Genomeditech (Shanghai) Co.,Ltd.
Order: +86 021-68455258/50432826/50432825
Toll-free: +86 400 627 9288
Email: service@genomeditech.com

Product Sheet

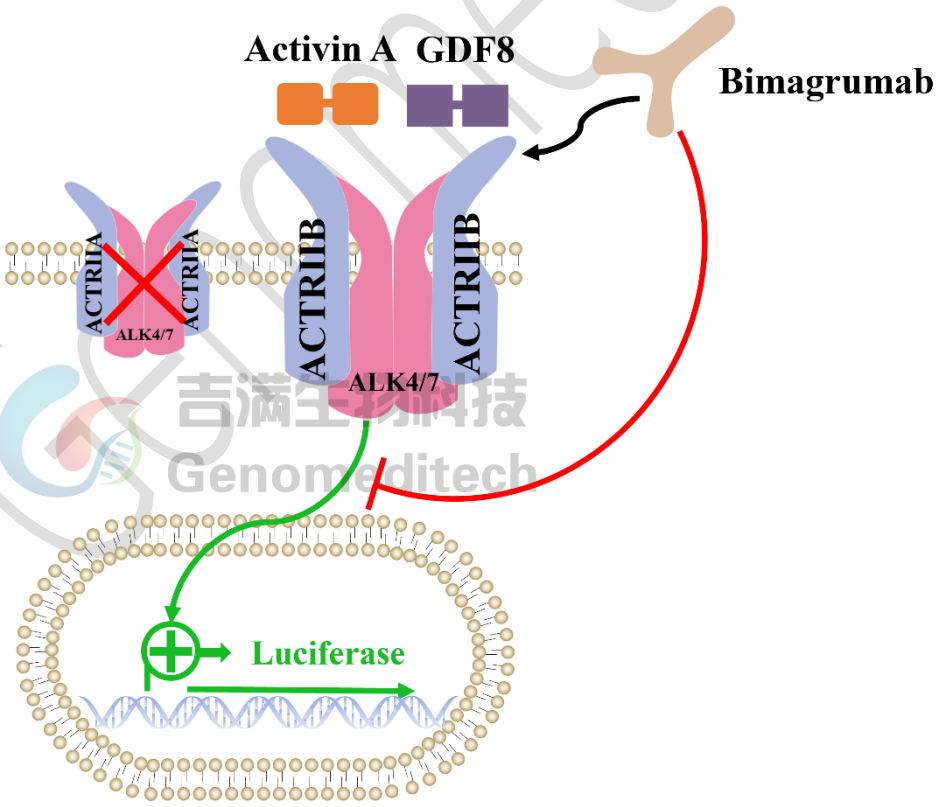
H_ACVR2B Reporter Cell Line

Catalog number: GM-C26076

Version 3.3.1.241120

The Activin type II receptors (ActRII) include two distinct receptors, ActRIIA (ACVR2A) and ActRIIB (ACVR2B), which are members of the transforming growth factor-beta (TGF-β) receptor family. They serve as type II receptors for other TGF-β family members, including Activin A, Activin B, BMP7, BMP9, BMP10, GDF1, GDF8 (myostatin), GDF11, and Nodal. ALK4 and ALK7 are the primary type I TGF-β receptor family members for Activin A and Activin B, respectively. Bimagrumab is a human monoclonal antibody that can block Activin type II receptors (ActRII), thereby regulating skeletal muscle growth. Research has shown that Bimagrumab has an affinity for both ActRIIB and ActRIIA, but its affinity for ActRIIB is at least 200 times stronger than for ActRIIA.

H_ACVR2B Reporter Cell Line is a clonal stable cell line that knockout ACVR2A and constitutive expression of human ACVR2B gene, along with signal-dependent expression of a luciferase reporter gene. When Activin A or GDF-8 binds to ACVR2B, 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 this signaling pathway.



