Genomeditech (Shanghai) Co.,Ltd.
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Product Sheet

H_GCGR Reporter HEK-293 DDX35™ Cell Line

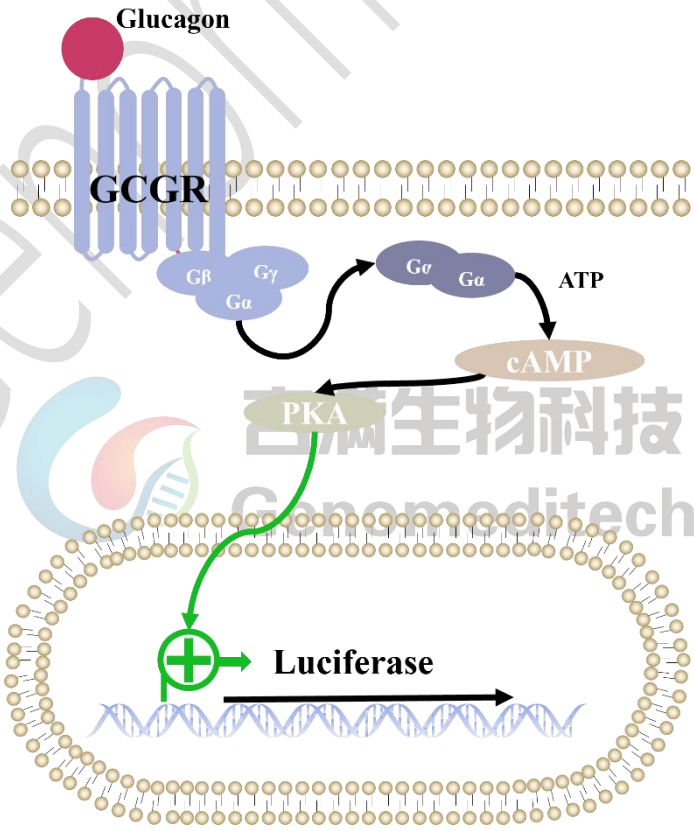
Catalog number: GM-C36999

Version 3.3.1.241226

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 α, β, and γ 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 DDX35™ Cell Line is a clonal stable HEK-293 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.

H_GCGR Reporter HEK-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+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
Puromycin	Genomeditech/GM-040401
Glucagon (1-29), bovine, human	MCE/HY-P0082
Anti-H_GCGR hIgG2 Antibody(volagidemab)	Genomeditech/GM-84555AB
GMOne-Step Luciferase Reporter Gene Assay Kit	Genomeditech/GM-040503

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Figures

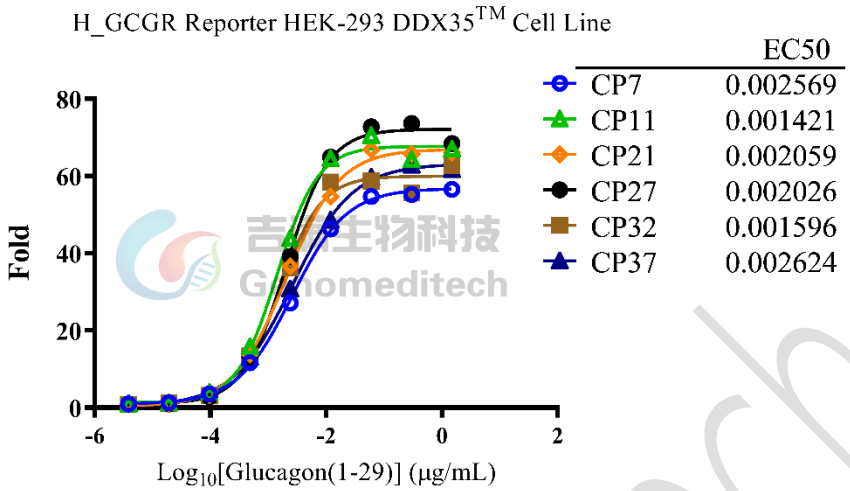


Figure 1 | The passage stability of response to Glucagon (1-29), bovine, human. The passage 7, 11, 21, 27, 32 and 37 of H_GCGR Reporter HEK-293 DDX35™ Cell Line (Cat. GM-C36999) at a concentration of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of Glucagon(1-29) (MCE/HY-P0082) in assay buffer (DMEM+1% FBS+1% P.S) for 16 hours. The firefly luciferase activity was measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). Data are shown by drug mass concentration.

