

# Product Sheet

## H\_ACVR2B Reporter DDX35™ Cell Line

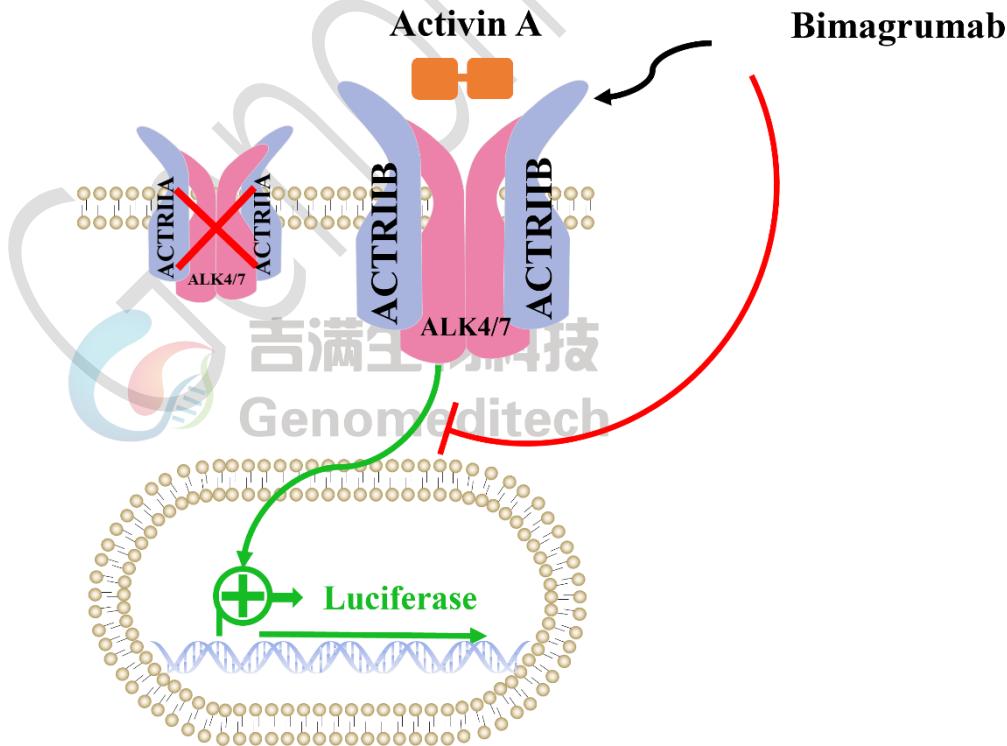
Catalog number: GM-C39211

Version 3.3.1.251228

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- $\beta$ ) receptor family. They serve as type II receptors for other TGF- $\beta$  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- $\beta$  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.

H\_ACVR2B Reporter DDX35™ 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 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.

H\_ACVR2B Reporter 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.



## Specifications

<b>Quantity</b>	5E6 Cells per vial, 1 mL
<b>Product Format</b>	1 vial of frozen cells
<b>Shipping</b>	Shipped on dry ice
<b>Storage Conditions</b>	Liquid nitrogen immediately upon receipt
<b>Recovery Medium</b>	DMEM+10% FBS+1% P.S
<b>Growth medium</b>	DMEM+10% FBS+1% P.S+4 µg/mL Blasticidin+125 µg/mL Hygromycin
<b>Note</b>	Cells contain G418 and Puromycin resistance genes.
<b>Freezing Medium</b>	90% FBS+10% DMSO
<b>Growth properties</b>	Adherent
<b>Growth Conditions</b>	37°C, 5% CO <sub>2</sub>
<b>Mycoplasma Testing</b>	The cell line has been screened to confirm the absence of Mycoplasma species.
<b>Safety considerations</b>	Biosafety Level 2
<b>Note</b>	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/ <a href="#">GM-040404</a>
Hygromycin	Genomeditech/ <a href="#">GM-040403</a>
Human Activin A Protein; His Tag	Genomeditech/ <a href="#">GM-87616RP</a>
Anti-ACVR2B hIgG1 Antibody(Bimagrumbab)	Genomeditech/ <a href="#">GM-51148AB</a>
Anti-ACVR2B hIgG1 Antibody(Fab-17G05)	Genomeditech/ <a href="#">GM-82350AB</a>
GMOne-Step 2.0 Luciferase Reporter Gene Assay Kit	Genomeditech/ <a href="#">GM-040513</a>