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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+125 μg/mL Hygromycin
Note	The cells are very sensitive to antibiotics and should be cultured using the cell growth medium provided by Genomeditech.These cells are constructed based on ACVR2A knockout parent cells, which contain G418 and Puromycin resistance genes.
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
Hygromycin	Genomeditech/GM-040403
Recombinant Human/Mouse/Rat GDF-8	Novoprotein/CJ43
Human Activin A Protein; His Tag	Genomeditech/GM-87616RP
Anti-ACVR2B hIgG1 Antibody(Bimagrumab)	Genomeditech/GM-51148AB
Anti-ACVR2B hIgG1 Antibody(Fab-17G05)	Genomeditech/GM-82350AB
GMOne-Step Luciferase Reporter Gene Assay Kit	Genomeditech/GM-040503

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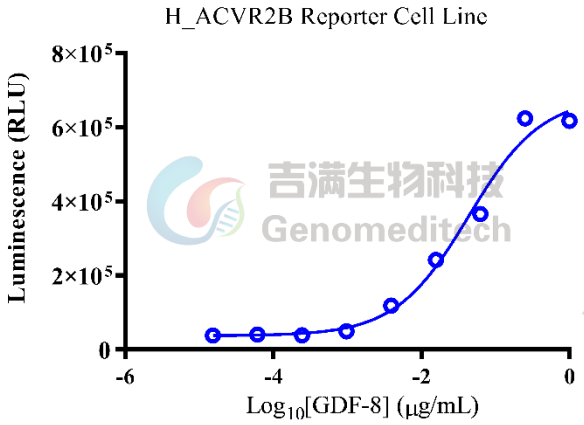
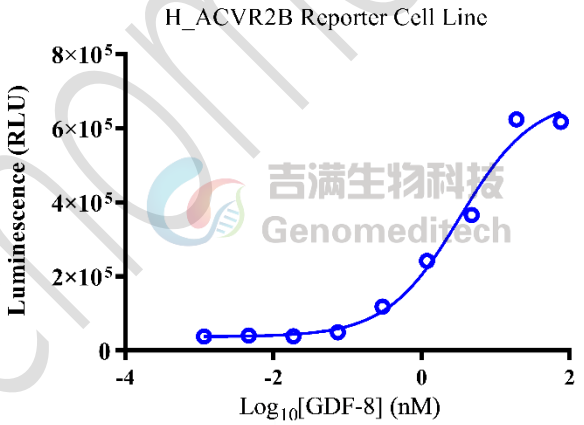
Figures



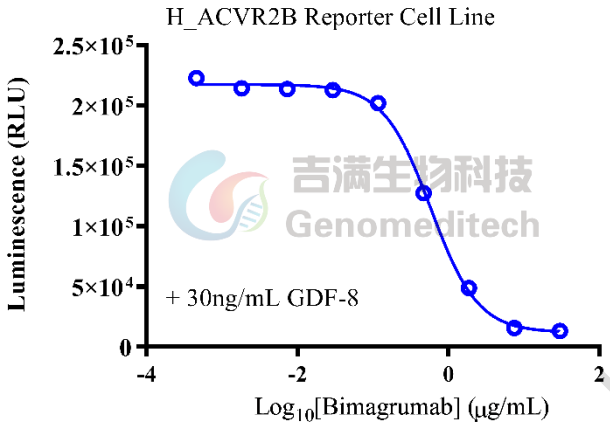
	H_ACVR2B Reporter Cell Line
EC50	0.04333

Figure 1 | Response to Recombinant Human/Mouse/Rat GDF-8. H_ACVR2B Reporter Cell Line (Cat. GM-C26076) at a concentration of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of Recombinant Human/Mouse/Rat GDF-8 (Novoprotein /CJ43) 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). The maximum induction fold was approximately [15.7]. Data are shown by drug mass concentration.



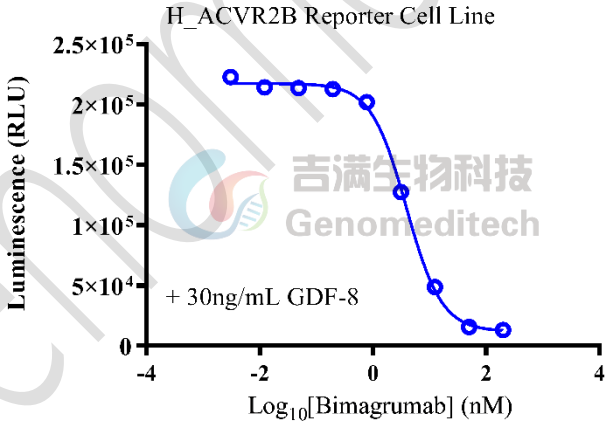
	H_ACVR2B Reporter Cell Line
EC50	3.308

Figure 2 | Response to Recombinant Human/Mouse/Rat GDF-8. H_ACVR2B Reporter Cell Line (Cat. GM-C26076) at a concentration of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of Recombinant Human/Mouse/Rat GDF-8 (Novoprotein /CJ43) 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). The maximum induction fold was approximately [15.7]. Data are shown by drug molar concentration.



