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

Product Sheet

H_IL-6 Reporter 293 Cell Line

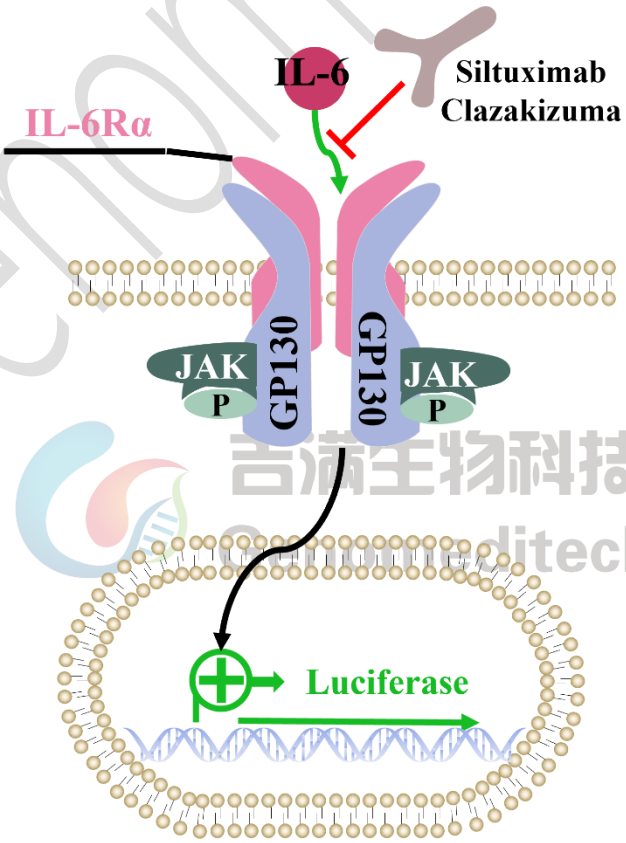
Catalog number: GM-C01951

Version 3.3.1.251022

Interleukin-6 (IL-6) is a crucial pro-inflammatory cytokine released during infections or tissue damage, boosting both innate and adaptive immune responses. Immune cells, like macrophages, quickly express IL-6 when encountering DAMPs and PAMPs. Although it aids in clearing infected cells, too much IL-6 can cause chronic inflammatory diseases, such as rheumatoid arthritis, and cytokine storms.

IL-6 signals through three pathways: classic signaling, trans-signaling, and trans-presentation. In classic signaling, IL-6 binds to mIL-6R and gp130, activating the JAK-STAT pathway. In trans-signaling, it interacts with soluble IL-6 receptor (sIL-6R) in body fluids. In trans-presentation, mIL-6R on dendritic cells presents IL-6 to T cells with gp130, crucial for activating T helper 17 (Th17) cells.

H_IL-6 Reporter 293 Cell Line is a stable clonal 293 cell line generated using lentiviral technology that constitutively expresses IL-6Rα and endogenously expresses GP130, along with signal-dependent expression of a luciferase reporter gene. Upon stimulation with IL-6, the IL-6/IL-6Rα/GP130 pathway activates a luciferase reporter gene, producing a measurable luminescent signal. Neutralizing antibodies against IL-6 or IL-6Rα can block this activation, making the luciferase signal a reliable indicator for evaluating the in vitro effects of drugs targeting the IL-6 signaling pathway.