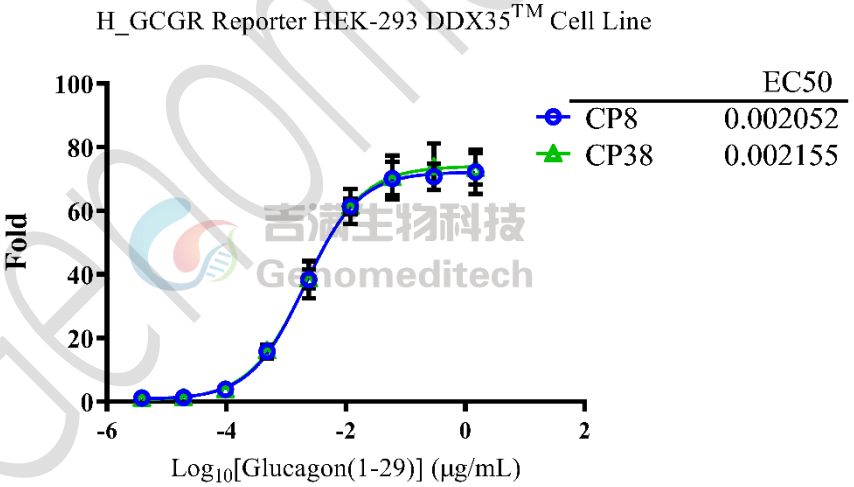
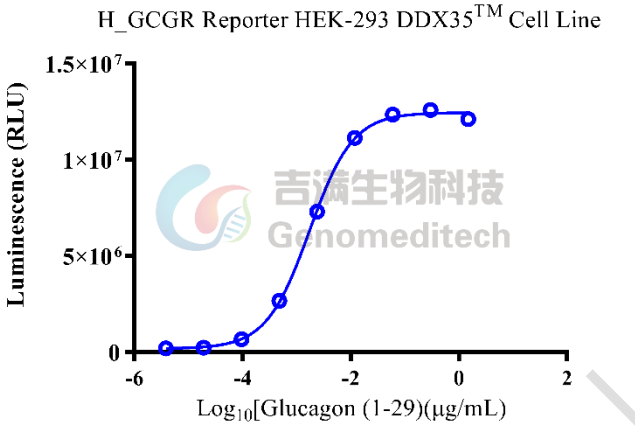
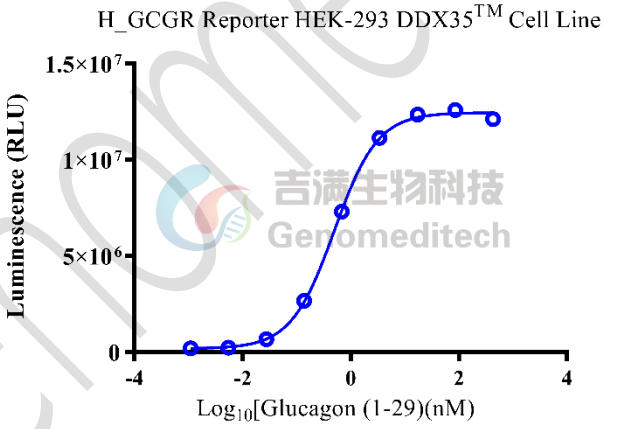


Figure 2 | The passage stability of response to Glucagon (1-29), bovine, human. The passage 8 and 38 of H_GCGR Reporter HEK-293 DDX35™ Cell Line (Cat. GM-C36999) at a concentration of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of Glucagon(1-29) (MCE/HY-P0082) in assay buffer (DMEM+1% FBS+1% P.S) for 16 hours, in triplicate. 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.



	H_GCGR Reporter HEK-293 DDX35TM
EC50	0.001745

Figure 3 | Response to Glucagon (1-29), bovine, human. The H_GCGR Reporter HEK-293 DDX35™ Cell Line (Cat. GM-C36999) 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 GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). The maximum induction fold was approximately [66.0]. Data are shown by drug mass concentration.



	H_GCGR Reporter HEK-293 DDX35TM
EC50	0.5011

Figure 4 | Response to Glucagon(1-29). The H_GCGR Reporter HEK-293 DDX35™ Cell Line (Cat. GM-C36999) 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 GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). The maximum induction fold was approximately [66.0]. Data are shown by drug molar concentration.

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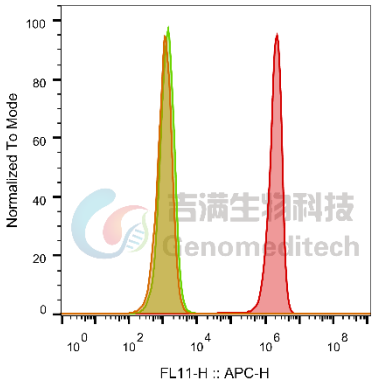

	SampleID	Geometric Mean : FL11-H
■	HEK-293 anti-GCGR+APC-2nd Ab	1087
■	H_GCGR Reporter 293 DDX35TM H_IgG+APC-2nd Ab	1307
■	H_GCGR Reporter 293 DDX35TM anti-GCGR+APC-2nd Ab	1.80E6

Figure 5 | H_GCGR Reporter HEK-293 DDX35™ Cell Line (Cat. GM-C36999) was determined by flow cytometry using Anti-H_GCGR hIgG2 Antibody(volagidemab)(Cat. GM-84555AB).

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+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	
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
H_GLP1R Reporter HEK-293 DDX35TM 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	H_CALCRL RAMP1 Reporter HEK-293 DDX35TM Cell Line
Cynomolgus_CALCRL RAMP1 HEK-293 Cell Line	H_CALCRL RAMP1 CHO-K1 Cell Line
H_CALCRL RAMP1 HEK-293 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	H_ACVR2B Reporter Cell Line
H_ACVR2B Reporter DDX35TM Cell Line	ACVR2B KO HEK-293 Cell Line
H_ACVR2A HEK-293(ACVR2B KO) Cell Line	H_ACVR2B CHO-K1 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 ACVR2A Protein; His-Avi Tag	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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