	H_ACVR2B Reporter Cell Line
IC50	0.5846

Figure 3 | Response to Anti-ACVR2B hIgG1 Antibody (Bimagrumab). Serial dilutions of antibodies were incubated with 1.5E4 cells/well of the H_ACVR2B Reporter Cell Line (Cat. GM-C26076) in a 96-well plate for 1 hour. Then, 30 ng/mL of Recombinant Human/Mouse/Rat GDF-8 (Novoprotein/CJ43) was added, and the incubation continued in assay buffer (DMEM + 1% FBS + 1% P.S) for 6 hours. Firefly luciferase activity is measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). The results indicated a maximum blocking fold of approximately [16.0]. Data are shown by drug mass concentration.



	H_ACVR2B Reporter Cell Line
IC50	3.897

Figure 4 | Response to Anti-ACVR2B hIgG1 Antibody (Bimagrumab). Serial dilutions of antibodies were incubated with 1.5E4 cells/well of the H_ACVR2B Reporter Cell Line (Cat. GM-C26076) in a 96-well plate for 1 hour. Then, 30 ng/mL of Recombinant Human/Mouse/Rat GDF-8 (Novoprotein/CJ43) was added, and the incubation continued in assay buffer (DMEM + 1% FBS + 1% P.S) for 6 hours. Firefly luciferase activity is measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). The results indicated a maximum blocking fold of approximately [16.0]. Data are shown by drug molar concentration.

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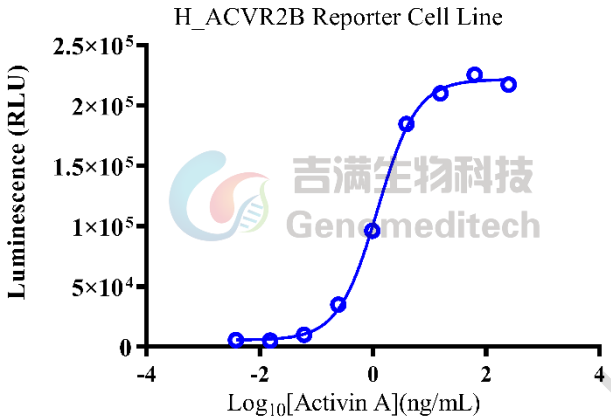
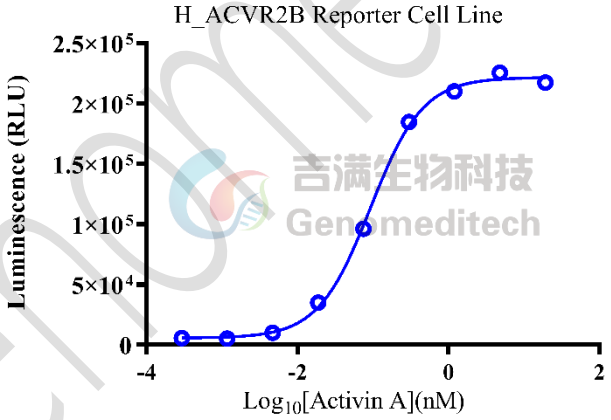


	H_ACVR2B Reporter Cell Line
EC50	1.209

Figure 5 | Response to Human Activin A Protein. H_ACVR2B Reporter Cell Line (Cat. GM-C26076) at a concentration of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of Human Activin A Protein (Cat. GM-87616RP) 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). The maximum induction fold was approximately [40.2]. Data are shown by drug mass concentration.



	H_ACVR2B Reporter Cell Line
EC50	0.09296

Figure 6 | Response to Human Activin A Protein. H_ACVR2B Reporter Cell Line (Cat. GM-C26076) at a concentration of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of Human Activin A Protein (Cat. GM-87616RP) 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). The maximum induction fold was approximately [40.2] Data are shown by drug molar concentration.

