Genomeditech (Shanghai) Co.,Ltd.
Order: +86 021-68455258/50432826/50432825
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Product Sheet

H_IL-1 Reporter 293 Cell Line

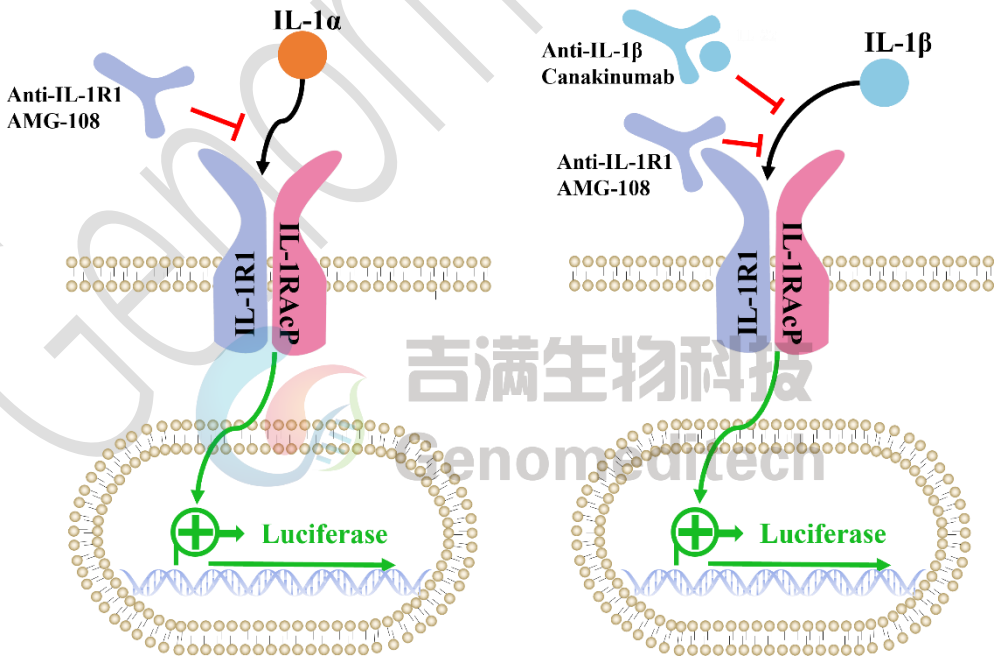
Catalog number: GM-C26463

Version 3.3.1.250909

Interleukin-1 (IL-1) is a key pro-inflammatory cytokine with two forms, IL-1α and IL-1β, produced mainly by macrophages and also by endothelial and epithelial cells. Their activities are generally indistinguishable, and signaling occurs through IL-1R1 (not IL-1R2), which recruits IL-1RAcP to form a high-affinity receptor complex.

IL-1α is rarely detected in circulation during disease, whereas IL-1β is a major mediator of inflammation and host defense. Elevated IL-1β is linked to atherosclerosis, type 2 diabetes, and autoimmune diseases such as rheumatoid arthritis, multiple sclerosis, and Crohn's disease; blocking IL-1 with a receptor antagonist can lessen autoinflammatory disease severity. IL-1β synthesis, release, and activity are tightly controlled via innate sensing of PAMPs and DAMPs.

H_IL-1 Reporter 293 Cell Line is a clonal stable cell line constructed using lentiviral technology, constitutive expression of human IL1RAP gene, and endogenously expression of the IL-1R1 gene, along with signal-dependent expression of a luciferase reporter gene. When IL-1α or IL-1β binds to IL-1R1 and IL-1RAcP, it activates downstream signaling pathways, leading to the expression of luciferase. Blockade antibodies can inhibit this signal transmission. 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-1.



