

# Product Sheet

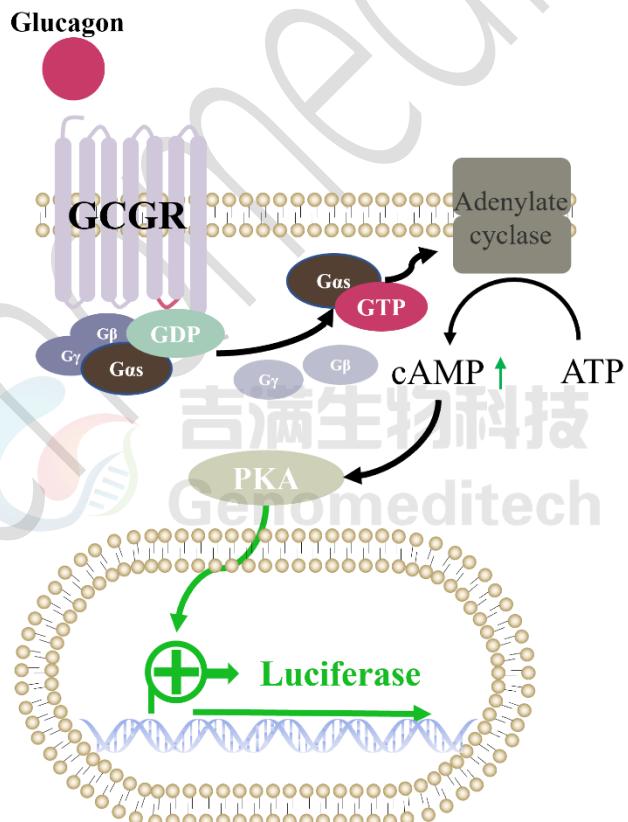
## H\_GCGR Reporter HEK-293 Cell Line

Catalog number: GM-C31698

Version 3.3.1.260114

The glucagon receptor (GCGR) is a 62 kDa protein activated by glucagon and belongs to the family of class B G protein-coupled receptors. It is primarily expressed in the liver and kidneys. When glucagon activates GCGR, it binds to the heterotrimer Gs (composed of  $\alpha$ ,  $\beta$ , and  $\gamma$  subunits), which triggers the activation of adenylate cyclase, increasing the levels of cAMP in the cytoplasm. cAMP then activates PKA, leading to the phosphorylation of regulatory gene transcription proteins, which causes them to relocate to the cell nucleus.

H\_GCGR Reporter HEK-293 Cell Line is a clonal stable cell line constructed using lentiviral technology, constitutive expression of the GCGR gene, along with signal-dependent expression of a luciferase reporter gene. When glucagon binds to GCGR, it activates downstream signaling pathways, leading to the expression of luciferase. The luciferase readout represents the activation level of the signaling pathway and can thus be used for evaluating the in vitro effects of related drugs of GCGR.



## 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+0.75 µg/mL Puromycin
<b>Note</b>	None
<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>
Puromycin	Genomeditech/ <a href="#">GM-040401</a>
Glucagon (Human, Rat, Mouse, Porcine, Bovine, Canine)	PHOENIX/028-02
Glucagon (1-29), bovine, human	MCE/HY-P0082
GMOne-Step 2.0 Luciferase Reporter Gene Assay Kit	Genomeditech/ <a href="#">GM-040513</a>
Anti-H <sub>2</sub> GCGR hIgG1 Antibody(H4H1327P)	Genomeditech/GM-84556AB

## Figures

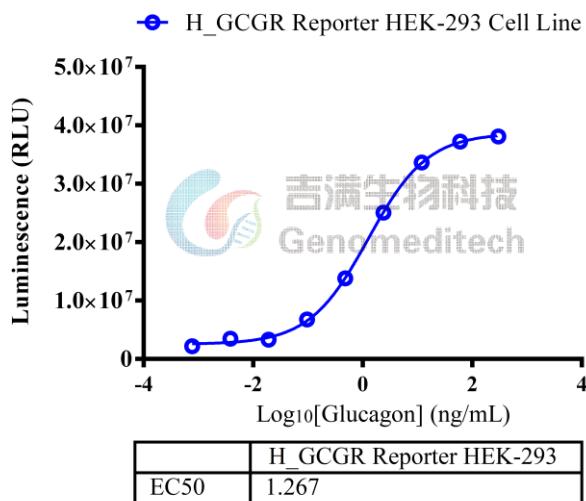


Figure 1 | Response to Glucagon (Human, Rat, Mouse, Porcine, Bovine, Canine). The H\_GCGR Reporter HEK-293 Cell Line (Cat. GM-C31698) at a concentration of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of Glucagon (PHOENIX/028-02) in assay buffer (DMEM + 1% FBS + 1% P.S) for 16 hours. The firefly luciferase activity was measured using the Luciferase Reporter Assay Kit (Genomeditech). The maximum induction fold was approximately [34.6]. Data are shown by drug mass concentration.

