Genomeditech (Shanghai) Co.,Ltd.
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Product Sheet

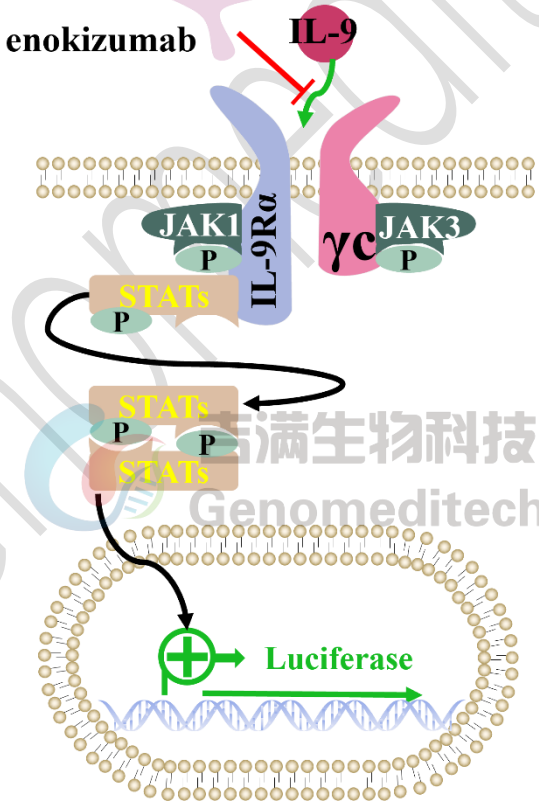
H_IL-9 Reporter 293 Cell Line

Catalog number: GM-C37820

Version 3.3.1.250123

IL-9 (Interleukin-9) is a cytokine secreted by lymphocytes and belongs to the type II cytokine family. Initially referred to as a T-cell growth factor or hematopoietic factor, it plays multiple roles in the immune system, primarily affecting T cells, B cells, mast cells, eosinophils, and other cell types.

H_IL-9 Reporter 293 Cell Line is a clonal stable 293 cell line constructed using lentiviral technology, constitutive expression of the IL-9Rα gene and IL2RG(γc) gene, along with signal-dependent expression of a luciferase reporter gene. When igand binds to IL-9, 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 effects of drugs related to IL-9.



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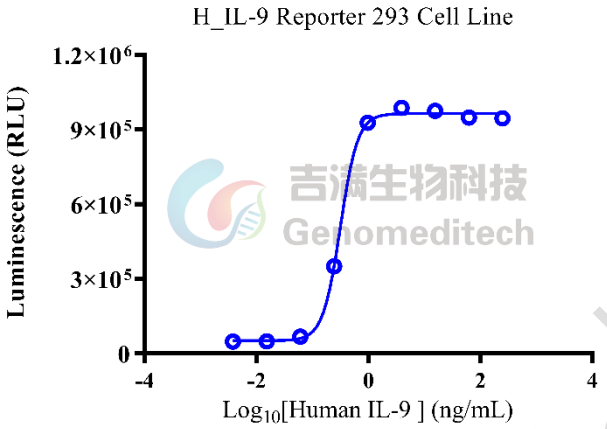
Specifications

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+150 μg/mL Bleomycin+400 μg/mL G418+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

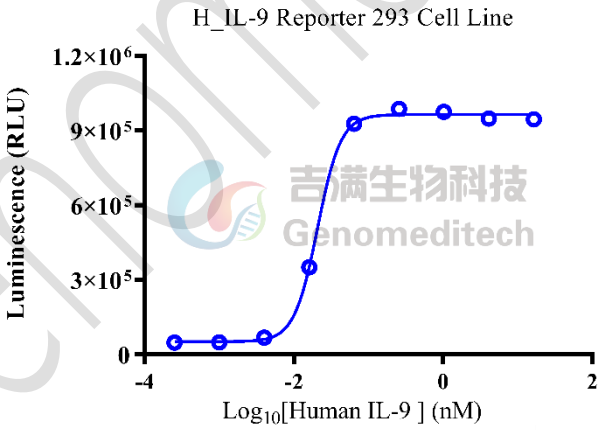
Reagent	Manufacturer/Catalogue No.
DMEM	Gibco/C11995500BT
Fetal Bovine Serum	Cegrogen biotech/A0500-3010
Pen/Strep	Thermo/15140-122
Blasticidin	Genomeditech/GM-040404
Bleomycin	Genomeditech/GM-040407
G418	Genomeditech/GM-040402
Puromycin	Genomeditech/GM-040401
Recombinant Human IL-9 (C-6His)	Novoprotein/CA17
Recombinant Mouse IL-9 (C-6His)	Novoprotein/CC22
Anti-IL-9 hlgG1 Antibody(enokizumab)	Genomeditech/GM-87796AB
Anti-CD132(IL2RG) hIgG4 Antibody(REGN7257)	Genomeditech/GM-52334AB
PE anti-human CD129 (IL-9 R) Antibody	Biolegend/31040
GMOne-Step Luciferase Reporter Gene Assay Kit	Genomeditech/GM-040503

