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

# Product Sheet

## H_GLP1R Reporter HEK-293 DDX35™ Cell Line

Catalog number: GM-C26019

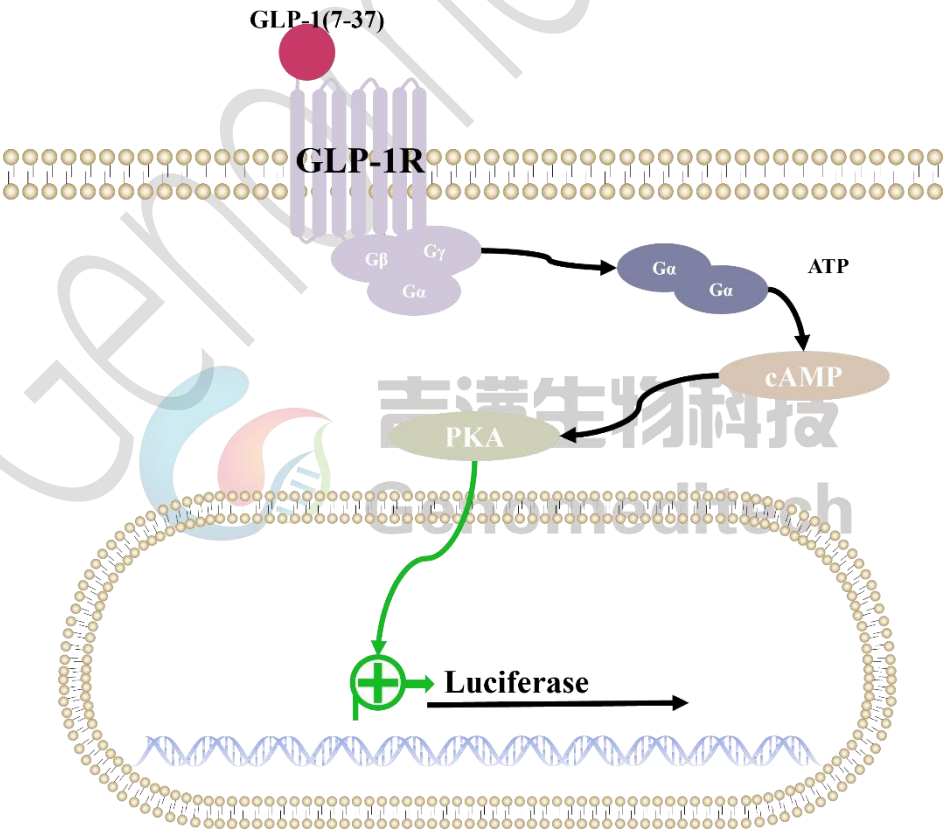
Version 3.3.1.241119

Glucagon-like peptide-1 receptor (GLP-1R) is a receptor protein found on pancreatic cells and brain neurons, made from the GLP1R gene on chromosome 6. As part of the glucagon receptor family of G protein-coupled receptors, GLP-1R, when activated, stimulates the adenylate cyclase pathway, boosting insulin synthesis and release. This makes it a target for diabetes medications called GLP-1R agonists, and it also helps regulate appetite in the brain.

GLP-1R recognizes specific ligands at its N-terminal and couples with various G proteins (Gαs, Gαi, Gαo, and Gαq/11) to influence cell pathways.

The H_GLP1R Reporter HEK-293 DDX35™ Cell Line is a clonal stable HEK-293 cell line constitutively expressing human GLP1R, along with signal-dependent expression of a luciferase reporter gene. The binding of the agonistic GLP-1 protein to GLP1R activates downstream reporter genes, leading to luciferase expression. Blockade antibodies of GLP1R can inhibit GLP1-GLP1R signal transmission. 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 GLP1R.

The H_GLP1R 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 |
| GLP-1(7-37) acetate | MCE/HY-P0055A |
| GMOne-Step Luciferase Reporter Gene Assay Kit | Genomeditech/GM-040503 |

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# Figures

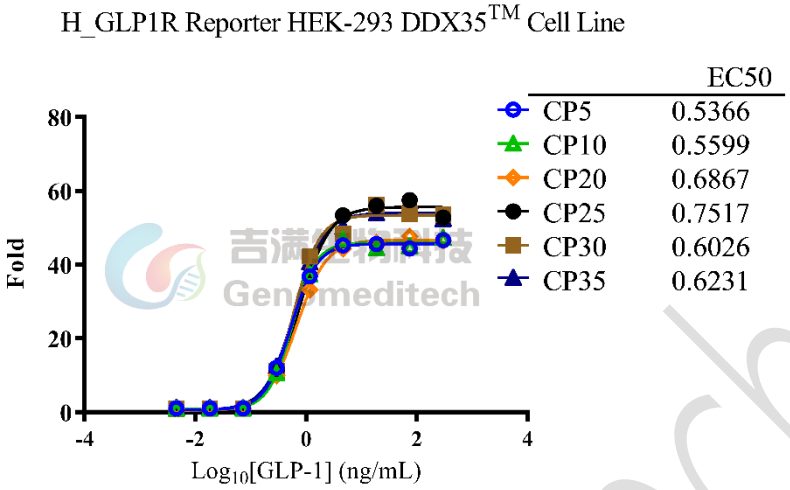



Figure 1 | The passage stability of response to Recombinant GLP-1(7-37) acetate. The passage 5, 10, 20, 25, 30 and 35 of H_GLP1R Reporter HEK-293 DDX35™ Cell Line (Cat. GM-C26019) at a concentration of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of GLP-1(7-37) (MCE/HY-P0055A) 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.

