Genomeditech (Shanghai) Co.,Ltd.
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Product Sheet

H_IL-36 Reporter 293 Cell Line

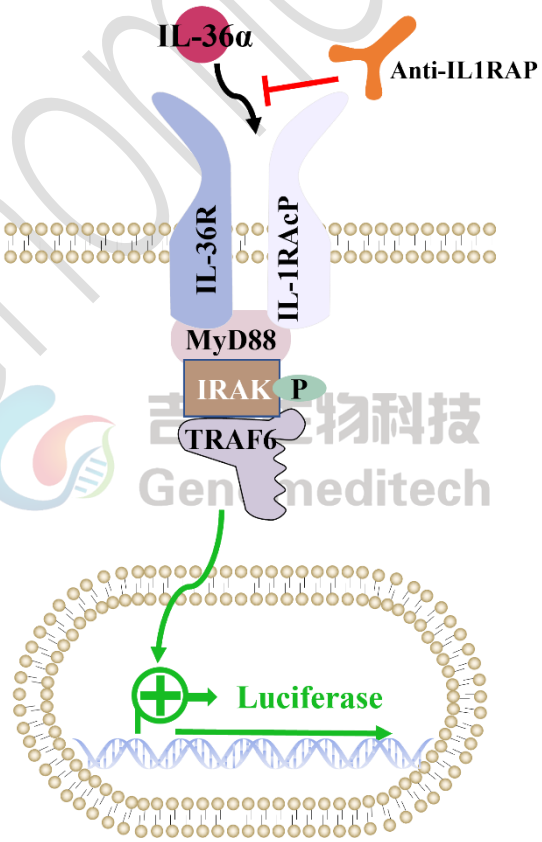
Catalog number: GM-C46318

Version 3.3.1.260512

IL-36 is a cytokine from the IL-1 family, mainly produced by keratinocytes and immune cells. It is crucial for immune responses in the skin and mucosa, especially in inflammation and autoimmune diseases. There are three subtypes: IL-36α, IL-36β, and IL-36γ, which activate inflammatory responses by binding to the IL-36 receptor.

The IL-36 signaling pathway is mediated by the IL-36 receptor (Heterodimer of IL-36R and IL-1RAcP), activating the MyD88-dependent pathway and transcription factors like NF-κB and MAPK. This leads to the production of pro-inflammatory cytokines such as IL-6 and TNF-α, enhancing inflammatory responses. The IL-36 pathway is significant in diseases like psoriasis and rheumatoid arthritis.

H_IL-36 Reporter 293 Cell Line is a clonal stable 293 cell line constructed using lentiviral technology, which constitutively expresses the IL-36R (IL1RL2) gene, endogenously expresses the IL1RAP gene, and signal-dependently expresses a luciferase reporter gene. The addition of IL-36 ligand protein agonists promotes ligand binding to IL-36R and IL1RAP, which activates downstream signaling and induces luciferase expression. This system can be used to evaluate the in vitro effects of drugs related to IL-36.



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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	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-36 alpha/IL-1F6 Protein	Sino Biological/10607-HNCE1
Anti-IL1RAP hIgG1 Antibody (48D2_VH5.GL_VL4)	Genomeditech/GM-88388AB
GMOne-Step 2.0 Luciferase Reporter Gene Assay Kit	Genomeditech/GM-040513

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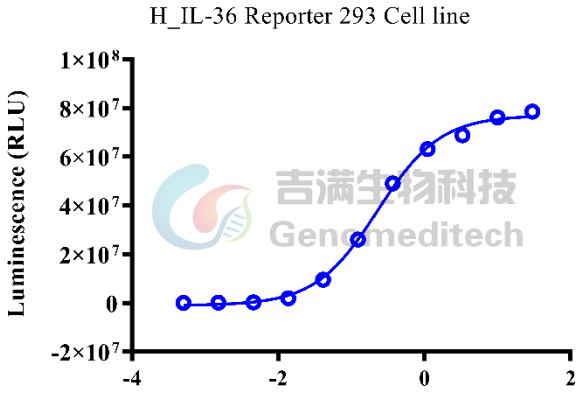
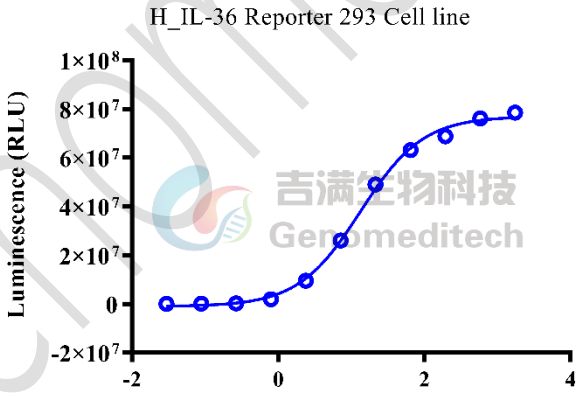
Figures



