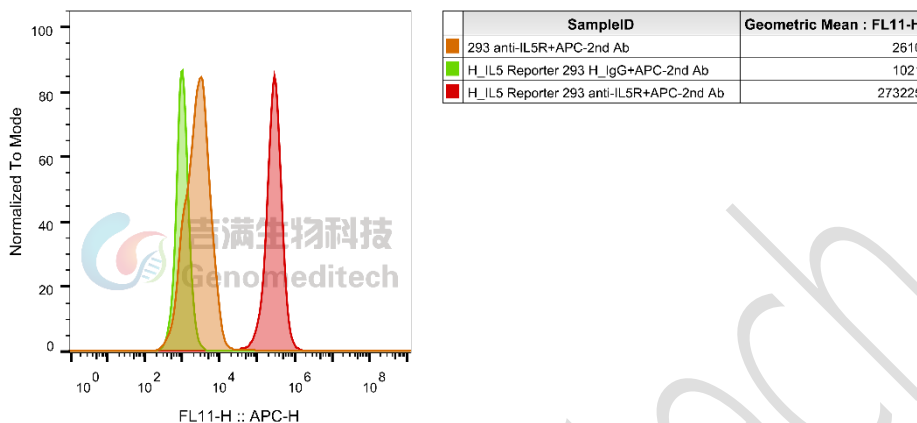


Figure 5 | H\_IL-5 Reporter 293 Cell Line (Cat. GM-C06723) was determined by flow cytometry using Anti-IL-5R hIgG1 Antibody(Benralizumab) (Cat. GM-46851AB).

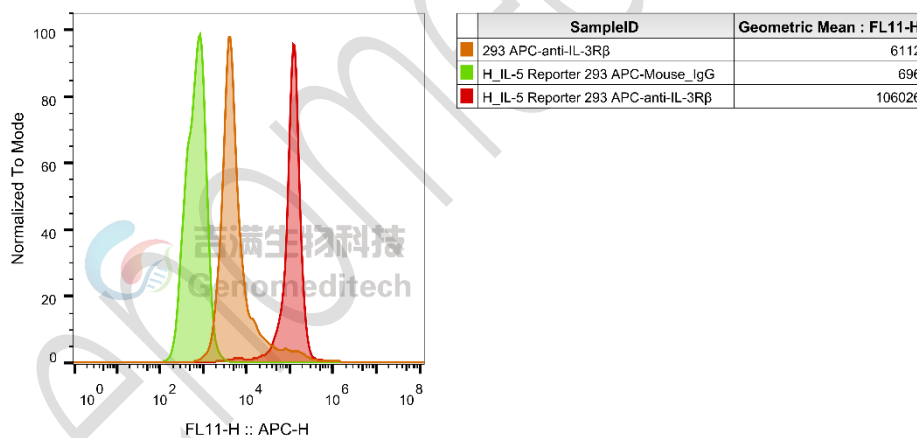


Figure 6 | H\_IL-5 Reporter 293 Cell Line (Cat. GM-C06723) was determined by flow cytometry using Anti-IL-3R Beta/IL3RB Antibody (APC) (SinoBiological/10516-MM01-A).

## 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<sub>2</sub> 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+400 µg/mL G418+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

- 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.
- Ensure that the cell density does not exceed 80%, as overcrowding may lead to reduced viability due to compression.

## Related Products

IL-4/IL-13	
IL-4 Reporter Cell Line	IL-4/IL-13 Reporter 293 Cell Line
IL-4/IL-13 Reporter 293 DDX35TM Cell Line	Cynomolgus_IL4R CHO-K1 Cell Line
H_IL4R CHO-K1 Cell Line	Mouse_IL4R CHO-K1 Cell Line
Anti-IL-4R hIgG1 Antibody(12B5)	Anti-IL4R hIgG4 Antibody(Dupilumab)
Anti-IL4R hIgG4 Reference Antibody (Dupbio)	
Biotinylated Human IL-4R alpha Protein; Avi-His Tag	Cynomolgus IL-4R alpha Protein; His Tag
Human IL-4 Protein; His Tag	Human IL-4R alpha Protein; hFc Tag
Human IL-4R alpha Protein; His Tag	Human IL-4R alpha Protein; mFc Tag
Mouse IL-13 Protein; His Tag	Mouse IL-4R alpha Protein; His Tag
Rat IL-4R alpha Protein; His Tag	
TSLP:TSLPR	
H_TSLP Reporter 293 Cell Line	H_TSLP Reporter Cell Line
H_TSLPR CHO-K1 Cell Line	
Anti-H_TSLPR hIgG1 Antibody	Anti-TSLP hIgG2 Antibody(Tezepelumab)
Anti-TSLP hIgG2 Reference Antibody (Tezbio)	
Biotinylated Human TSLP Protein; His-Avi Tag	Cynomolgus TSLP Protein; His Tag
Human TSLP Protein; hFc Tag	Human TSLP Protein; His Tag
Human TSLPR Protein; His Tag	
IL-5	
H_IL-5RA CHO-K1 Cell Line	H_IL-5RA HEK-293 Cell Line
Anti-IL5 hIgG4 Antibody(Reslizumab)	Anti-IL-5R hIgG1 Antibody(Benralizumab)

## 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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