Genomeditech (Shanghai) Co.,Ltd.
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Product Sheet

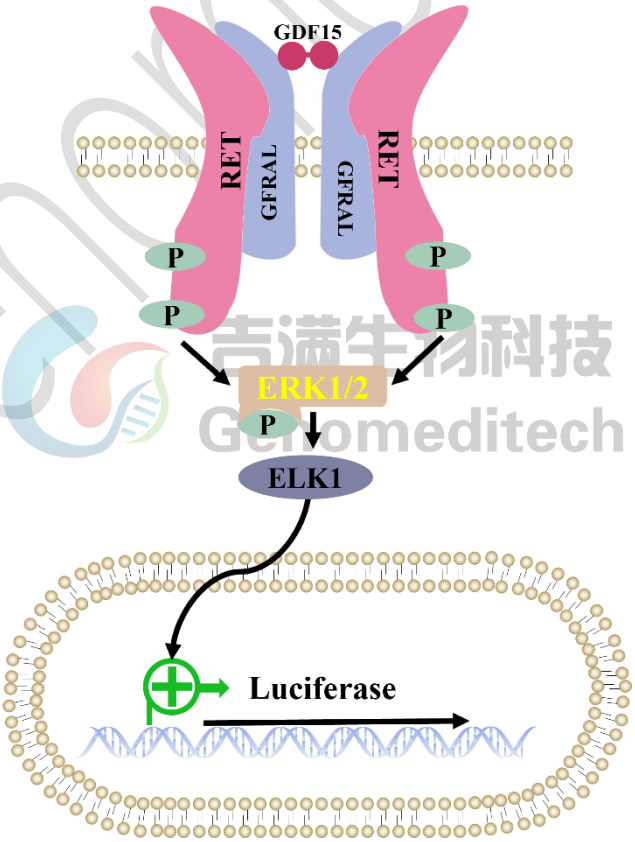
H_GDF15 Reporter 293 Cell Line (High Basal)

Catalog number: GM-C43937

Version 3.3.1.260603

Growth differentiation factor 15 (GDF15), a distant member of the transforming growth factor-β (TGF-β) superfamily, is a secreted protein. It binds exclusively to the glial cell line-derived neurotrophic factor family receptor alpha (GFRAL), which serves as its sole receptor. This interaction recruits the co-receptor RET, activating downstream signaling pathways characteristic of the GDNF family, including phosphorylation of RET, AKT, ERK, and PLC-γ1.

H_GDF15 Reporter 293 Cell Line is a clonal stable HEK-293 cell line constructed using lentiviral technology, constitutive expression of the human GFRAL gene and human RET, along with signal-dependent expression of a luciferase reporter gene. The H_GDF15 Reporter 293 cell line (high basal luciferase activity, Cat. GM-C43937) and the H_GDF15 Reporter 293 cell line (low basal luciferase activity, Cat. GM-C06718) are both luciferase reporter cell lines constructed based on the RET-ERK signaling pathway. The core difference between them lies in the level of basal luciferase activity: the former is a cell line with high basal luciferase activity, while the latter is a cell line with low basal luciferase activity. When GDF15 binds to GFRAL-RET, it activates downstream signaling pathways, leading to the expression of luciferase. 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+1.5 μg/mL Blasticidin+400 μg/mL G418+125 μg/mL Hygromycin+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	ExCell/FSP500
Pen/Strep	Thermo/15140-122
Blasticidin	Genomeditech/GM-040404
G418	Genomeditech/GM-040402
Hygromycin	Genomeditech/GM-040403
Puromycin	Genomeditech/GM-040401
Human GDF15 Protein; His Tag	Genomeditech/GM-87627RP
Anti-H_GFRAL hIgG4 Antibody(QUEL-0301)	Genomeditech/GM-71276AB
Anti-GDF15 hIgG1 Reference Antibody(Ponsbio)	Genomeditech/GM-87500MAB
GMOne-Step 2.0 Luciferase Reporter Gene Assay Kit	Genomeditech/GM-040513

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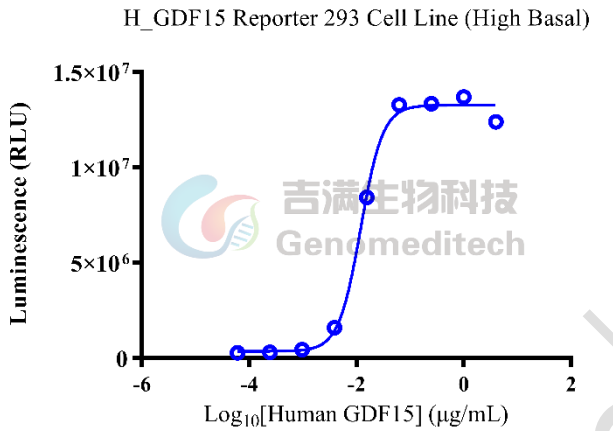
Figures



	H_GDF15 Reporter 293 (High Basal)
EC50	0.01208

Figure 1 | Response to Human GDF15 Protein; His Tag. The H_GDF15 Reporter 293 Cell Line (High Basal) (Cat. GM-C43937) at a concentration of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of Human GDF15 Protein; His Tag (Cat. GM-87627RP) 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.