## Figures

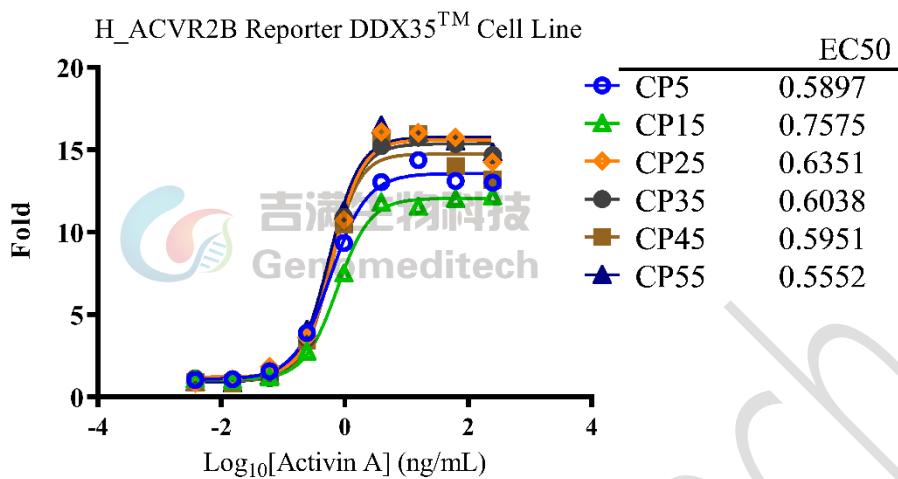


Figure 1 | The passage stability of response to Human Activin A Protein; His Tag. The passage 5, 15, 25, 35, 45 and 55 of H\_ACVR2B Reporter DDX35™ Cell Line (Cat. GM-C39211) at a concentration of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of Human Activin A Protein; His Tag (Cat. GM-87616RP) 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). Data are shown by drug mass concentration.

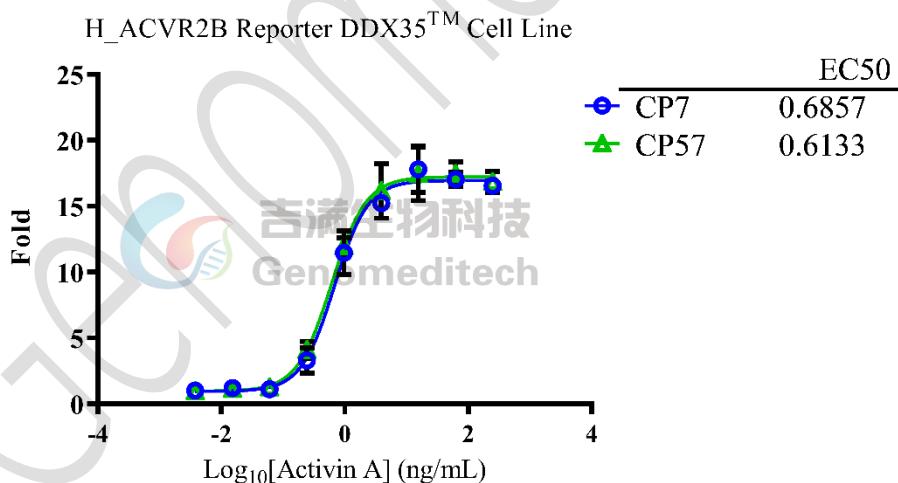


Figure 2 | The passage stability of response to Human Activin A Protein; His Tag. The passage 5 and 57 of H\_ACVR2B Reporter DDX35™ Cell Line (Cat. GM-C39211) at a concentration of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of Human Activin A Protein; His Tag (Cat. GM-87616RP) in assay buffer (DMEM+1% FBS+1% P.S) for 6 hours, in triplicate. The firefly luciferase activity was measured using the Luciferase Reporter Assay Kit (Genomeditech). Data are shown by drug mass concentration.