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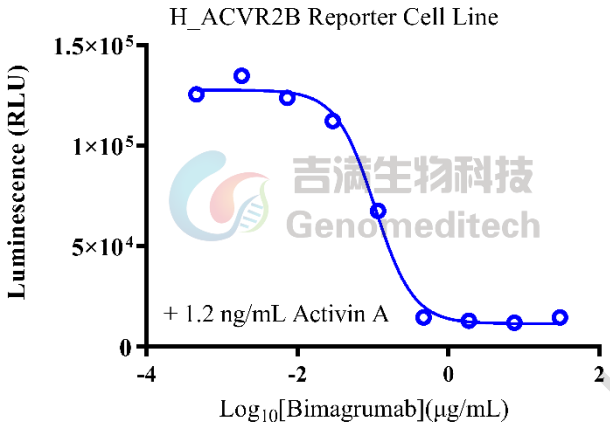
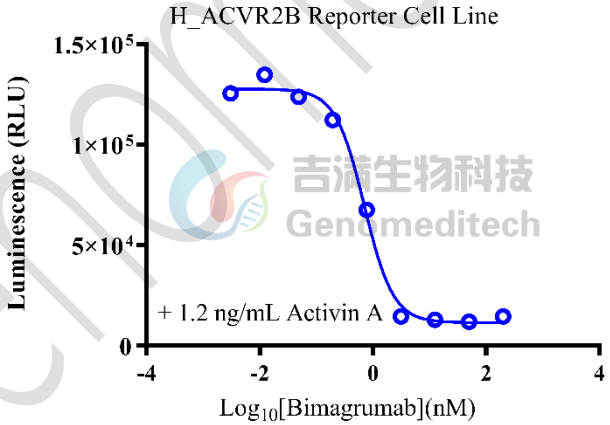


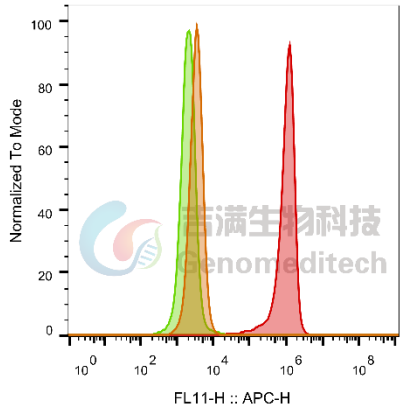
	H_ACVR2B Reporter Cell Line
IC50	0.1060

Figure 7 | Response to Anti-ACVR2B hIgG1 Antibody (Bimagrumab). Serial dilutions of antibodies were incubated with 1.5E4 cells/well of the H_ACVR2B Reporter Cell Line (Cat. GM-C26076) in a 96-well plate for 1 hour. Then, 1.2 ng/mL of Human Activin A Protein (Cat. GM-87616RP) was added, and the incubation continued in assay buffer (DMEM + 1% FBS + 1% P.S) for 6 hours. Firefly luciferase activity is measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). The results indicated a maximum blocking fold of approximately [9.0]. Data are shown by drug mass concentration.



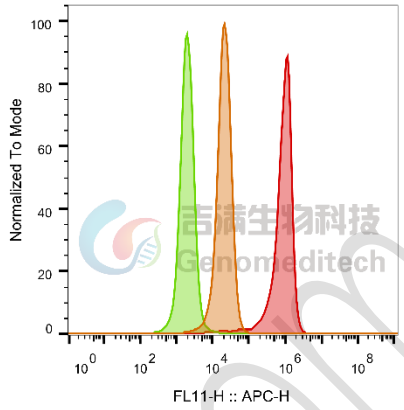
	H_ACVR2B Reporter Cell Line
IC50	0.7064

Figure 8 | Response to Anti-ACVR2B hIgG1 Antibody (Bimagrumab). Serial dilutions of antibodies were incubated with 1.5E4 cells/well of the H_ACVR2B Reporter Cell Line (Cat. GM-C26076) in a 96-well plate for 1 hour. Then, 1.2 ng/mL of Human Activin A Protein (Cat. GM-87616RP) was added, and the incubation continued in assay buffer (DMEM + 1% FBS + 1% P.S) for 6 hours. Firefly luciferase activity is measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). The results indicated a maximum blocking fold of approximately [9.0]. Data are shown by drug molar concentration.