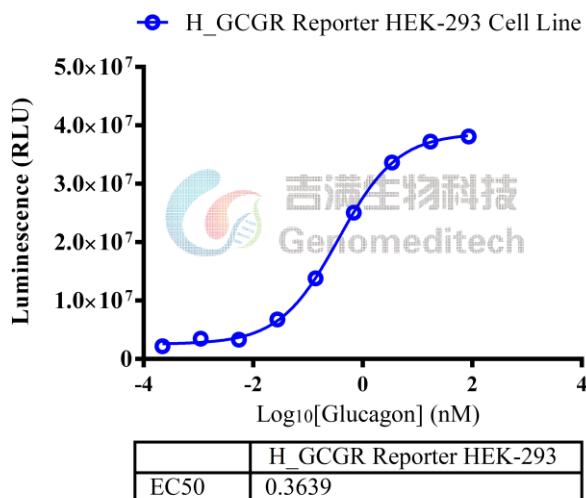


Figure 2 | Response to Glucagon (Human, Rat, Mouse, Porcine, Bovine, Canine). The H\_GCGR Reporter HEK-293 Cell Line (Cat. GM-C31698) at a concentration of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of Glucagon (PHOENIX/028-02) in assay buffer (DMEM + 1% FBS + 1% P.S) for 16 hours. The firefly luciferase activity was measured using the Luciferase Reporter Assay Kit (Genomeditech). The maximum induction fold was approximately [34.6]. Data are shown by drug molar concentration.

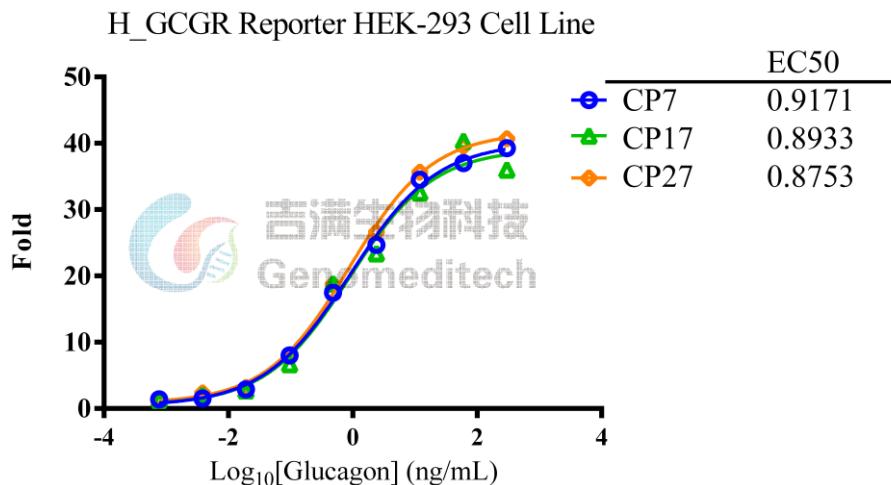


Figure 3 | The passage stability of response to Glucagon (1-29), bovine, human. The passage 7, 17 and 27 of H\_GCGR Reporter HEK-293 Cell Line (Cat. GM-C31698) at a concentration of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of Glucagon (1-29) (MCE/HY-P0082) in assay buffer (DMEM + 1% FBS + 1% P.S) for 16 hours. The firefly luciferase activity was measured using the Luciferase Reporter Assay Kit (Genomeditech). Data are shown by drug mass concentration.

