

Product Sheet

H_IL-23 Reporter 293 Cell Line

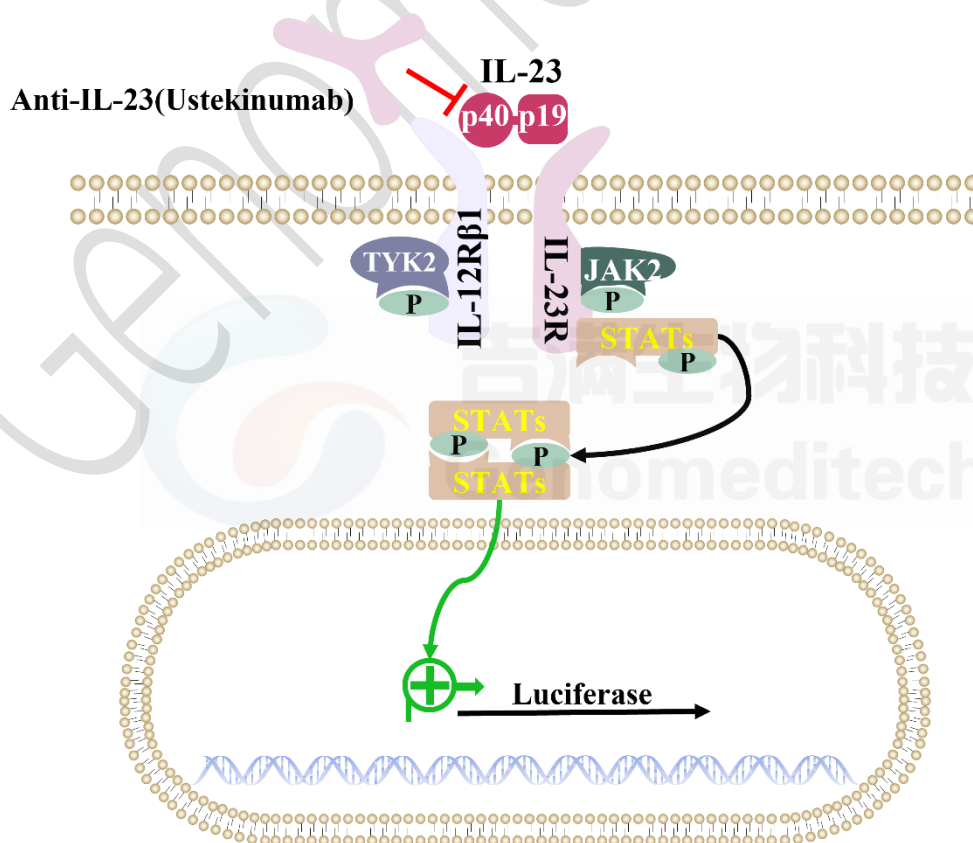
Catalog number: GM-C06722

Version 3.3.1.241205

Interleukin-23 (IL-23) is an inflammatory cytokine that belongs to the IL-12 cytokine family. It consists of two subunits: IL-12B (IL-12p40) and IL-23A (IL-23p19). It's mainly secreted by activated dendritic cells, macrophages, or monocytes. IL-23 is a crucial cytokine for the maintenance and expansion of T helper 17 cells (Th17 cells), enabling them to release their effector cytokines that mediate autoimmune responses.

The functional receptor for IL-23 (IL-23 receptor) is a heterodimer formed between IL-12R β 1 and IL-23R. IL-23 is associated with autoimmune inflammatory diseases such as colitis, gastritis, psoriasis, and arthritis. Ustekinumab is a monoclonal antibody targeting this cytokine, acting on the P40 subunit, and is used to treat certain autoimmune diseases.

H_IL-23 Reporter 293 Cell Line is a clonal stable HEK-293 cell line constructed using lentiviral technology, constitutive expression of human IL-12R β 1, IL-23R, along with signal-dependent expression of a luciferase reporter gene. It serves two primary purposes: firstly, when an agonist drug is added, it activates downstream signaling in the reporter gene cells, and luciferase expression is measured to screen or validate agonist drugs targeting IL-12R β 1 and IL-23R. Secondly, by introducing an IL-23 antagonist antibody to block the signaling activated by IL-23 and measuring luciferase expression, the cell line can be used to screen or validate antagonist drugs targeting IL-23.