Figures



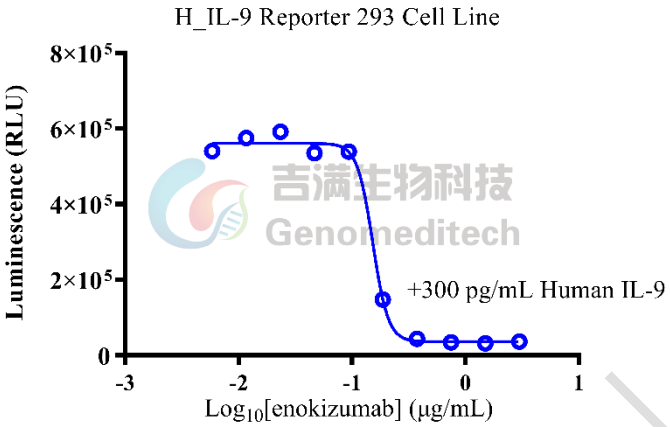
	H_IL-9 Reporter 293 Cell Line
EC50	0.3160

Figure 1 | Response to Human IL-9 protein. The H_IL-9 Reporter 293 Cell Line (Cat. GM-C37820) at a concentration of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of Recombinant Human IL-9(C-6His) (novoprotein/CA17) in assay buffer (DMEM + 1% FBS + 1% P.S) for 6 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 [19.8]. Data are shown by drug mass concentration.



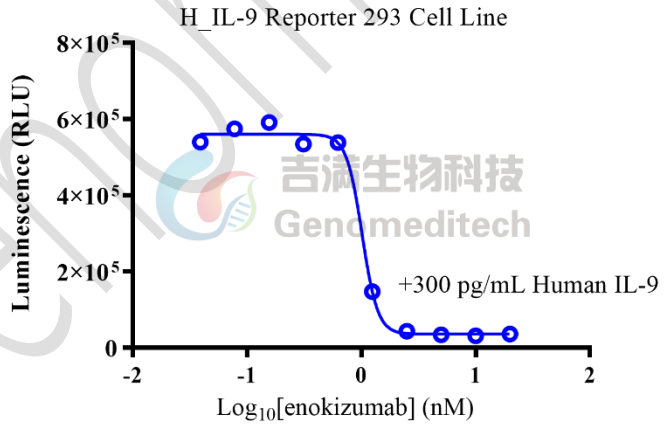
	H_IL-9 Reporter 293 Cell Line
EC50	0.02085

Figure 2 | Response to Human IL-9 protein. The H_IL-9 Reporter 293 Cell Line (Cat. GM-C37820) at a concentration of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of Recombinant Human IL-9(C-6His) (novoprotein/CA17) in assay buffer (DMEM + 1% FBS + 1% P.S) for 6 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 [19.8]. Data are shown by drug molar concentration.



	H_IL-9 Reporter 293 Cell Line
IC50	0.1526

Figure 3 | Response to Anti-IL-9 hlgG1 Antibody(enokizumab). Serial dilutions of Anti-IL-9 hlgG1 Antibody(enokizumab) (Cat. GM-87796AB) was incubated with 30 Pg/well of Recombinant Human IL-9 (C-6His) (Novoprotein/CA17) for 1 hour in assay buffer (DMEM+1% FBS+1% P.S). After pre-incubation, add the mixture to the H_IL-9 Reporter 293 Cell Line (Cat. GM-C37820) at a density of 1.5E4 cells/well in a 96-well format, and incubate for 6 hours. Firefly luciferase activity was then measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). The results indicated maximum blocking folds of approximately [15.0]. Data are shown by drug mass concentration.



	H_IL-9 Reporter 293 Cell Line
IC50	1.017

Figure 4 | Response to Anti-IL-9 hlgG1 Antibody(enokizumab). Serial dilutions of Anti-IL-9 hlgG1 Antibody(enokizumab) (Cat. GM-87796AB) was incubated with 30 pg/well of Recombinant Human IL-9 (C-6His) (Novoprotein/CA17) for 1 hour in assay buffer (DMEM+1% FBS+1% P.S). After pre-incubation, add the mixture to the H_IL-9 Reporter 293 Cell Line (Cat. GM-C37820) at a density of 1.5E4 cells/well in a 96-well format, and incubate for 6 hours. Firefly luciferase activity was then measured using the GMOne-Step Luciferase Reporter Gene Assay Kit

(Cat. GM-040503). The results indicated maximum blocking folds of approximately [15.0]. Data are shown by drug molar concentration.