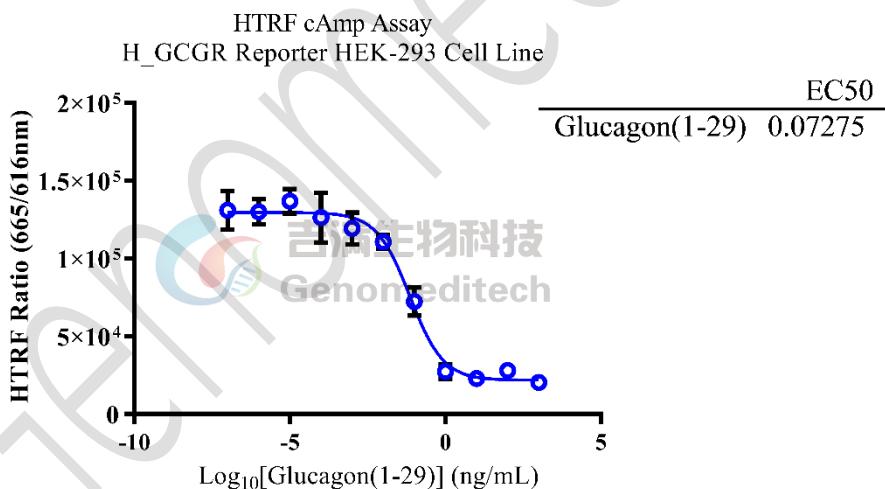


Figure 4 | H\_GCGR Reporter HEK-293 cells were seeded at a density of 7500 cells per well in white 384-well microplates (5  $\mu$ L per well). Gradient-diluted human Glucagon (1-29) solutions were then added, and the cells were incubated at room temperature for 30 minutes. The HTRF cAMP Gs Dynamic Detection Kit (Revvi, Cat. No. 62AM4PEB) was used according to the manufacturer's instructions. Fluorescence signals were measured using a Molecular Devices i3x multi-mode plate reader with excitation at 340 nm and emissions detected at 616 nm and 665 nm. The data were expressed as the 665 nm/616 nm  $\times$  100,000 (HTRF Ratio) and used to calculate the EC50 value.

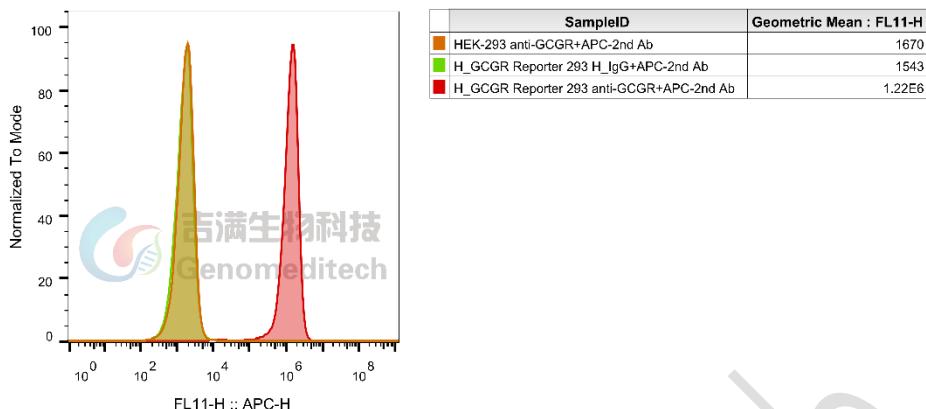


Figure 5 | H\_GCGR Reporter HEK-293 Cell Line (Cat. GM-C31698) was determined by flow cytometry using Anti-H\_GCGR hIgG1 Antibody(H4H1327P) (Cat. GM-84556AB).

## 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.
- 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.
- Resuspend cell pellet with the recommended recovery medium. And dispense into appropriate culture dishes.
- 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

- Centrifuge at 176 x g for 3 minutes to collect cells.
- Resuspend the cells in pre-cooled freezing medium and adjust the cell density to 5E6 cells/mL.
- Aliquot 1 mL into each vial.
- 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+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**

## 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 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>	
Human GCGR Protein; His Tag	Mouse GCGR Protein; His Tag
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="#">Rat_GLP1R HEK-293 Cell Line</a>
<a href="#">Anti-GLP1R hIgG1 Antibody(mAb-36986)</a>	<a href="#">Anti-H_GLP1R hIgG1 Antibody(glutazumab)</a>
<b>FGFR1</b>	
<a href="#">H_FGF21 Reporter HEK-293 Cell Line</a>	
<a href="#">Human FGF-21 Protein; His Tag</a>	
<b>CALCA(CGRP):CALCRL RAMP</b>	
<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>	
<b>GIPR</b>	
<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>	
<b>ACVR2A:ACTRIIB:Active A</b>	
<a href="#">ACVR2A KO HEK-293 Cell Line</a>	<a href="#">ACVR2B 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="#">H_ACVR2B Reporter DDX35TM 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(Bimagrumab)</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="#">Cynomolgus latent GDF-8 Protein; His 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>
<a href="#">Mouse latent GDF-8 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_THR<math>\beta</math> Reporter HEK-293 Cell Line</a>	
	<a href="#">MC4R</a>
<a href="#">H_MC4R Reporter HEK-293 Cell Line</a>	
	<a href="#">ASGR1</a>
<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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