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+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 | Cegrogen biotech/A0500-3010 |
| Pen/Strep | Thermo/15140-122 |
| Blasticidin | Genomeditech/ GM-040404 |
| Hygromycin | Genomeditech/ GM-040403 |
| Puromycin | Genomeditech/ GM-040401 |
| Recombinant Human IL-23 (C-6His) | Novoprotein/CJ40 |
| Anti-IL-12/23(p40) hIgG1 antibody(Ustekinumab) | Genomeditech/ GM-46333AB |
| GMOne-Step Luciferase Reporter Gene Assay Kit | Genomeditech/ GM-040503 |

Figures

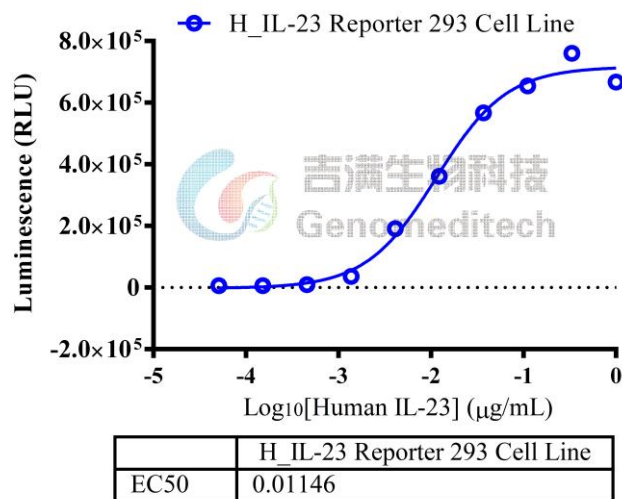


Figure 1 | Response to Human IL-23 protein. The H_IL-23 Reporter 293 Cell Line (Cat. GM-C06722) at a concentration of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of Recombinant Human IL-23 (C-6His) (Novoprotein/CJ40) in assay buffer (DMEM + 1% FBS + 1% P.S) for 16 hours. The firefly luciferase activity was measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). The maximum induction fold was approximately [118.6]. Data are shown by drug mass concentration.

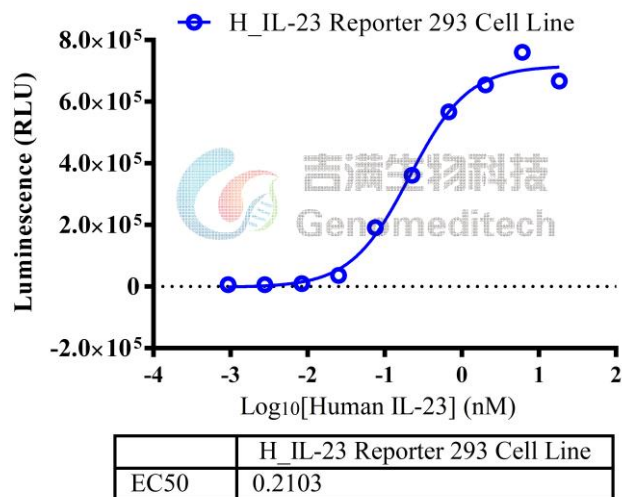


Figure 2 | Response to Human IL-23 protein. The H_IL-23 Reporter 293 Cell Line (Cat. GM-C06722) at a concentration of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of Recombinant Human IL-23 (C-6His) (Novoprotein/CJ40) in assay buffer (DMEM + 1% FBS + 1% P.S) for 16 hours. The firefly luciferase activity was measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). The maximum induction fold was approximately [118.6]. Data are shown by drug molar concentration.

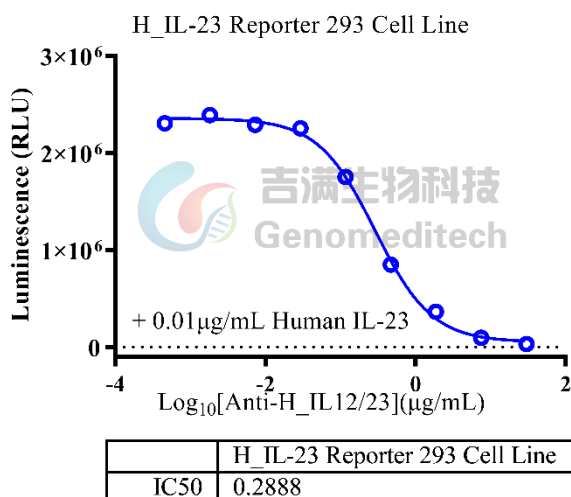


Figure 3 | Response to Anti-H_IL-12/23 hlgG1 antibody. Serial dilutions of Anti-H_IL-12/23(p40) hlgG1 antibody(Ustekinumab) (Cat. [GM-46333AB](#)) was incubated with 1 ng/well of Recombinant Human IL-23 (C-6His) (Novoprotein/CJ40) for 1 hour in assay buffer (DMEM + 1% FBS + 1% P.S). After pre-incubation, add the mixture to the H_IL-23 Reporter 293 Cell Line (Cat. GM-C06722) 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 GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. [GM-040503](#)). The results indicated maximum blocking folds of approximately [78.9]. Data are shown by drug mass concentration.