SampleID	Geometric Mean : FL11-H
Null anti-ACVR2B+APC-2nd Ab	3371
H_ACVR2B Reporter H_IgG+APC-2nd Ab	2035
H_ACVR2B Reporter anti-ACVR2B+APC-2nd Ab	9.70E5

Figure 9 | H_ACVR2B Reporter Cell Line (Cat. GM-C26076) was determined by flow cytometry using Anti-ACVR2B hIgG1 Antibody(Fab-17G05) (Cat. GM-82350AB).



SampleID	Geometric Mean : FL11-H
Null anti-ACVR2B+APC-2nd Ab	20282
H_ACVR2B Reporter H_IgG+APC-2nd Ab	1973
H_ACVR2B Reporter anti-ACVR2B+APC-2nd Ab	7.89E5

Figure 10 | H_ACVR2B Reporter Cell Line (Cat. GM-C26076) was determined by flow cytometry using Anti-ACVR2B hIgG1 Antibody(Bimagrumab) (Cat. GM-51148AB).

Figure 11 | The Sanger sequencing of the H_ACVR2B Reporter Cell Line showed successful knockout of ACVR2A.

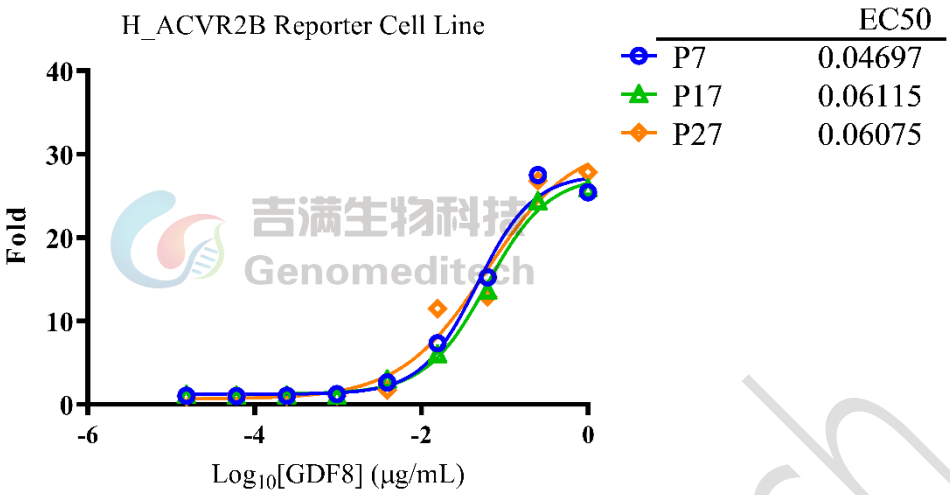


Figure 12 | The passage stability of response to Recombinant Human/Mouse/Rat GDF-8. The passage 7, 17 and 27 of H_ACVR2B Reporter Cell Line (Cat. GM-C26076) at a concentration of 1.5E4 cells/well (96-well format) were stimulated with serial dilutions of Recombinant Human/Mouse/Rat GDF-8 (Novoprotein/CJ43) 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.