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

Specifications

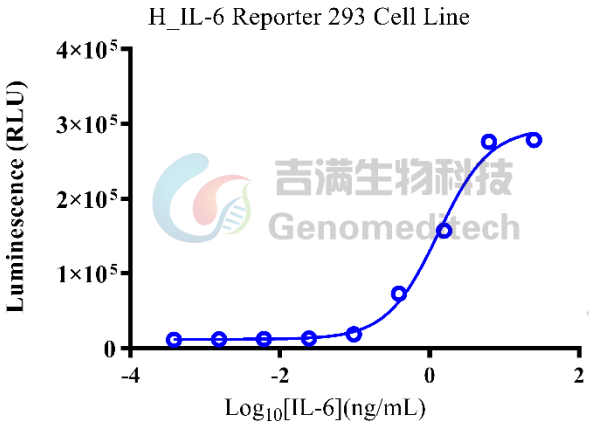
Quantity	5E6 Cells per vial,1 mL
Product Format	1 vial of frozen cells
Shipping	Shipped on dry ice
Storage Conditions	Liquid nitrogen immediately upon receipt
Recovery Medium	DMEM+10% FBS+1% P.S
Growth medium	DMEM+10% FBS+1% P.S+4 μg/mL Blasticidin+0.75 μg/mL Puromycin
Note	None
Freezing Medium	90% FBS+10% 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
Recombinant Human IL-6	PEPROTECH/200-06
Anti-IL-6 hIgG1 Reference Antibody (Siltubio)	Genomeditech/GM-88118MAB
Anti-IL-6 hIgG1 Reference Antibody (Clazabio)	Genomeditech/GM-88119MAB
Anti-gp130/IL6ST Antibody (PE), Mouse Monoclonal	SinoBiological/10974-MM11-P
Anti-IL6R hIgG1 Antibody(tocilizumab)	Genomeditech/GM-87897AB
GMOne-Step 2.0 Luciferase Reporter Gene Assay Kit	Genomeditech/GM-040513

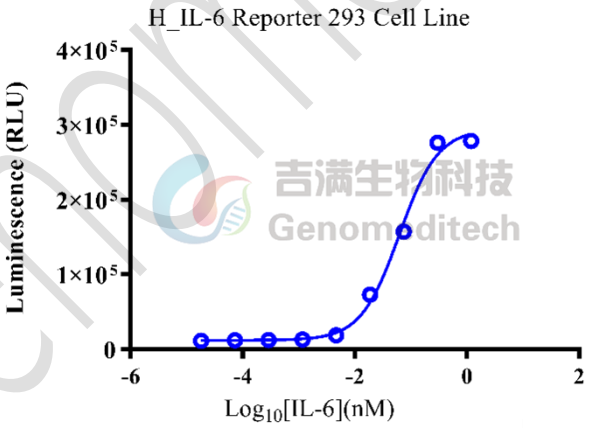
Genomeditech (Shanghai) Co.,Ltd.
Order: +86 021-68455258/50432826/50432825
Toll-free: +86 400 627 9288
Email: service@genomeditech.com

Figures



	H_IL-6 Reporter 293 Cell Line
EC50	1.309

Figure 1 | Response to Recombinant Human IL-6. The H_IL-6 Reporter 293 Cell Line (Cat. GM-C01951) at a concentration of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of Recombinant Human IL-6 (PEPROTECH/200-06) 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 [25.1]. Data are shown by drug mass concentration.



	H_IL-6 Reporter 293 Cell Line
EC50	0.06263

Figure 2 | Response to Recombinant Human IL-6. The H_IL-6 Reporter 293 Cell Line (Cat. GM-C01951) at a concentration of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of Recombinant Human IL-6 (PEPROTECH/200-06) 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 [25.1]. Data are shown by drug molar concentration.

