Genomeditech (Shanghai) Co.,Ltd.
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Product Sheet

H_IL12 Reporter 293 DDX35™ Cell Line

Catalog number: GM-C26022

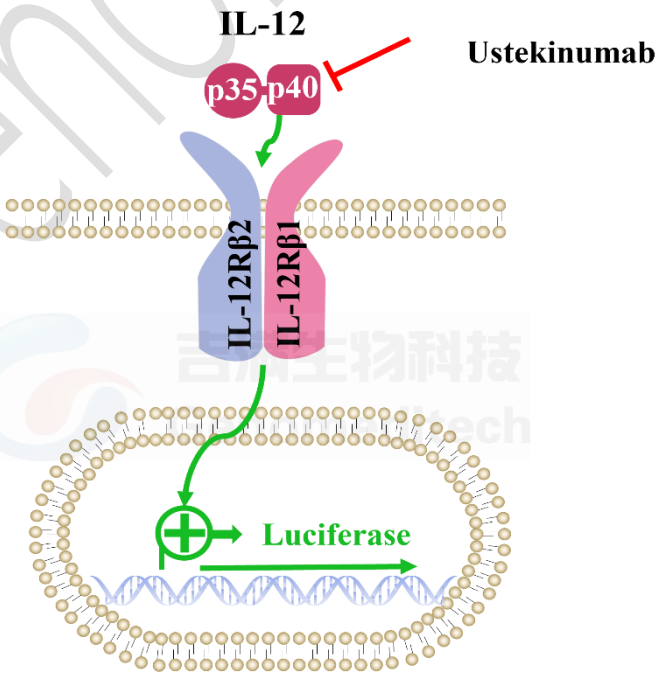
Version 3.3.1.241212

Interleukin-12 (IL-12) is a cytokine produced by macrophages and dendritic cells, composed of two subunits, p35 and p40, which form the active dimer IL-12p70. It plays a crucial role in immune responses by activating and proliferating T cells and natural killer (NK) cells, and it boosts interferon-gamma (IFN-γ) production to enhance immunity against viruses and tumors.

IL-12 binds to its receptor IL-12R, which has two subunits (IL-12Rβ1 and IL-12Rβ2), activating the tyrosine kinases JAK2 and TYK2. This activation leads to the phosphorylation of the STAT4 transcription factor, which then regulates the expression of genes, including IFN-γ production.

H_IL12 Reporter 293 DDX35™ Cell Line is a clonal stable HEK-293 cell line constructed using lentiviral technology, constitutive expression of the IL-12Rβ1 and IL-12Rβ2 gene, along with signal-dependent expression of a luciferase reporter gene. It is activated when IL-12 binds to its receptors. Blockade antibodies can inhibit this signaling. Measuring luciferase activity reflects the activation level of the signaling pathway, allowing for the evaluation of in vitro effects of IL-12-related drugs

The H_IL12 Reporter 293 DDX35™ Cell Line was obtained through extensive monoclonal screening and multiple rounds of monoclonal selection. It possesses high stability, high sensitivity, and high amplification properties, meeting the standards for customers' batch library construction and release experiments.



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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+400 μg/mL G418+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
G418	Genomeditech/GM-040402
Hygromycin	Genomeditech/GM-040403
Puromycin	Genomeditech/GM-040401
Recombinant Human IL-12 Protein (His Tag)	Sino Biological/CT011-H08H
GMOne-Step Luciferase Reporter Gene Assay Kit	Genomeditech/GM-040503