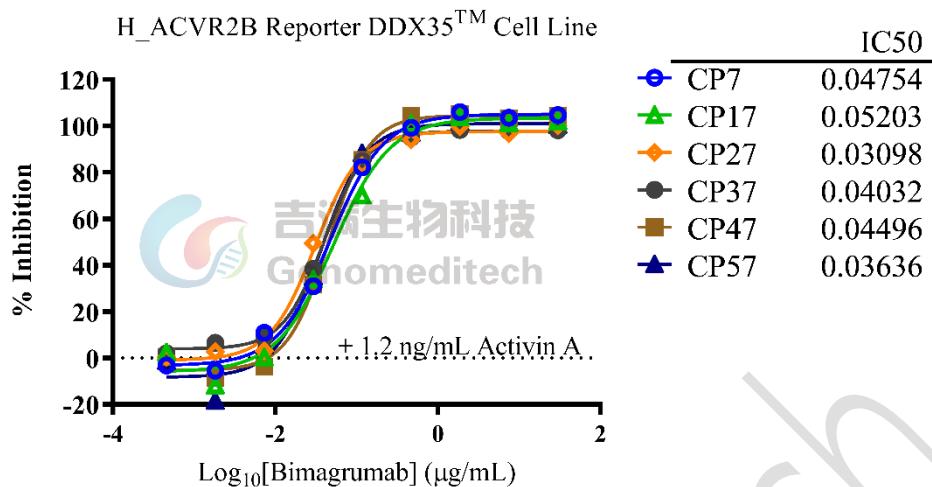


Figure 3 | Passage-to-passage stability of the response to anti-ACVR2B hIgG1 antibody (bimagrumab). Serial dilutions of the antibody were incubated for 1 hour in 96-well plates with 1.5E4 cells/well of the H\_ACVR2B Reporter DDX35™ Cell Line (Cat. GM-C39211) at passages 5, 15, 25, 35, 45, and 55. Then, 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. The firefly luciferase activity was measured using the Luciferase Reporter Assay Kit (Genomeditech). Data are shown by drug mass concentration.

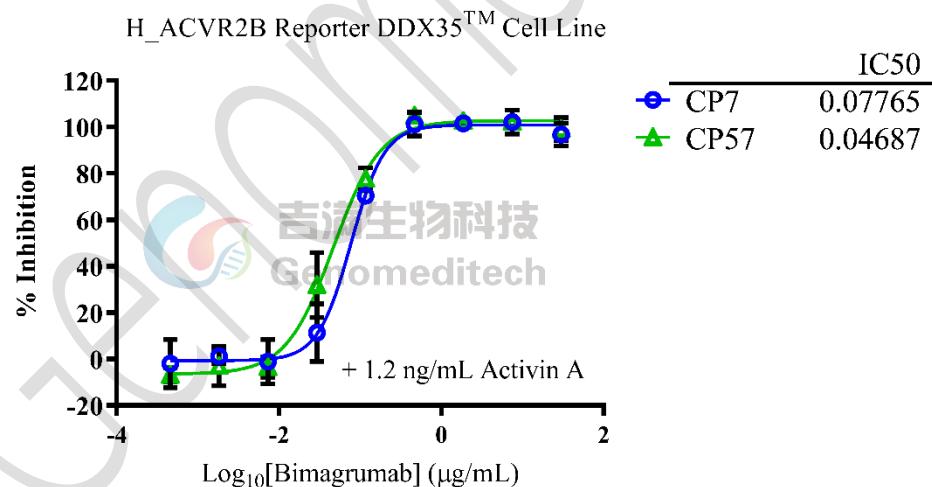


Figure 4 | Passage-to-passage stability of the response to anti-ACVR2B hIgG1 antibody (bimagrumab). Serial dilutions of the antibody were incubated for 1 hour in 96-well plates with 1.5E4 cells/well of the H\_ACVR2B Reporter DDX35™ Cell Line (Cat. GM-C39211) at passages 5, 15, 25, 35, 45, and 55. Then, 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, in triplicate. The firefly luciferase activity was measured using the Luciferase Reporter Assay Kit (Genomeditech). Data are shown by drug mass concentration.