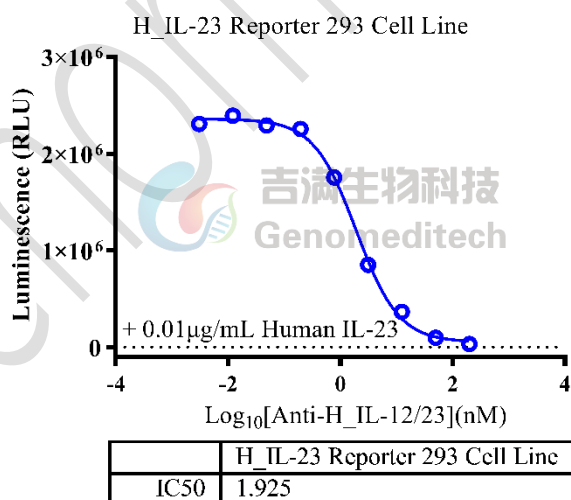


Figure 4 | Response to Anti-H_IL-12/23 hlgG1 antibody. Serial dilutions of Anti-H_IL-12/23(p40) hlgG1 antibody(Ustekinumab) (Cat. [GM-46333AB](#)) was incubated with 1 ng/well of Recombinant Human IL-23 (C-6His) (Novoprotein/CJ40) for 1 hour in assay buffer (DMEM + 1% FBS + 1% P.S). After pre-incubation, add the mixture to the H_IL-23 Reporter 293 Cell Line (Cat. GM-C06722) 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 GMOne-Step Luciferase Reporter Gene

Assay Kit (Cat. [GM-040503](#)). The results indicated maximum blocking folds of approximately [78.9]. Data are shown by drug molar concentration.

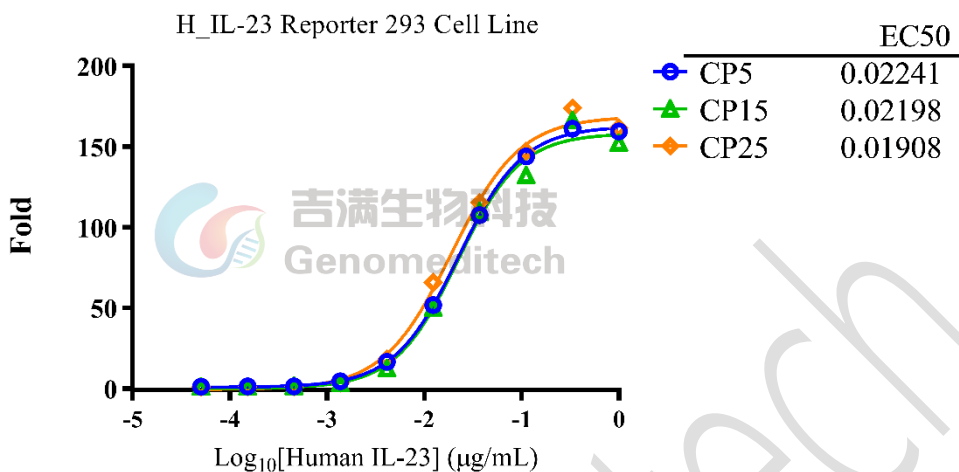


Figure 5 | The passage stability of response to Recombinant Human IL-23 (C-6His). The passage 5, 15 and 25 of H_IL-23 Reporter 293 Cell Line (Cat. GM-C06722) at a concentration of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of Recombinant Human IL-23 (C-6His) (Novoprotein/CJ40) in assay buffer (DMEM + 1% FBS + 1% P.S) for 16 hours. The firefly luciferase activity was measured using the GOne-Step Luciferase Reporter Gene Assay Kit (Cat. [GM-040503](#)). Data are shown by drug mass concentration.

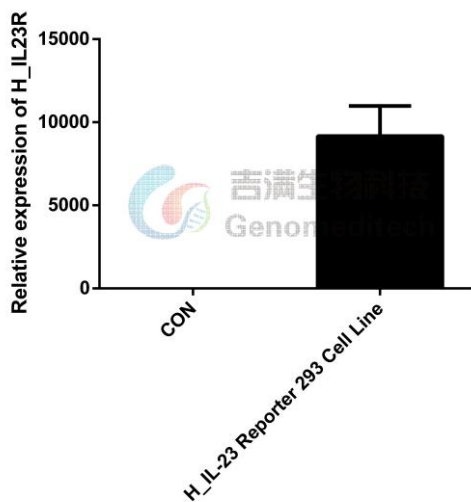


Figure 6 | The mRNA expression levels of H_IL-23R in the H_IL-23 Reporter 293 Cell Line (Cat. GM-C06722) were determined by RT-qPCR.