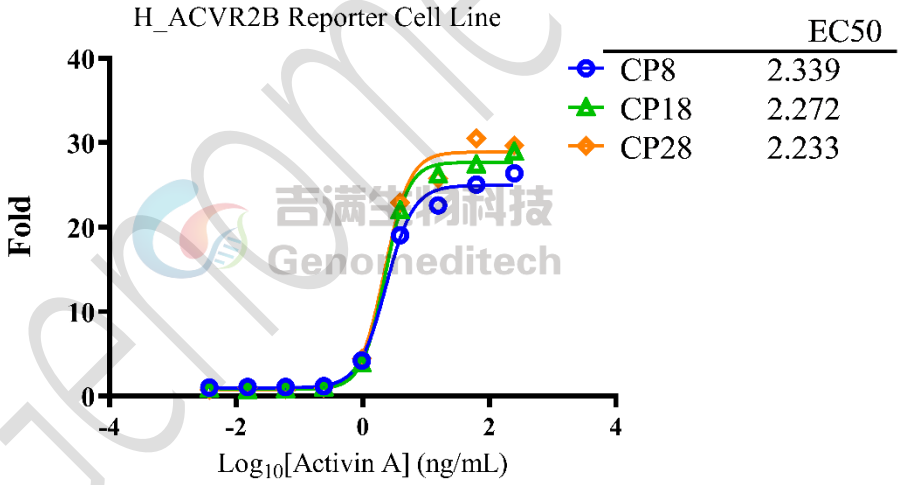


Figure 13 | The passage stability of response to Human Activin A Protein. The passage 8, 18 and 28 of H_ACVR2B Reporter Cell Line (Cat. GM-C26076) at a concentration of 1.5E4 cells/well (96-well format) were stimulated with serial dilutions of Human Activin A Protein (Cat. GM-87616RP) in assay buffer (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.

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+125 μg/mL Hygromycin

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

GCGR	
H_GCGR Reporter CHO-K1 Cell Line	H_GCGR Reporter HEK-293 Cell Line
H_GCGR CHO-K1 Cell Line	H_GCGR HEK-293 Cell Line
Mouse_GCGR HEK-293 Cell Line	
Anti-H_GCGR hIgG2 Antibody(volagidemab)	
GLP1R	
H_GLP1R Reporter CHO-K1 Cell Line	H_GLP1R Reporter HEK-293 Cell Line
Cynomolgus_GLP1R HEK-293 Cell Line	H_GLP1R CHO-K1 Cell Line
H_GLP1R HEK-293 Cell Line	Mouse_GLP1R HEK-293 Cell Line
Anti-GLP1R hIgG1 Antibody(mAb-36986)	Anti-H_GLP1R hIgG1 Antibody(glutazumab)
FGF21:FGFR	
H_FGF21 Reporter HEK-293 Cell Line	
CALCA(CGRP)：CALCRL RAMP	
H_CALCRL RAMP1 Reporter HEK-293 Cell Line	Cynomolgus_CALCRL RAMP1 HEK-293 Cell Line
H_CALCRL RAMP1 CHO-K1 Cell Line	H_CALCRL RAMP1 HEK-293 Cell Line
H_CALCRL RAMP2(AM1) CHO-K1 Cell Line	H_CALCRL RAMP3(AM2) CHO-K1 Cell Line
Anti-CALCRL RAMP1 hIgG2 Antibody(Erenumab)	
GIP:GIPR	
H_GIPR Reporter CHO-K1 Cell Line	H_GIPR Reporter HEK-293 Cell Line
H_GIPR Reporter HEK-293 DDX35TM Cell Line	Cynomolgus_GIPR HEK-293 Cell Line
H_GIPR CHO-K1 Cell Line	H_GIPR HEK-293 Cell Line
Mouse_GIPR HEK-293 Cell Line	
Anti-H_GIPR hIgG1 Antibody(AMG-133)	
ACVR2A：ACTRIIB：Active A	
ACVR2A KO HEK-293 Cell Line	Activin A Reporter Cell Line
H_ACVR2A Reporter Cell Line	ACVR2B KO HEK-293 Cell Line

H_ACVR2A HEK-293(ACVR2B KO) Cell Line	H_ACVR2B HEK-293(ACVR2A KO) Cell Line
Anti-ACVR2B hIgG1 Antibody(Bimagrumab)	Anti-ACVR2B hIgG1 Antibody(Fab-17G05)
Anti-ACVR2B mIgG2a Antibody(Bimagrumab)	Anti-H_ACVR2B hIgG1 Reference Antibody(Bimbio)
Biotinylated Human ACVR2B Protein; His-Avi Tag	Biotinylated Mouse ACVR2A Protein; His-Avi Tag
Biotinylated Mouse ACVR2B Protein; His-Avi Tag	Human Activin A Protein; His Tag
Human Activin B Protein; His Tag	Human ACVR2A Protein; hFc Tag
Human ACVR2A Protein; His Tag	Human ACVR2B Protein; hFc Tag
Human ACVR2B Protein; His Tag	Mouse ACVR2B Protein; His Tag
AMY：CALCR RAMP	
H_CALCR RAMP3(AMY3) Reporter CHO-K1 Cell Line	H_CALCR Reporter CHO-K1 Cell Line

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