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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
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
Recombinant Human IL-1 alpha/IL1A Protein	Sino Biological/10128-HNCH
Human IL-1 beta / IL1B Protein	Sino Biological/10139-HNAE
Anti-IL-1β hIgG1 Antibody(Canakinumab)	Genomeditech/GM-51764AB
Anti-IL1R1 hIgG1 Antibody(AMG 108)	Genomeditech/GM-51990AB
Anti-IL1RAP hIgG1 Antibody (Nadunolimab)	Genomeditech/GM-88204AB
GMOne-Step 2.0 Luciferase Reporter Gene Assay Kit	Genomeditech/GM-040513

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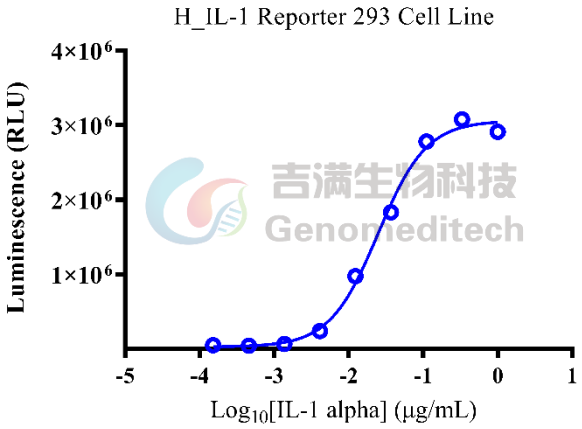
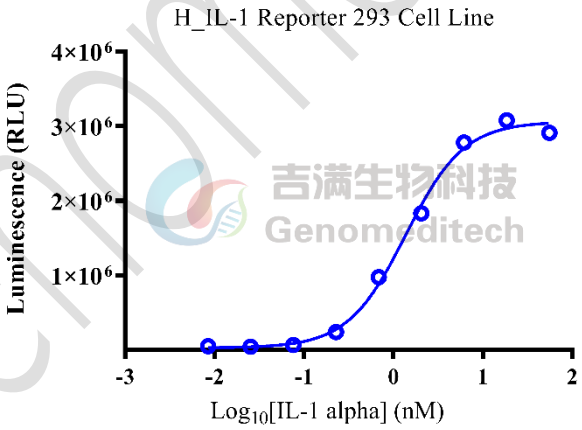
Figures



	H_IL-1 Reporter 293 Cell Line
EC50	0.02481

Figure 1 | Response to Human IL-1 alpha/IL1A. The H_IL-1 Reporter 293 Cell Line (Cat. GM-C26463) at a concentration of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of Recombinant Human IL-1 alpha/IL1A Protein (Sino Biological/10128-HNCH) 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 [71.8]. Data are shown by drug mass concentration.



	H_IL-1 Reporter 293 Cell Line
EC50	1.378

Figure 2 | Response to Human IL-1 alpha/IL1A. The H_IL-1 Reporter 293 Cell Line (Cat. GM-C26463) at a concentration of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of Recombinant Human IL-1 alpha/IL1A Protein (Sino Biological/10128-HNCH) 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 [71.8]. Data are shown by drug molar concentration.

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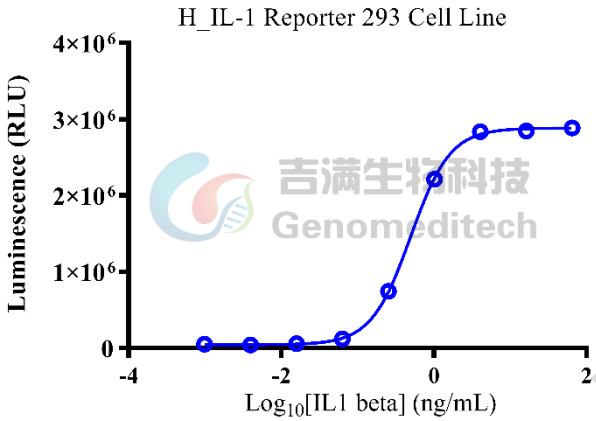
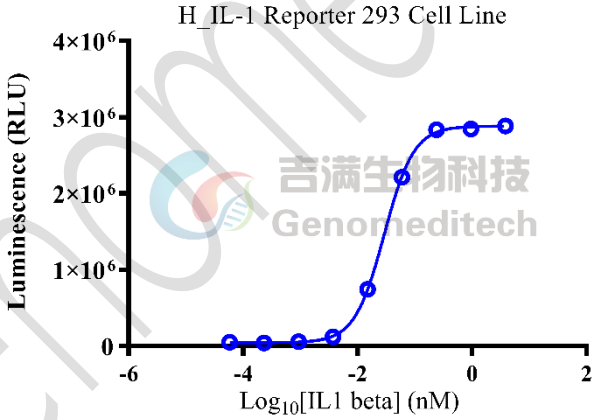


	H_IL-1 Reporter 293 Cell Line
EC50	0.4978

Figure 3 | Response to Human IL-1 beta / IL1B Protein. The H_IL-1 Reporter 293 Cell Line (Cat. GM-C26463) at a concentration of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of Human IL-1 beta / IL1B Protein (Sino Biological/10139-HNAE) 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 [75.4]. Data are shown by drug mass concentration.