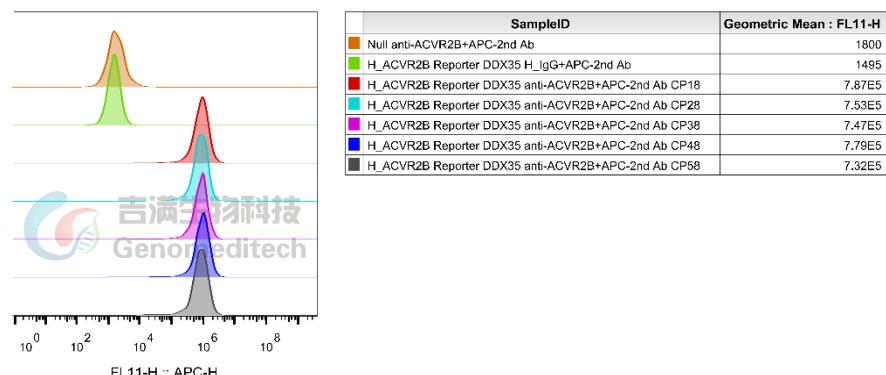


Figure 5 | The passage stability of the H\_ACVR2B Reporter DDX35™ Cell Line (Cat. GM-C39211) was determined by flow cytometry using Anti-ACVR2B hIgG1 Antibody(Fab-17G05) (Cat. [GM-82350AB](#)).

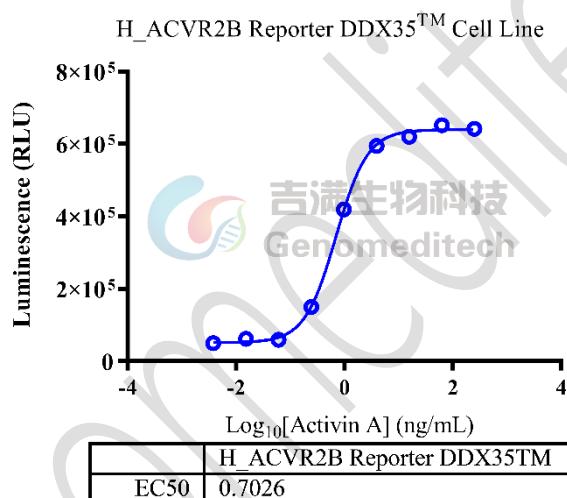


Figure 6 | Response to Human Activin A Protein. H\_ACVR2B Reporter DDX35™ Cell Line (Cat. GM-C39211) at a concentration of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of Human Activin A Protein (Cat. [GM-87616RP](#)) in assay buffer (DMEM+1% FBS+1% P.S) for 6 hours. The firefly luciferase activity was measured using the Luciferase Reporter Assay Kit (Genomeditech). The maximum induction fold was approximately [16.3]. Data are shown by drug mass concentration.

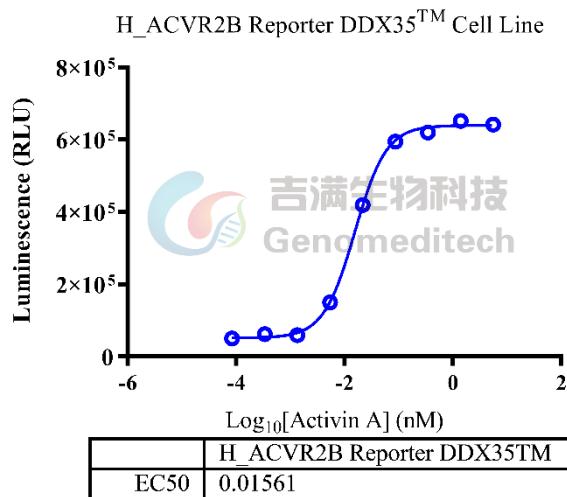


Figure 7 | Response to Human Activin A Protein. H\_ACVR2B Reporter DDX35<sup>TM</sup> Cell Line (Cat. GM-C39211) at a concentration of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of Human Activin A Protein (Cat. GM-87616RP) in assay buffer (DMEM+1% FBS+1% P.S) for 6 hours. The firefly luciferase activity was measured using the Luciferase Reporter Assay Kit (Genomeditech). The maximum induction fold was approximately [16.3]. Data are shown by drug molar concentration.

