Genomeditech (Shanghai) Co.,Ltd.
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Product Sheet

H_IL18 Reporter 293 Cell Line

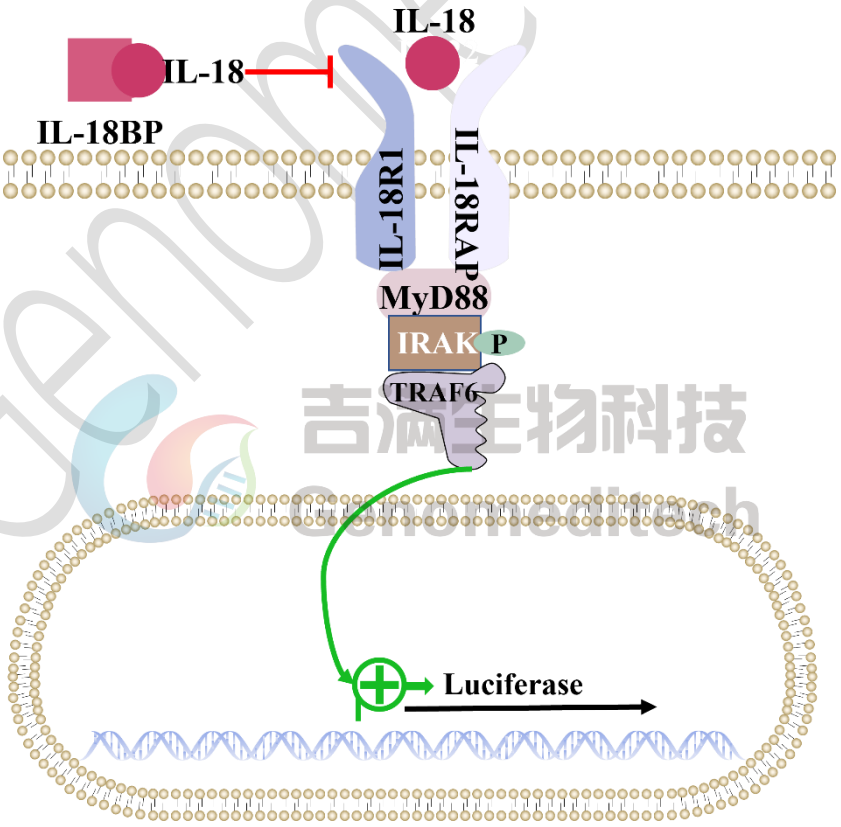
Catalog number: GM-C21147

Version 3.3.1.241113

Interleukin 18 (IL-18), also known as interferon-gamma inducing factor, is a protein encoded by the IL18 gene in the human body. The protein encoded by this gene is a pro-inflammatory cytokine. Many types of cells, including both hematopoietic and non-hematopoietic cells, have the potential to produce IL-18.

Free IL-18 binds to a specific heterodimeric cell surface receptor, which is a member of the IL-1 receptor/Toll-like receptor superfamily,composed of IL-18Rα (IL-18R1) and IL-18Rβ (IL-18RAP) subunits. This binding recruits the MyD88 adaptor protein, leading to the activation of IRAK, which then interacts with TRAF6 to initiate downstream signaling pathways.

H_IL18 Reporter 293 Cell Line is a clonal stable 293 cell line constructed using lentiviral technology, constitutively expressing the IL-18R1 and IL-18RAP, along with signal-dependent expression of a luciferase reporter gene. When IL18 protein binds to IL-18R1 and IL-18RAP, 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 activity of drugs related to IL18.