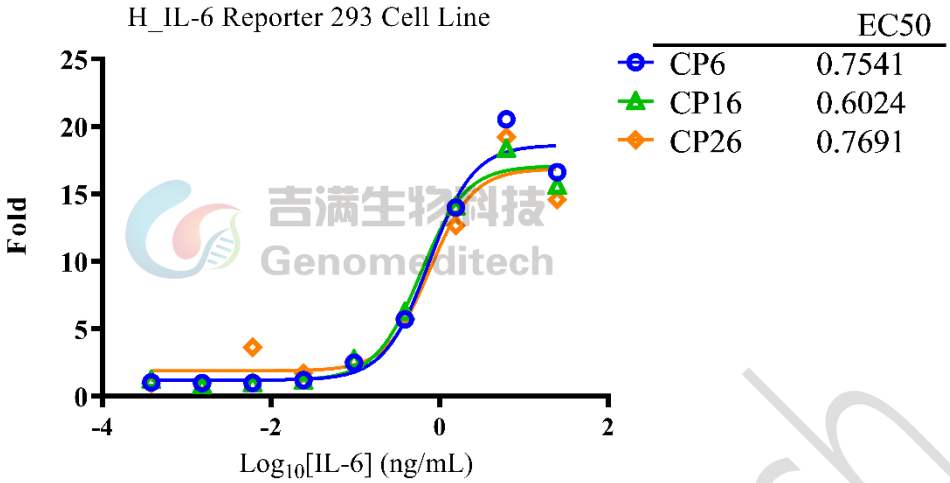
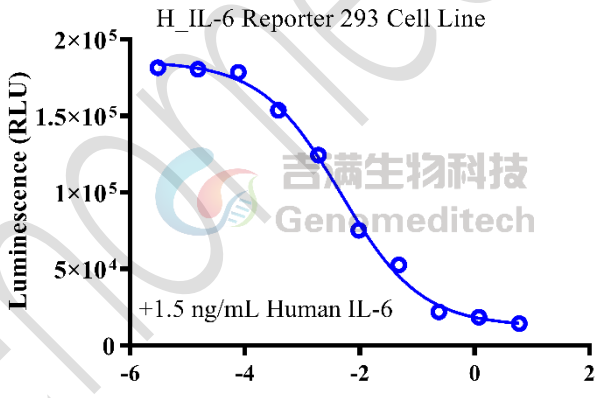
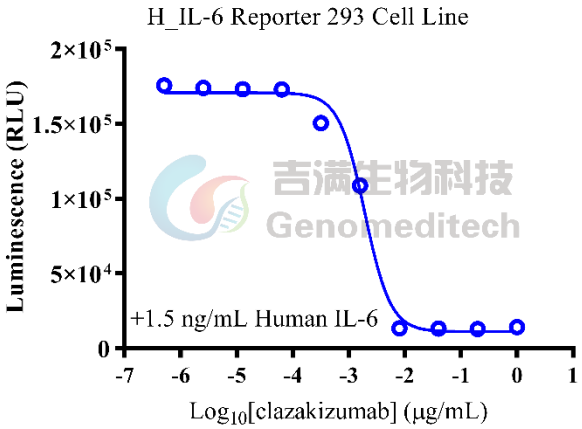


Figure 3 | The passaging stability of response to Recombinant Human IL-6. The passage 6, 16 and 26 of H_IL-6 Reporter 293 Cell Line (Cat. GM-C01951) at a concentration of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of Recombinant Human IL-6 (PEPROTECH/200-06) in assay buffer (DMEM + 1% FBS + 1% P.S) for 16 hours. The firefly luciferase activity was measured using the Luciferase Reporter Assay Kit (Genomeditech). Data are shown by drug mass concentration.



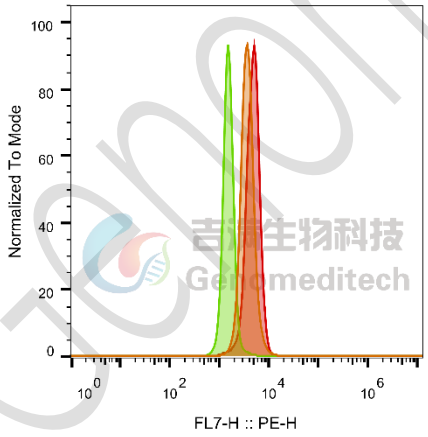
	H_IL-6 Reporter 293 Cell Line
IC50	0.004714

Figure 4 | Response to Anti-IL-6 hIgG1 Reference Antibody (Siltubio) (Cat. GM-88118MAB). Serial dilutions of Anti-IL-6 hIgG1 Reference Antibody (Siltubio) were incubated with 0.15 ng/well of Recombinant Human IL-6 (Peprotech/200-06) for 1 hour in assay buffer (DMEM + 1% FBS + 1% P.S). After pre-incubation, add the mixture to the H_IL-6 Reporter 293 Cell Line (Cat. GM-C01951) at a density of 1.5E4 cells/well in a 96-well format, and incubate for 16 hours. Firefly luciferase activity was measured using the Luciferase Reporter Assay Kit (Genomeditech). The results indicated maximum blocking folds of approximately [12.2]. Data are shown by drug mass concentration.