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Figures

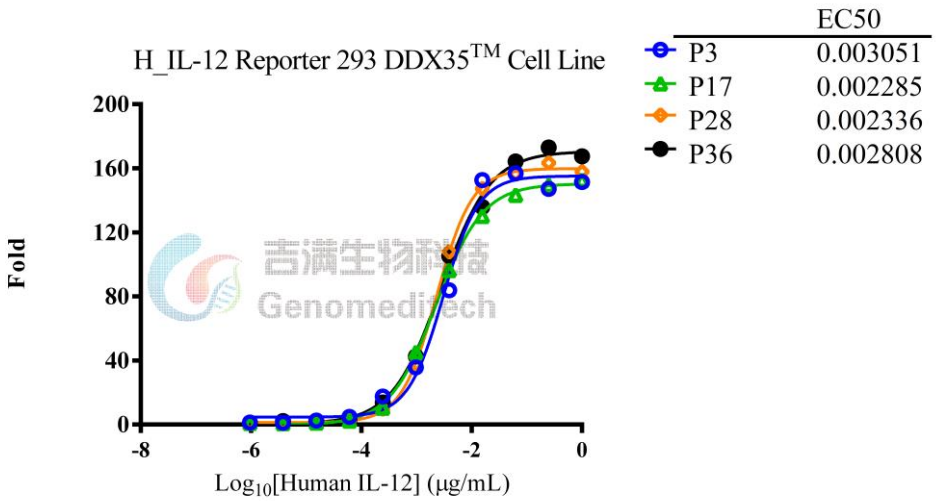

Figure 1 | The passage stability of response to Recombinant Human IL-12 Protein. The passage 3, 17, 28 and 36 of H_IL12 Reporter 293 DDX35™ Cell Line (Cat. GM-C26022) at a concentration of 1.5E4 cells/well (96-well format) were stimulated with serial dilutions of Recombinant Human IL-12 Protein (Sino Biological/CT011-H08H) in assay buffer (DMEM + 1% FBS + 1% P.S) for 7 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.

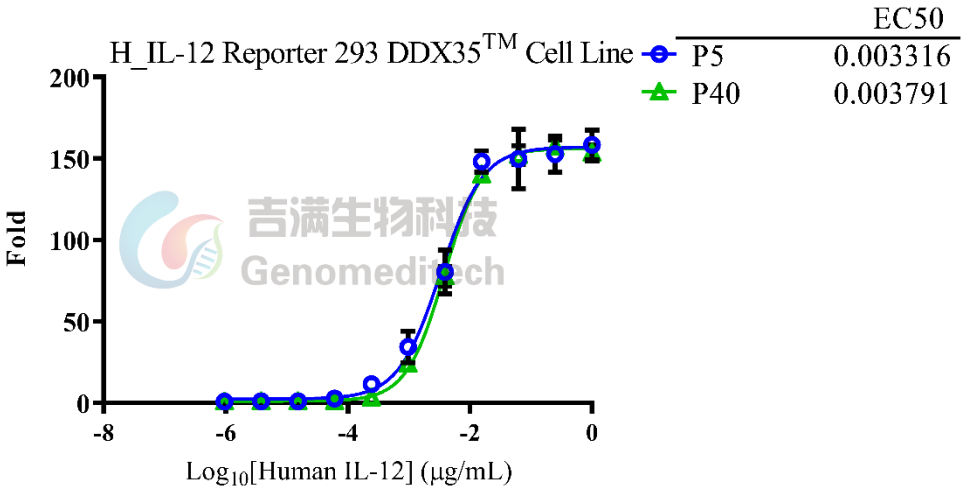

Figure 2 | The passage stability of response to Recombinant Human IL-12 Protein. The passage 5 and 40 of H_IL12 Reporter 293 DDX35™ Cell Line (Cat. GM-C26022) at a concentration of 1.5E4 cells/well (96-well format) were stimulated with serial dilutions of Recombinant Human IL-12 Protein (Sino Biological/CT011-H08H) in assay buffer (DMEM + 1% FBS + 1% P.S) for 7 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.

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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.

Cell passage

Growth medium: DMEM+10% FBS+1% P.S+4 μg/mL Blasticidin+400 μg/mL G418+125 μg/mL Hygromycin+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.

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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-12	
H_IL12 Reporter 293 Cell Line	
Anti-IL-12/23(p40) hIgG1 antibody(Ustekinumab)	

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