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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	EMEM(ATCC)+10% FBS+1% P.S
Growth medium	EMEM(ATCC)+10% FBS+1% P.S+3 μg/mL Blasticidin+400 μg/mL G418+1.5 μg/mL Puromycin
Note	Cells should be cultured using 30-2003 EMEM medium from ATCC or the Growth medium purchased from Genomeditech. The serum should be the same as specified in the instructions or sourced from Gibco.
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.
EMEM	ATCC/30-2003
Fetal Bovine Serum	Cegrogen biotech/A0500-3010
Pen/Strep	Thermo/15140-122
Blasticidin	Genomeditech/GM-040404
G418	Genomeditech/GM-040402
Puromycin	Genomeditech/GM-040401
Human IL-18 Protein	KACTUS/IL1-HE018
Recombinant Human IL-18 BPa Fc Chimera Protein	R&D SYSTEMS/119-BP-100
IL18R1 Antibody (APC), Mouse Mab	Sino Biological/11102-MM17-A
Firely Luciferase Assay Reagent(the Kit is replaced by GMOne-Step 2.0)	Genomeditech/G0483M002

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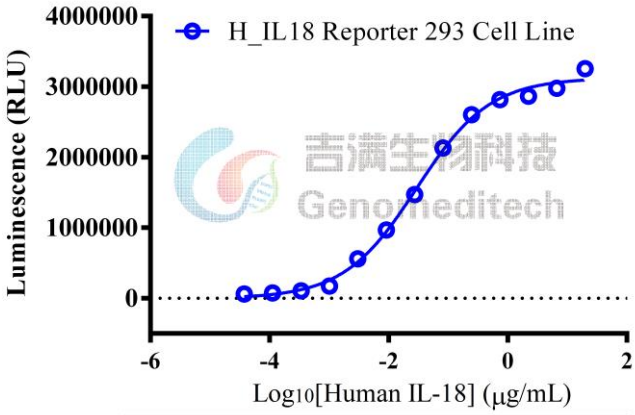
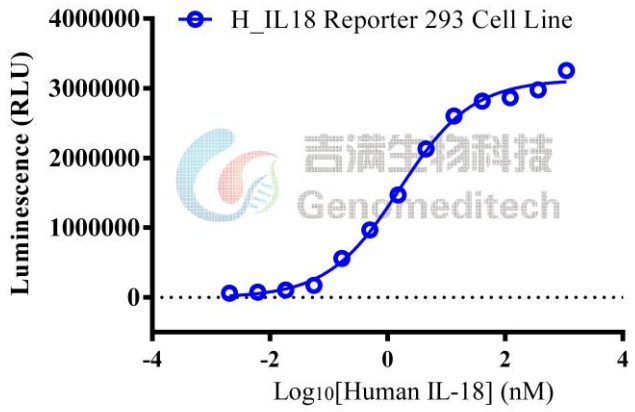
Figures



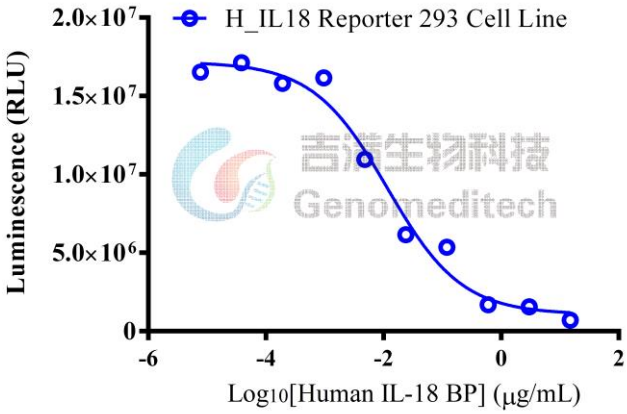
	H_IL18 Reporter 293 Cell Line
EC50	0.02959

Figure 1 | Response to Human IL-18 Protein. The H_IL18 Reporter 293 Cell Line (Cat. GM-C21147) at a concentration of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of Human IL-18 Protein (KACTUS/IL1-HE018) in assay buffer (EMEM(ATCC) + 1% FBS + 1% P.S) for 16 hours. The firefly luciferase activity was measured using the Firely Luciferase Assay Reagent (Cat. G0483M002). The maximum induction fold was approximately [82.0]. Data are shown by drug mass concentration.