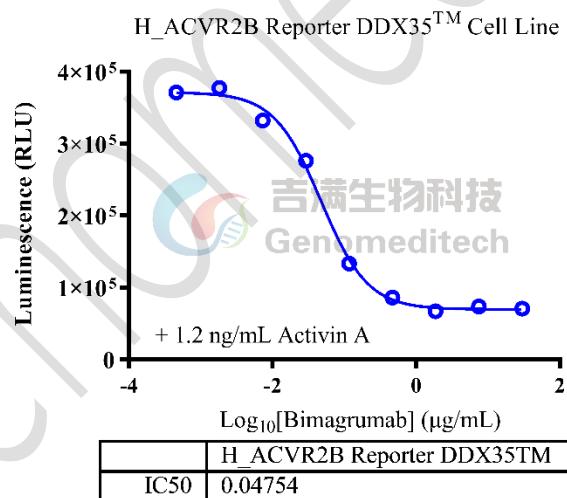


Figure 8 | Response to Anti-ACVR2B hIgG1 Antibody (Bimagrumab). Serial dilutions of antibodies were incubated with 1.5E4 cells/well of the H\_ACVR2B Reporter DDX35<sup>TM</sup> Cell Line (Cat. GM-C39211) in a 96-well plate for 1 hour. Then, 1.2 ng/mL of Human Activin A Protein (Cat. GM-87616RP) was added, and the incubation continued in assay buffer (DMEM+1% FBS+1% P.S) for 6 hours. Firefly luciferase activity is measured using the Luciferase Reporter Assay Kit (Genomeditech). The results indicated a maximum blocking fold of approximately [5.2]. Data are shown by drug mass concentration.

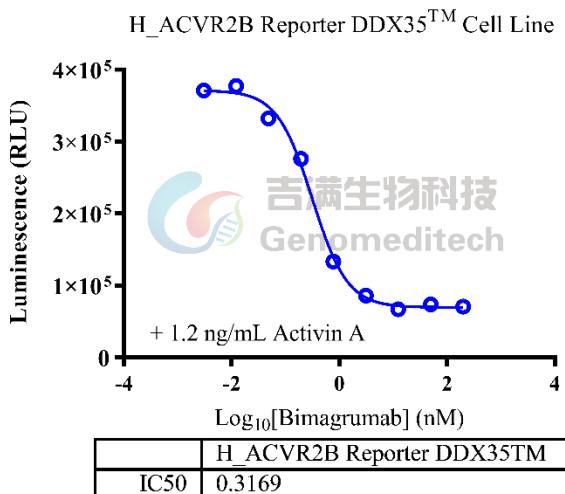


Figure 9 | Response to Anti-ACVR2B hIgG1 Antibody (Bimagrumab). Serial dilutions of antibodies were incubated with 1.5E4 cells/well of the H\_ACVR2B Reporter DDX35™ Cell Line (Cat. GM-C39211) in a 96-well plate for 1 hour. Then, 1.2 ng/mL of Human Activin A Protein (Cat. GM-87616RP) was added, and the incubation continued in assay buffer (DMEM+1% FBS+1% P.S) for 6 hours. Firefly luciferase activity is measured using the Luciferase Reporter Assay Kit (Genomeditech). The results indicated a maximum blocking fold of approximately [5.2]. Data are shown by drug molar concentration.