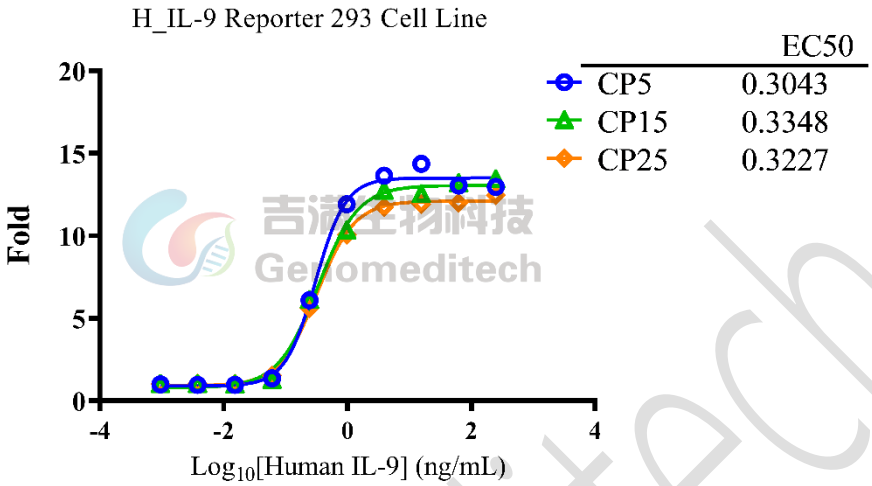
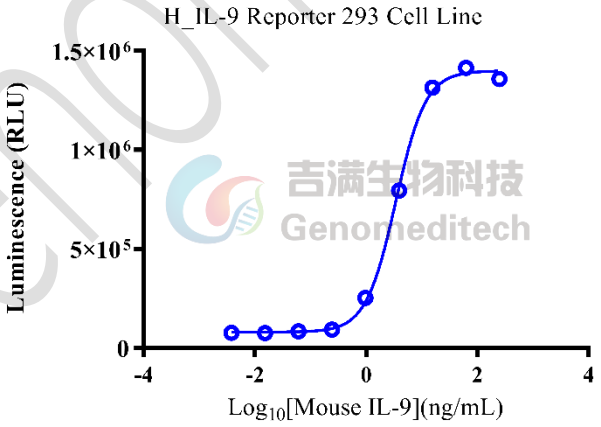
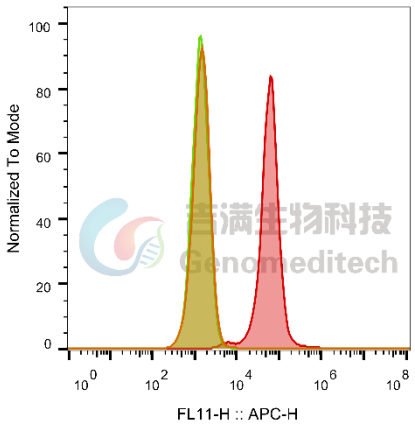


Figure 5 | The passage stability of response to Recombinant Human IL-9 (C-6His). The passage 5, 15, and 25 of H_IL-9 Reporter 293 Cell Line (Cat. GM-C37820) at a concentration of 1.5E4 cells/well (96-well format) were stimulated with serial dilutions of Recombinant Human IL-9 (C-6His) (Novoprotein/CA17) in assay buffer (DMEM+1% FBS+1% P.S) for 6 hours. The firefly luciferase activity was measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). Data are shown by drug mass concentration.



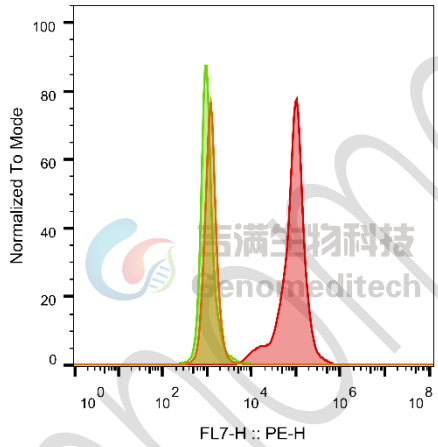
	H_IL-9 Reporter 293 Cell Line
EC50	3.428

Figure 6 | Response to Recombinant Mouse IL-9 (C-6His). The H_IL-9 Reporter 293 Cell Line (Cat. GM-C37820) at a concentration of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of Recombinant Mouse IL-9 (novoprotein/CC22) in assay buffer (DMEM + 1% FBS + 1% P.S) for 6 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 [16.9]. Data are shown by drug mass concentration.



SampleID	Geometric Mean : FL11-H
Null anti-CD132+APC-2nd Ab	1470
H_IL9 Reporter H_IgG+APC-2nd Ab	1390
H_IL9 Reporter anti-CD132+APC-2nd Ab	57945

Figure 7 | H_IL-9 Reporter 293 Cell Line (Cat. GM-C37820) was determined by flow cytometry using Anti-CD132(IL2RG) hIgG4 Antibody(REGN7257) (Cat. GM-52334AB).



SampleID	Geometric Mean : FL7-H
Null PE-anti-CD129 Ab	1244
H_IL-9 Reporter PE-M_IgG Ab	1057
H_IL-9 Reporter PE-anti-CD129 Ab	82860

Figure 8 | H_IL-9 Reporter 293 Cell Line (Cat. GM-C37820) was determined by flow cytometry using PE anti-human CD129 (IL-9 R) Antibody (Biolegend/31040).

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).

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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: DMEM+10% FBS+1% P.S+4 μg/mL Blasticidin+150 μg/mL Bleomycin+400 μg/mL G418+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

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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.

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