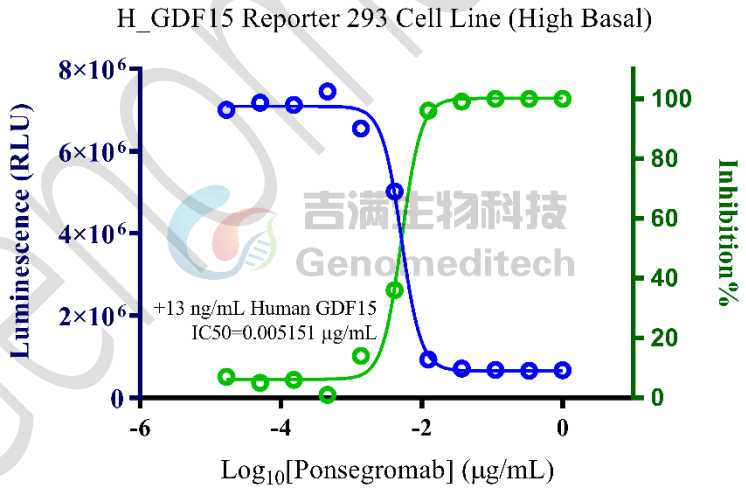
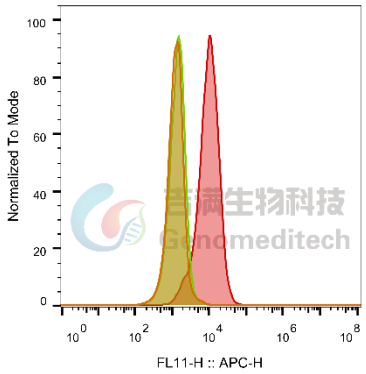


Figure 2 | Inhibition of Human GDF15 protein-induced reporter activity by Anti-GDF15 hIgG1 Reference Antibody(Ponsbio). Serial dilutions of Anti-GDF15 hIgG1 Reference Antibody(Ponsbio) (Cat. GM-87500MAB) was incubated with 1.3 ng/well of Human GDF15 Protein; His Tag (GM-87627RP) for 1 hour in assay buffer (DMEM+1% FBS+1% P.S). After pre-incubation, add the mixture to the H_GDF15 Reporter 293 Cell Line (High Basal) (Cat. GM-C43937) at a density of 1.5E4 cells/well in a 96-well format, and incubate for 16 hours. Firefly luciferase activity was then measured using the Luciferase Reporter Assay Kit (Genomeditech) (left Y-axis, relative luminescence units), with inhibition percentages shown on the right Y-axis. Data are shown by drug mass concentration.



SampleID	Geometric Mean : FL11-H
293 anti-H_GFRAL+APC-2nd Ab	1234
H_GDF15 Reporter 293(High Basal) H_IgG+APC-2nd Ab	1339
H_GDF15 Reporter 293(High Basal) anti-H_GFRAL+APC-2nd Ab	9547

Figure 3 | H_GDF15 Reporter 293 Cell Line (High Basal) (Cat. GM-C43937) was determined by flow cytometry using Anti-H_GFRAL Antibody(QUEL-0301) (Cat. GM-71276AB).

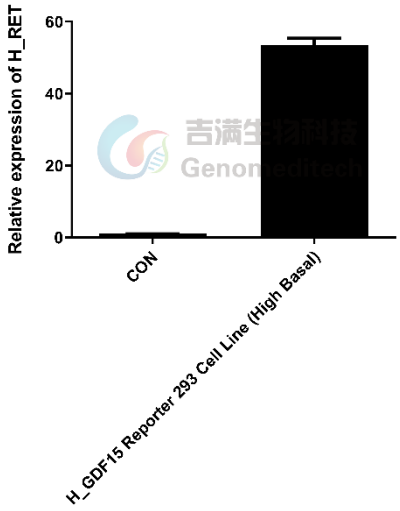


Figure 4 | The mRNA expression levels of H_RET in the H_GDF15 Reporter 293 Cell Line (High Basal) (Cat. GM-C43937) were determined by RT-qPCR.

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).

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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+1.5 μg/mL Blasticidin+400 μg/mL G418+125 μg/mL Hygromycin+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

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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

GDF15:GFRAL	
H_GDF15 Reporter 293 Cell Line	H_GDF15 Reporter 293 DDX35TM Cell Line
Mouse_GDF15 Reporter 293 Cell Line	H_GDF15 MC38 Cell Line
H_GFRAL CHO-K1 Cell Line	H_GFRAL HEK-293 Cell Line
Membrane Bound H_GDF15 CHO-K1 Cell Line	
Anti-GDF15 hIgG1 Reference Antibody(Ponsbio)	Anti-GDF15 hIgG4 Reference Antibody (Visubio)
Anti-GFRAL hIgG1 Antibody(NGM-120)	Anti-H_GDF15 hIgG4 Antibody(CTL-002)
Anti-H_GFRAL hIgG4 Antibody(QUEL-0301)	
Biotinylated Human GFRAL Protein; His-Avi Tag	Cynomolgus GFRAL Protein; His Tag
Human GDF15 Protein; His Tag	Human GFRAL Protein; hFc Tag
Human GFRAL Protein; His Tag	Human pro-GDF15 Protein; His Tag

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