	H_IL-1 Reporter 293 Cell Line
EC50	0.02928

Figure 4 | Response to Human IL-1 beta / IL1B Protein. The H_IL-1 Reporter 293 Cell Line (Cat. GM-C26463) at a concentration of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of Human IL-1 beta / IL1B Protein (Sino Biological/10139-HNAE) 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 [75.4]. Data are shown by drug molar concentration.

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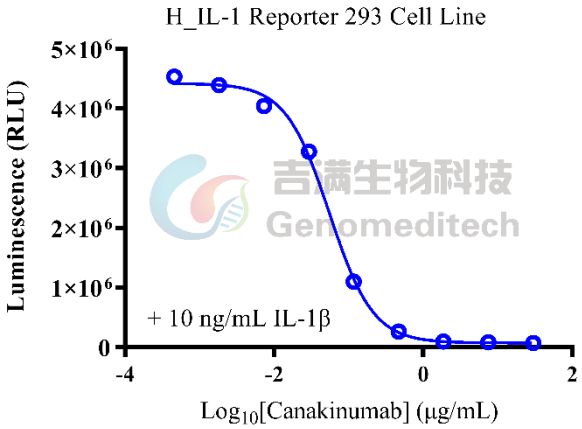
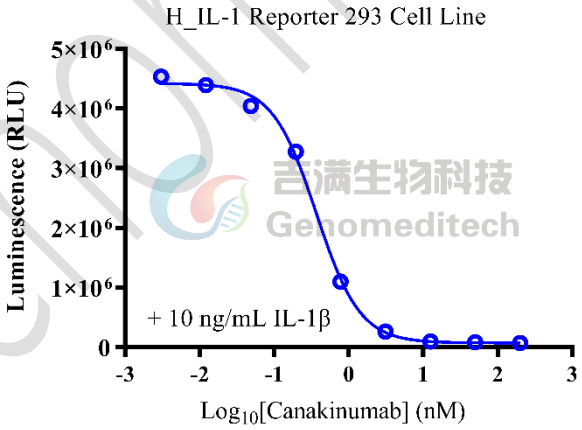


	H_IL-1 Reporter 293 Cell Line
IC50	0.05528

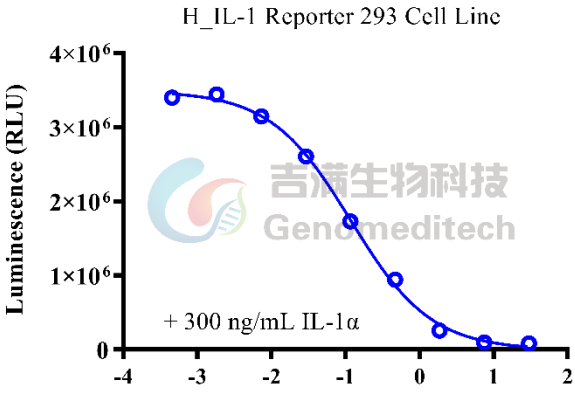
Figure 5 | Response to Anti-IL-1β hIgG1 Antibody(Canakinumab). Serial dilutions of Anti-IL-1β hIgG1 Antibody(Canakinumab) (Cat. GM-51764AB) was incubated with 1 ng/well of Human IL-1 beta / IL1B Protein (Sino Biological/10139-HNAE) for 1 hour in assay buffer (DMEM + 1% FBS + 1% P.S). After pre-incubation, add the mixture to the H_IL-1 Reporter 293 Cell Line (Cat. GM-C26463) at a density of 1.5E4 cells/well in a 96-well format, and incubate for 7 hours. Firefly luciferase activity is then measured using the Luciferase Reporter Assay Kit (Genomeditech). The results indicated maximum blocking folds of approximately [66.9]. Data are shown by drug mass concentration.