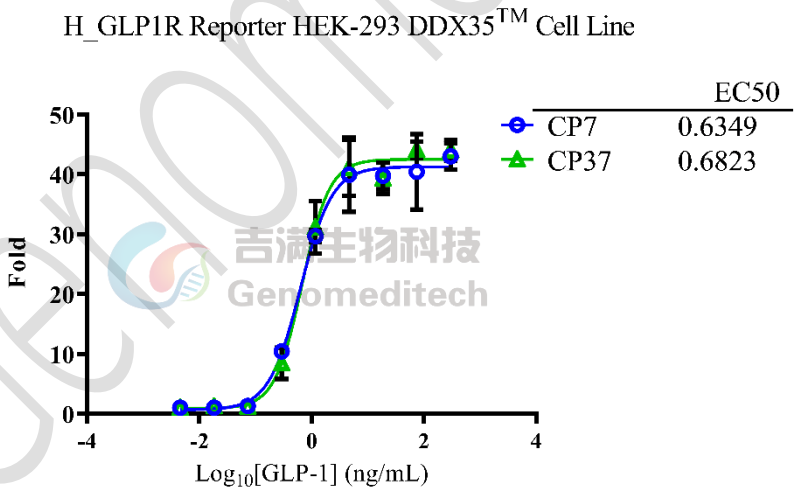
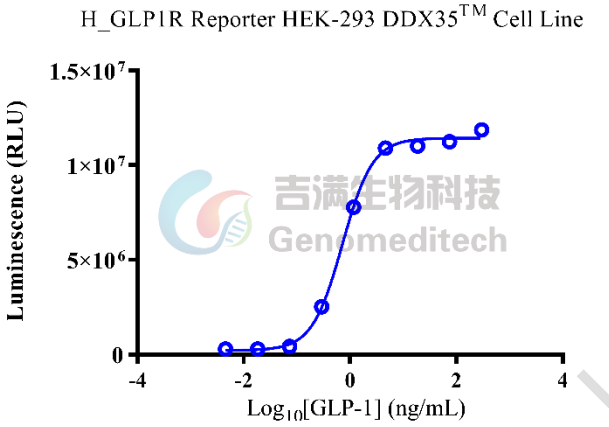
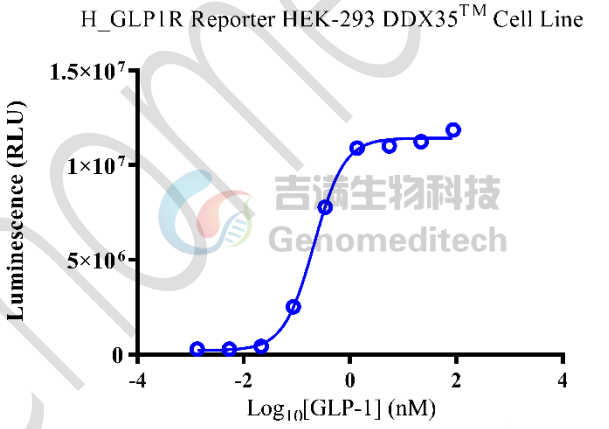



Figure 2 | The passage stability of response to GLP-1(7-37) acetate. The passage 7 and 37 of H_GLP1R Reporter HEK-293 DDX35™ Cell Line (Cat. GM-C26019) at a concentration of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of GLP-1(7-37) (MCE/HY-P0055A) in assay buffer (DMEM+1% FBS+1% P.S) for 7 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_GLP1R Reporter HEK-293 DDX35TM |
|---|---|
| EC50 | 0.7227 |

Figure 3 | Response to GLP-1(7-37) acetate. The H_GLP1R Reporter HEK-293 DDX35™ Cell Line (Cat. GM-C26019) at a concentration of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of GLP-1(7-37) (MCE/HY-P0055A) 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 [45.4]. Data are shown by drug mass concentration.




| | H_GLP1R Reporter HEK-293 DDX35TM |
|---|---|
| EC50 | 0.2116 |

Figure 4 | Response to GLP-1(7-37) acetate. The H_GLP1R Reporter HEK-293 DDX35™ Cell Line (Cat. GM-C26019) at a concentration of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of GLP-1(7-37) (MCE/HY-P0055A) 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 [45.4]. Data are shown by drug molar concentration.

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## 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 |
| 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 DDX35™ 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 |

| | |
|---|---|
| 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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