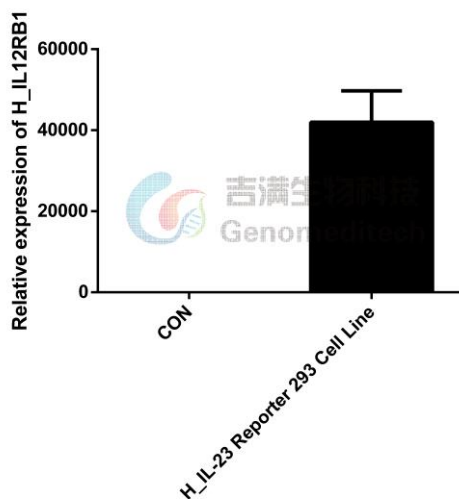


Figure 7 | The mRNA expression levels of H_IL-12RB1 in the H_IL-23 Reporter 293 Cell Line (Cat. GM-C06722) were determined by RT-qPCR.

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.

- 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).
- 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.
- Transfer the vial contents to a centrifuge tube containing 5.0 mL complete culture medium and spin at approximately $176 \times g$ for 5 minutes. Discard supernatant.
- Resuspend cell pellet with the recommended recovery medium. And dispense into appropriate culture dishes.
- 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

- Centrifuge at $176 \times g$ for 3 minutes to collect cells.
- Resuspend the cells in pre-cooled freezing medium and adjust the cell density to $5\text{E}6$ cells/mL.
- 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 $\mu\text{g}/\text{mL}$ Blasticidin+125 $\mu\text{g}/\text{mL}$ Hygromycin+0.75 $\mu\text{g}/\text{mL}$ Puromycin

For the first 1 to 2 passages post-resuscitation, use the recovery medium. Once the cells have stabilized, switch to a growth medium.

- 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.
- Remove and discard culture medium.
- Briefly rinse the cell layer with PBS to remove all traces of serum that contains trypsin inhibitor.
- 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).
- 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.
- Add 2.0 mL of growth medium to mix well and aspirate cells by gently pipetting.
- After centrifugation, resuspend the pellet and add appropriate aliquots of the cell suspension to new culture vessels.
- 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-23 | |
|--|--|
| H_IL-23R HEK-293 Cell Line | |
| TNF:TNFR2:TNFR1 | |
| H_TNFR2 Null Reporter Cell Line | H_TNFR2 Reporter Jurkat Cell Line |
| H_TNFR2 Reporter V2 Cell Line | Cynomolgus_TNFRSF1B(TNFR2) CHO-K1 Cell Line |
| H_TNFRSF1B(TNFR2) CHO-K1 Cell Line | H_TNFRSF1B(TNFR2) HEK-293 Cell Line |
| Membrane Bound H_TNFα CHO-K1 Cell Line | Membrane Bound H_TNFα(cleavage-resistant) CHO-K1 Cell Line |
| Anti-H_TNFR2 hIgG1 Antibody(1H10) | Anti-H_TNFRSF1B(TNFR2) hIgG1 Antibody(UC2.3.8) |

| | |
|--|---|
| Anti-TNFR1 hIgG1 Antibody(Atrosab) | Anti-TNF- α hIgG1 Antibody (CT-P17) |
| TL1A:DR3(TNFRSF25) | |
| H_TNFRSF25(DR3) Reporter Jurkat Cell Line | H_TNFSF15(TL1A) Reporter Cell Line |
| Mouse_TNFRSF25(DR3) Reporter Jurkat Cell Line | Cynomolgus_TNFSF15(TL1A) HEK-293 Cell Line |
| H_TNFRSF25(DR3) CHO-K1 Cell Line | H_TNFRSF25(DR3) HEK-293 Cell Line |
| H_TNFSF15(TL1A) CHO-K1 Cell Line | H_TNFSF15(TL1A) HEK-293 Cell Line |
| Mouse_TNFSF15(TL1A) HEK-293 Cell Line | |
| Anti-H_TNFSF15(TL1A) hIgG1 Antibody(PF-06480605) | Anti-H_TNFSF15(TL1A) hIgG1 Antibody(Tulisokibart、PRA-023) |
| Anti-H_TNFSF15(TL1A) hIgG4 Antibody | Anti-TL1A hIgG1 Reference Antibody (Duvbio) |
| Anti-TL1A hIgG1 Reference Antibody (Tulbio) | Anti-TNFRSF25(DR3) hIgG1 Antibody(PTX-35) |
| Cynomolgus TL1A Protein; His Tag | Human TL1A Protein; His Tag |

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