	H_IL-1 Reporter 293 Cell Line
IC50	0.3685

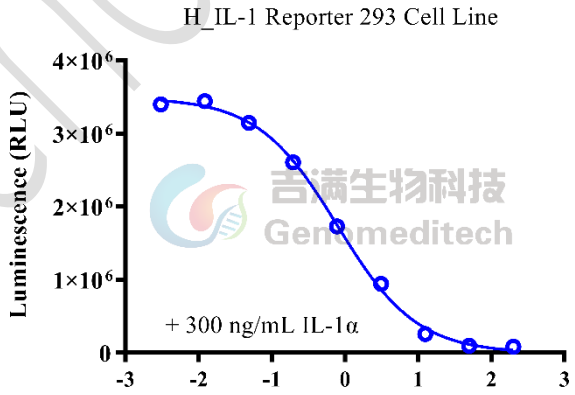
Figure 6 | Response to Anti-IL-1β hIgG1 Antibody(Canakinumab). Serial dilutions of Anti-IL-1β hIgG1 Antibody(Canakinumab) (Cat. GM-51764AB) was incubated with 1 ng/well of Human IL-1 beta / IL1B Protein (Sino Biological/10139-HNAE) for 1 hour in assay buffer (DMEM + 1% FBS + 1% P.S). After pre-incubation, add the mixture to the H_IL-1 Reporter 293 Cell Line (Cat. GM-C26463) at a density of 1.5E4 cells/well in a 96-well format, and incubate for 7 hours. Firefly luciferase activity is then measured using the Luciferase Reporter Assay Kit

(Genomeditech). The results indicated maximum blocking folds of approximately [66.9]. Data are shown by drug molar concentration.



	H_IL-1 Reporter 293 Cell Line
IC50	0.1182

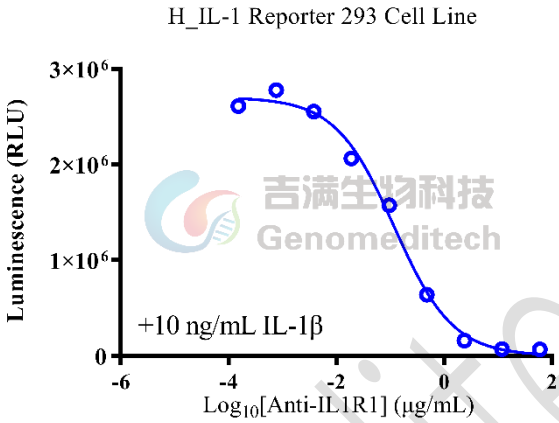
Figure 7 | Response to Anti-IL1R1 hIgG1 Antibody(AMG 108). Serial dilutions of the Anti-IL1R1 hIgG1 Antibody(AMG 108) (Cat. GM-51990AB) was incubated with 1.5E4 cells/well of the H_IL-1 Reporter 293 Cell Line (Cat. GM-C26463) in a 96-well plate for 1 hour in assay buffer (DMEM + 1% FBS + 1% P.S). Subsequently, the Human IL-1 alpha/IL1A Protein (Sino Biological/10128-HNCH) at a concentration of 30 ng/well was added, and the coculture proceeded for an additional 7 hours. Firefly luciferase activity is then measured using the Luciferase Reporter Assay Kit (Genomeditech). The results indicated maximum blocking folds of approximately [41.6]. Data are shown by drug mass concentration.



	H_IL-1 Reporter 293 Cell Line
IC50	0.7878

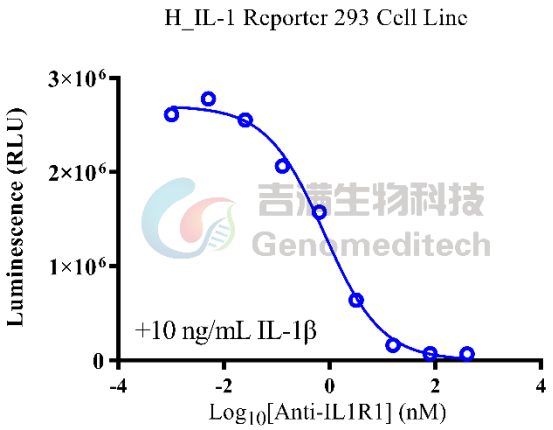
Figure 8 | Response to Anti-IL1R1 hIgG1 Antibody(AMG 108). Serial dilutions of the Anti-IL1R1 hIgG1 Antibody(AMG 108) (Cat. GM-51990AB) was incubated with 1.5E4 cells/well of the H_IL-1 Reporter 293 Cell Line (Cat. GM-C26463) in a 96-well plate for 1 hour in assay buffer (DMEM + 1% FBS + 1% P.S). Subsequently, the Human