	H_IL-36 Reporter 293 Cell line
EC50	0.2302

Figure 1 | Response to Recombinant Human IL-36 alpha/IL-1F6 Protein. H_IL-36 Reporter 293 Cell Line (Cat. GM-C46318) at a concentration of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of Human IL-36 alpha (Sinobiological/10607-HNCE1) in assay buffer (DMEM+1% FBS+1% P.S) for 6 hours. The firefly luciferase activity was measured using the Luciferase Reporter Assay Kit (Genomeditech). The maximum induction fold was approximately [1238.4]. Data are shown by drug mass concentration.



	H_IL-36 Reporter 293 Cell line
EC50	13.39

Figure 2 | Response to Recombinant Human IL-36 alpha/IL-1F6 Protein. H_IL-36 Reporter 293 Cell Line (Cat. GM-C46318) at a concentration of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of Human IL-36 alpha (Sinobiological/10607-HNCE1) in assay buffer (DMEM+1% FBS+1% P.S) for 6 hours. The firefly luciferase activity was measured using the Luciferase Reporter Assay Kit (Genomeditech). The maximum induction fold was approximately [1238.4]. Data are shown by drug molar concentration.

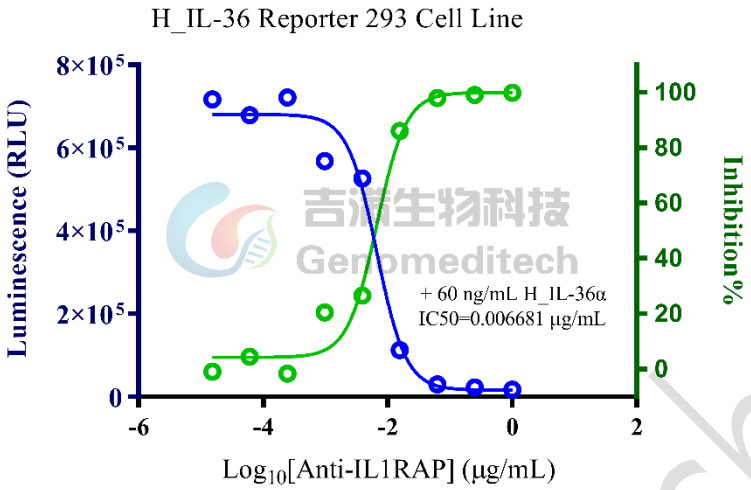


Figure 3 | Inhibition of Recombinant Human IL-36 alpha/IL-1F6 Protein-induced reporter activity by Anti-IL1RAP hIgG1 Antibody (48D2_VH5.GL_VL4). Serial dilutions of the Anti-IL1RAP hIgG1 Antibody (48D2_VH5.GL_VL4) (Cat. GM-88388AB) was incubated with 1.5E4 cells/well of the H_IL-36 Reporter 293 Cell Line (Cat. GM-C46318) in a 96-well plate for 1 hour in assay buffer (DMEM +1% FBS+1% P.S). Subsequently, the Human IL-36 alpha (Sinobiological/10607-HNCE1) at a concentration of 6 ng/well was added, and the coculture proceeded for an additional 6 hours. Firefly luciferase activity is then measured using the Luciferase Reporter Assay Kit (Genomeditech)(left Y-axis, relative luminescence units), with inhibition percentages shown on the right Y-axis.

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 complete medium. And 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.

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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+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) 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 3 days

Notes

a) After initial thawing, a higher proportion of dead cells is normal. The cell culture generally improves noticeably after approximately one week of adaptation. Once the culture stabilizes, the percentage of dead cells decreases with subsequent passages, and the cell proliferation rate becomes more consistent.

b) It is important to maintain the cell density below 80%, as exceeding this threshold can lead to decreased cell viability and metabolic activity due to overcrowding.

c) FBS requires heat inactivation at 56°C for 30 minutes, which can inactivate complement and some viruses, but does not significantly affect the activity of most growth factors and cytokines.

Related Products

IL-36	
H_IL-36 Reporter 293 Cell Line (old version)	

Anti-IL1RL2(IL-36R) hIgG1 Antibody(recibokibart)	Anti-IL1RL2(IL-36R) hIgG1 Antibody(spesolimab)

License Agreement:

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