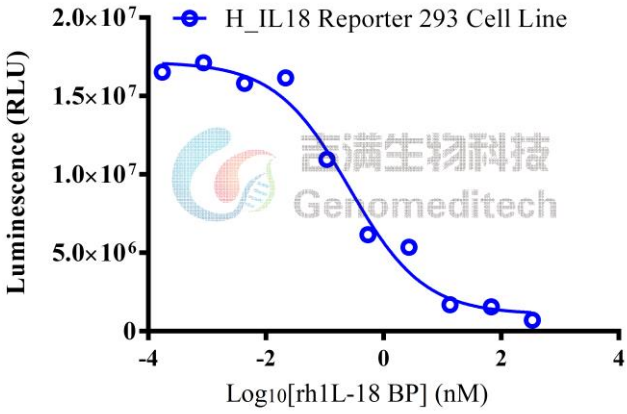
	H_IL18 Reporter 293 Cell Line
EC50	1.626

Figure 2 | Response to Human IL-18 Protein. The H_IL18 Reporter 293 Cell Line (Cat. GM-C21147) at a concentration of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of Human IL-18 Protein (KACTUS/IL1-HE018) in assay buffer (EMEM(ATCC) + 1% FBS + 1% P.S) for 16 hours. The firefly luciferase activity was measured using the Firely Luciferase Assay Reagent (Cat. G0483M002). The maximum induction fold was approximately [82.0]. Data are shown by drug molar concentration.



	H_IL18 Reporter 293 Cell Line
IC50	0.01166

Figure 3 | Response to Recombinant Human IL-18 Bpa Fc Chimera Protein. Serial dilutions of Recombinant Human IL-18 Bpa Fc Chimera Protein (R&D SYSTEMS/119-BP) was incubated with 0.21 μg/mL of Human IL-18 Protein (KACTUS/IL1-HE018) for 1 hour in assay buffer (EMEM(ATCC) + 1% FBS + 1% P.S). After pre-incubation, add the mixture to the H_IL18 Reporter 293 Cell Line (Cat. GM-C21147) at a density of 1.5E4 cells/well in a 96-well format, and incubate for 15 hours. Firefly luciferase activity is then measured using the Firely Luciferase Assay Reagent (Cat. G0483M002). The results indicated maximum blocking folds of approximately [24.5], respectively. Data are shown by drug mass concentration.



	H_IL18 Reporter 293 Cell Line
IC50	0.2638

Figure 4 | Response to Recombinant Human IL-18 BPa Fc Chimera Protein. Serial dilutions of Recombinant Human IL-18 BPa Fc Chimera Protein (R&D SYSTEMS/119-BP) was incubated with 0.21 μg/mL of Human IL-18 Protein (KACTUS/IL1-HE018) for 1 hour in assay buffer (EMEM(ATCC) + 1% FBS + 1% P.S). After pre-incubation, add the mixture to the H_IL18 Reporter 293 Cell Line (Cat. GM-C21147) at a density of 1.5E4 cells/well in a 96-well format, and incubate for 15 hours. Firefly luciferase activity is then measured using the Firely Luciferase Assay Reagent

(Cat. G0483M002). The results indicated maximum blocking folds of approximately [24.5], respectively. Data are shown by drug molar concentration.