IL-1 alpha/IL1A Protein (Sino Biological/10128-HNCH) at a concentration of 30 ng/well was added, and the coculture proceeded for an additional 7 hours. Firefly luciferase activity is then measured using the Luciferase Reporter Assay Kit (Genomeditech). The results indicated maximum blocking folds of approximately [41.6]. Data are shown by drug molar concentration.



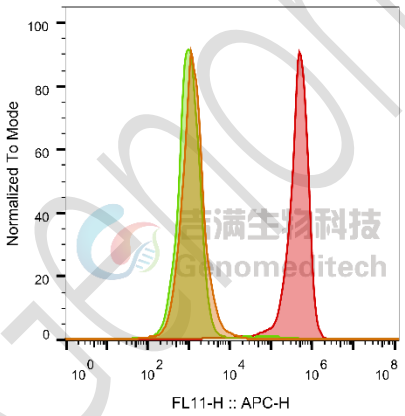
	H_IL-1 Reporter 293 Cell Line
IC50	0.1189

Figure 9 | Response to Anti-IL1R1 hIgG1 Antibody(AMG 108). Serial dilutions of the Anti-IL1R1 hIgG1 Antibody(AMG 108) (Cat. GM-51990AB) was incubated with 1.5E4 cells/well of the H_IL-1 Reporter 293 Cell Line (Cat. GM-C26463) in a 96-well plate for 1 hour in assay buffer (DMEM + 1% FBS + 1% P.S). Subsequently, the Human IL-1 beta / IL1B Protein (Sino Biological/10139-HNAE) at a concentration of 1 ng/well was added, and the coculture proceeded for an additional 7 hours. Firefly luciferase activity is then measured using the Luciferase Reporter Assay Kit (Genomeditech). The results indicated maximum blocking folds of approximately [42.5]. Data are shown by drug mass concentration.



	H_IL-1 Reporter 293 Cell Line
IC50	0.7927

Figure 10 | Response to Anti-IL1R1 hIgG1 Antibody(AMG 108). Serial dilutions of the Anti-IL1R1 hIgG1 Antibody(AMG 108) (Cat. GM-51990AB) was incubated with 1.5E4 cells/well of the H_IL-1 Reporter 293 Cell Line (Cat. GM-C26463) in a 96-well plate for 1 hour in assay buffer (DMEM + 1% FBS + 1% P.S). Subsequently, the Human IL-1 beta / IL1B Protein (Sino Biological/10139-HNAE) at a concentration of 1 ng/well was added, and the coculture proceeded for an additional 7 hours. Firefly luciferase activity is then measured using the Luciferase Reporter Assay Kit (Genomeditech). The results indicated maximum blocking folds of approximately [42.5]. Data are shown by drug molar concentration.



SampleID	Geometric Mean : FL11-H
293 anti-IL1RAP+APC-2nd Ab	1256
H_IL-1 Reporter 293 H_IgG+APC-2nd Ab	1095
H_IL-1 Reporter 293 anti-IL1RAP+APC-2nd Ab	4.07E5

Figure 11 | H_IL-1 Reporter 293 Cell Line (Cat. GM-C26463) was determined by flow cytometry using Anti-IL1RAP hIgG1 Antibody (Nadunolimab) (Cat. GM-88204AB).

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

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

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-1	
Cynomolgus_IL-1R HEK-293 Cell Line	H_IL-1R CHO-K1 Cell Line
H_IL-1R HEK-293 Cell Line	H_IL1RAP HEK-293 Cell Line
Anti-IL1R1 hIgG1 Antibody(AMG 108)	Anti-IL1RAP hIgG1 Antibody (Nadunolimab)
Anti-IL-1β hIgG1 Antibody(Canakinumab)	

License Agreement:

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