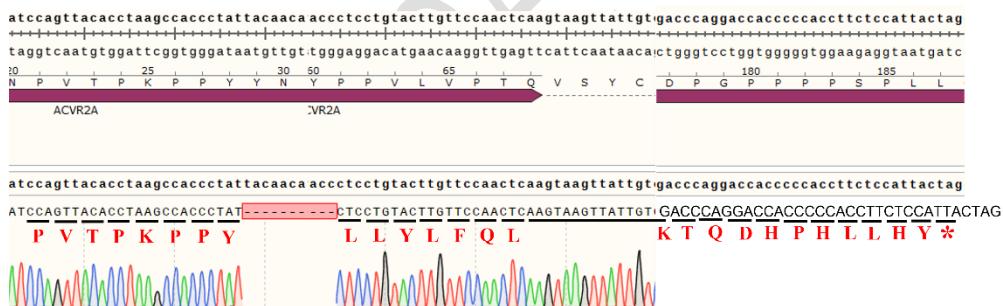


Figure 10 | The Sanger sequencing results showed that ACVR2A was successfully knocked out in the parental cells.

## 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.

- 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).
- 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<sub>2</sub> 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	
<a href="#">H_GCGR Reporter CHO-K1 Cell Line</a>	<a href="#">H_GCGR Reporter HEK-293 Cell Line</a>
<a href="#">H_GCGR Reporter HEK-293 DDX35TM Cell Line</a>	<a href="#">Cynomolgus_GCGR HEK-293 Cell Line</a>
<a href="#">H_GCGR CHO-K1 Cell Line</a>	<a href="#">H_GCGR HEK-293 Cell Line</a>
<a href="#">Mouse_GCGR HEK-293 Cell Line</a>	
<a href="#">Anti-H_GCGR hIgG2 Antibody(volagidemab)</a>	
GLP1R	
<a href="#">H_GLP1R Reporter CHO-K1 Cell Line</a>	<a href="#">H_GLP1R Reporter HEK-293 Cell Line</a>
<a href="#">H_GLP1R Reporter HEK-293 DDX35TM Cell Line</a>	<a href="#">H_GLP1R β-Arrestin Reporter CHO-K1 Cell Line</a>
<a href="#">Cynomolgus_GLP1R GIPR CHO-K1 Cell Line</a>	<a href="#">Cynomolgus_GLP1R HEK-293 Cell Line</a>
<a href="#">H_GLP1R CHO-K1 Cell Line</a>	<a href="#">H_GLP1R GIPR CHO-K1 Cell Line</a>
<a href="#">H_GLP1R HEK-293 Cell Line</a>	<a href="#">Mouse_GLP1R GIPR CHO-K1 Cell Line</a>
<a href="#">Mouse_GLP1R HEK-293 Cell Line</a>	
<a href="#">Anti-GLP1R hIgG1 Antibody(mAb-36986)</a>	<a href="#">Anti-H_GLP1R hIgG1 Antibody(glutazumab)</a>
FGFR1	
<a href="#">H_FGF21 Reporter HEK-293 Cell Line</a>	
<a href="#">Human FGF-21 Protein; His Tag</a>	
CALCA(CGRP):CALCRL RAMP	
<a href="#">H_CALCRL RAMP1 Reporter HEK-293 Cell Line</a>	<a href="#">H_CALCRL RAMP1 Reporter HEK-293 DDX35TM Cell Line</a>
<a href="#">Cynomolgus_CALCRL RAMP1 HEK-293 Cell Line</a>	<a href="#">H_CALCRL RAMP1 CHO-K1 Cell Line</a>
<a href="#">H_CALCRL RAMP1 HEK-293 Cell Line</a>	
<a href="#">Anti-CALCRL RAMP1 hIgG2 Antibody(Erenumab)</a>	
GIPR	
<a href="#">H_GIPR Reporter CHO-K1 Cell Line</a>	<a href="#">H_GIPR Reporter HEK-293 Cell Line</a>
<a href="#">H_GIPR Reporter HEK-293 DDX35TM Cell Line</a>	<a href="#">Cynomolgus_GIPR CHO-K1 Cell Line</a>
<a href="#">Cynomolgus_GIPR HEK-293 Cell Line</a>	<a href="#">H_GIPR CHO-K1 Cell Line</a>
<a href="#">H_GIPR HEK-293 Cell Line</a>	<a href="#">Mouse_GIPR CHO-K1 Cell Line</a>
<a href="#">Mouse_GIPR HEK-293 Cell Line</a>	
<a href="#">Anti-H_GIPR hIgG1 Antibody(AMG-133)</a>	
ACVR2A:ACTRIIB:Active A	
<a href="#">ACVR2A KO HEK-293 Cell Line</a>	<a href="#">Activin A Reporter Cell Line</a>
<a href="#">BRE Reporter 293 Cell Line</a>	<a href="#">H_ACVR2A Reporter Cell Line</a>
<a href="#">H_ACVR2B Reporter Cell Line</a>	<a href="#">ACVR2B KO HEK-293 Cell Line</a>
<a href="#">H_ACVR2A HEK-293(ACVR2B KO) Cell Line</a>	<a href="#">H_ACVR2B CHO-K1 Cell Line</a>
<a href="#">H_ACVR2B HEK-293(ACVR2A KO) Cell Line</a>	
<a href="#">Anti-ACVR2B hIgG1 Antibody(Bimagrumbab)</a>	<a href="#">Anti-ACVR2B hIgG1 Antibody(Fab-17G05)</a>
<a href="#">Anti-ACVR2B mIgG2a Antibody(Bimagrumbab)</a>	<a href="#">Anti-H_ACVR2B hIgG1 Reference Antibody(Bimbo)</a>