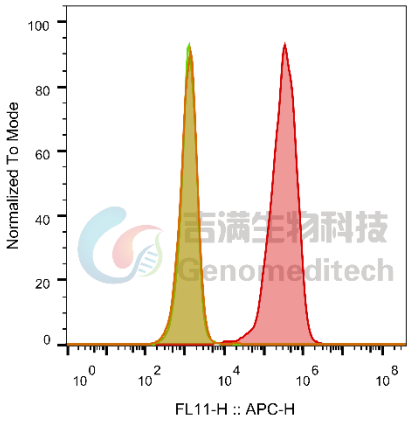
	H_IL-6 Reporter 293 Cell Line
IC50	0.001929

Figure 5 | Response to Anti-IL-6 hIgG1 Reference Antibody (Clazabio) (Cat. GM-88119MAB). Serial dilutions of Anti-IL-6 hIgG1 Reference Antibody (Clazabio) were incubated with 0.15 ng/well of Recombinant Human IL-6 (Peprotech/200-06) for 1 hour in assay buffer (DMEM + 1% FBS + 1% P.S). After pre-incubation, add the mixture to the H_IL-6 Reporter 293 Cell Line (Cat. GM-C01951) at a density of 1.5E4 cells/well in a 96-well format, and incubate for 16 hours. Firefly luciferase activity was measured using the Luciferase Reporter Assay Kit (Genomeditech). The results indicated maximum blocking folds of approximately [12.2]. Data are shown by drug mass concentration.



SampleID	Geometric Mean : FL7-H
293 PE-Anti-GP130 Ab	3677
H_IL6 Reporter 293 PE-M_IgG Ab	1540
H_IL6 Reporter 293 PE-Anti-GP130 Ab	4695

Figure 6 | H_IL-6 Reporter 293 Cell Line (Cat. GM-C01951) was determined by flow cytometry using Anti-gp130/IL6ST Antibody (sinobiological/10974-MM11-P).



SampleID	Geometric Mean : FL11-H
293 Anti-IL6R+APC-2nd Ab	1253
H_IL6 Reporter 293 H_IgG+APC-2nd Ab	1251
H_IL6 Reporter 293 Anti-IL6R+APC-2nd Ab	299516

Figure 7 | H_IL-6 Reporter 293 Cell Line (Cat. GM-C01951) was determined by flow cytometry using Anti-IL6R hIgG1 Antibody(tocilizumab) (Cat. GM-87897AB).

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.

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

Cell passage

Growth medium: DMEM+10% FBS+1% P.S+4 μg/mL Blasticidin+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.

Related Products

IL-6	
GP130 Reporter 293 Cell Line	
Anti-IL-6 hIgG1 Reference Antibody (Clazabio)	Anti-IL-6 hIgG1 Reference Antibody (Siltubio)
Anti-IL6R hIgG1 Antibody(tocilizumab)	
OSM	
H_OSMR Reporter 293 Cell Line	
Human OSMR beta Protein; His Tag	

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

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.
- Users and their contractors engaged for their benefit may use this material and its derivatives only within the agreed research scope; modification of the material is not permitted, nor may it be distributed, sold, transferred, or otherwise provided to any other entity (including affiliates).
- If use beyond the above scope is required, prior written permission from Genomeditech (Shanghai) Co.,Ltd. must be obtained. For details, please contact Genomeditech (Shanghai) Co.,Ltd.