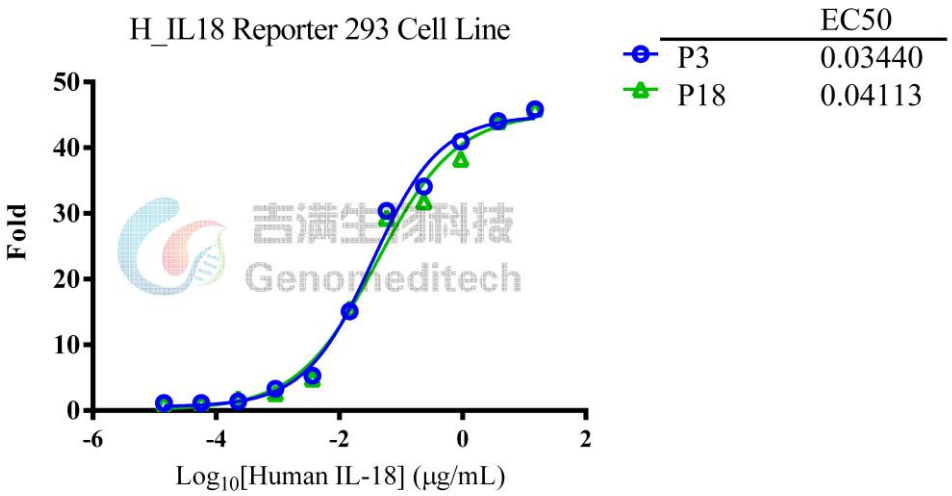


Figure 5 | The passage stability of response to Human IL-18 Protein. The passage 3 and 18 of H_IL18 Reporter 293 Cell Line (Cat. GM-C21147) at a concentration of 1.5E4 cells/well (96-well format) were stimulated with serial dilutions of Human IL-18 Protein (KACTUS/IL1-HE018) in assay buffer (EMEM(ATCC) + 1% FBS + 1% P.S) for 16 hours. The firefly luciferase activity was measured using the Firely Luciferase Assay Reagent (Cat. G0483M002). Data are shown by drug mass concentration.

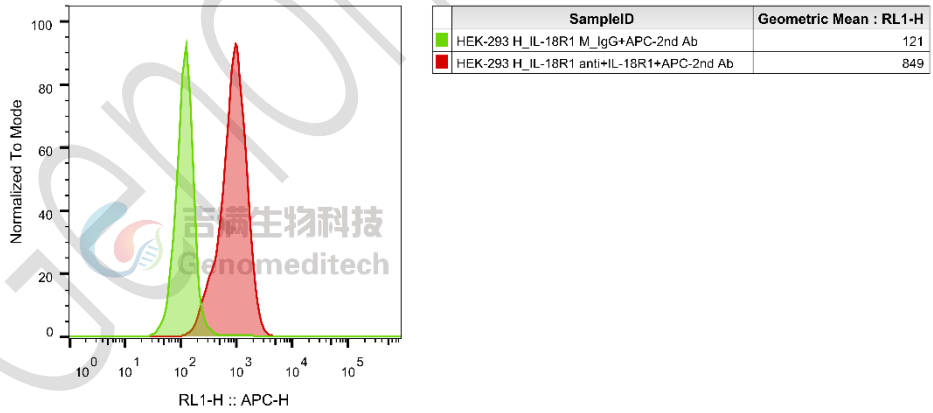


Figure 6 | H_IL18 Reporter 293 Cell Line (Cat. GM-C21147) was determined by flow cytometry using IL18R1 Antibody (APC), Mouse MAb (Sino Biologica/11102-MM17-A).

Cell Recovery

Recovery Medium: EMEM(ATCC)+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: EMEM(ATCC)+10% FBS+1% P.S+3 μg/mL Blasticidin+400 μg/mL G418+1.5 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) Remove and discard culture medium.
b) Briefly rinse the cell layer with PBS to remove all traces of serum that contains trypsin inhibitor.
c) 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).
d) 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.
e) Add 2.0 mL of growth medium to mix well and aspirate cells by gently pipetting.
f) After centrifugation, resuspend the pellet and add appropriate aliquots of the cell suspension to new culture vessels.
g) Incubate cultures at 37°C.

Subcultivation Ratio: A subcultivation ratio of 1:3 - 1:4 is recommended

Medium Renewal: Every 2 to 3 days

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Notes

a) Upon initial revival, a higher number of dead cells and poor adherence are observed, which is normal. Adherence typically recovers within 2 - 3 days. After 2 - 3 passages, the proportion of adherent cells increases, and the cells begin to spread normally.

b) After each passage, there may be 5 - 10% dead cells; however, as the number of passages increases, the recovery rate accelerates, the proportion of dead cells decreases, and the cell growth rate stabilizes.

c) It is recommended to retain cell images after revival and during each observation to assist in assessing cell status. In case of abnormalities, promptly communicate with Genomeditech sales.

Related Products

IL-18	
Mouse_IL18 Reporter 293 Cell Line	

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