<a href="#">Biotinylated Human ACVR2A Protein; His-Avi Tag</a>	<a href="#">Biotinylated Human ACVR2B Protein; His-Avi Tag</a>
<a href="#">Biotinylated Mouse ACVR2A Protein; His-Avi Tag</a>	<a href="#">Biotinylated Mouse ACVR2B Protein; His-Avi Tag</a>
<a href="#">Human Activin A Protein; His Tag</a>	<a href="#">Human Activin A Protein; His Tag (CHO)</a>
<a href="#">Human Activin B Protein; His Tag</a>	<a href="#">Human ACVR2A Protein; hFc Tag</a>
<a href="#">Human ACVR2A Protein; hFc Tag (Sotatercept)</a>	<a href="#">Human ACVR2A Protein; His Tag</a>
<a href="#">Human ACVR2B Protein; hFc Tag</a>	<a href="#">Human ACVR2B Protein; His Tag</a>
<a href="#">Human latent GDF-8 Protein; His Tag</a>	<a href="#">Mouse ACVR2A Protein; His Tag</a>
<a href="#">Mouse ACVR2B Protein; His Tag</a>	
<b>AMY:CALCR RAMP</b>	
<a href="#">H_CALCR RAMP3(AMY3) Reporter CHO-K1 Cell Line</a>	<a href="#">H_CALCR RAMP3(AMY3) β-Arrestin Reporter CHO-K1 Cell Line</a>
<a href="#">H_CALCR Reporter CHO-K1 Cell Line</a>	<a href="#">H_CALCR β-Arrestin Reporter CHO-K1 Cell Line</a>
<a href="#">Rat_CALCR RAMP3(AMY3) Reporter COS-7 Cell Line</a>	<a href="#">Rat_CALCR Reporter COS-7 Cell Line</a>
<b>THRB</b>	
<a href="#">H_THRβ Reporter HEK-293 Cell Line</a>	
<b>MC4R</b>	
<a href="#">H_MC4R Reporter HEK-293 Cell Line</a>	
<b>ASGR1</b>	
<a href="#">H_ASGR1 CHO-K1 Cell Line</a>	<a href="#">H_ASGR1 HEK-293 Cell Line</a>
<a href="#">Cynomolgus ASGR1 Protein; His Tag</a>	<a href="#">Human ASGR1 Protein; His Tag</a>

## License Agreement:

**By purchasing and using this cell line product, the user voluntarily agrees to accept and abide by the following policies:**

- This cell line product is restricted to research use only and shall not be used for any commercial purposes.
- This product is strictly prohibited from being used in the diagnosis or treatment of human or animal diseases, and shall not be directly used in experiments involving humans.
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