

# Product Sheet

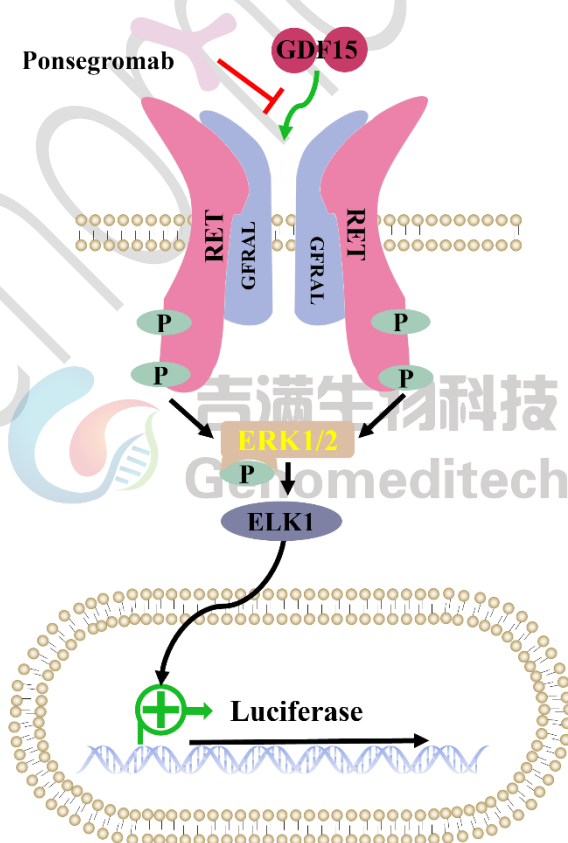
## H\_GDF15 Reporter 293 Cell Line

Catalog number: GM-C06718

Version 3.3.1.260603

Growth differentiation factor 15 (GDF15), a distant member of the transforming growth factor- $\beta$  (TGF- $\beta$ ) 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- $\gamma$ 1.

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 (low basal luciferase activity, Cat. GM-C06718) and the H\_GDF15 Reporter 293 cell line (high basal luciferase activity, Cat. GM-C43937) 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.



## 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+1.5 µg/mL Blasticidin+400 µg/mL G418+125 µg/mL Hygromycin+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>
G418	Genomeditech/ <a href="#">GM-040402</a>
Hygromycin	Genomeditech/ <a href="#">GM-040403</a>
Puromycin	Genomeditech/ <a href="#">GM-040401</a>
Human GDF15 Protein; His Tag	Genomeditech/ <a href="#">GM-87627RP</a>
Anti-GDF15 hIgG1 Reference Antibody(Ponsbio)	Genomeditech/ <a href="#">GM-87500MAB</a>
Anti-H_GFRAL hIgG4 Antibody(QUEL-0301)	Genomeditech/ <a href="#">GM-71276AB</a>
Anti-H_GDF15 hIgG4 Antibody(CTL-002)	Genomeditech/ <a href="#">GM-71274AB</a>
Anti-GFRAL hIgG1 Antibody(NGM-120)	Genomeditech/ <a href="#">GM-77399AB</a>
GMOne-Step 2.0 Luciferase Reporter Gene Assay Kit	Genomeditech/ <a href="#">GM-040513</a>

## Figures

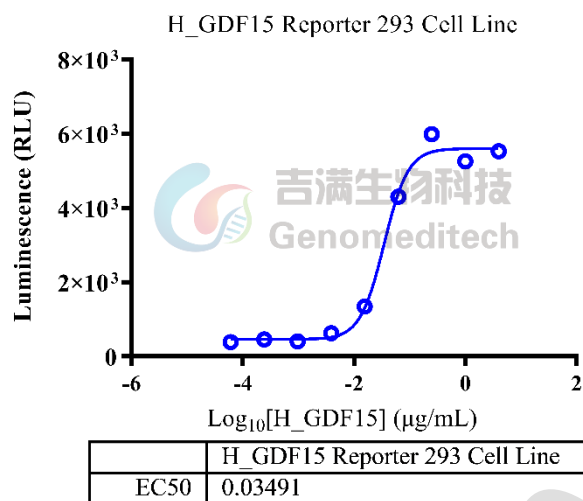


Figure 1 | Response to Human GDF15 Protein; His Tag. The H\_GDF15 Reporter 293 Cell Line (Cat. GM-C06718) at a concentration of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of Human GDF15 Protein; His Tag (Cat. GM-87627RP) in assay buffer (DMEM+1% FBS+1% P.S) for 16 hours. The firefly luciferase activity was measured using the Luciferase Reporter Assay Kit (Genomeditech). The maximum induction fold was approximately [14.0]. Data are shown by drug mass concentration.

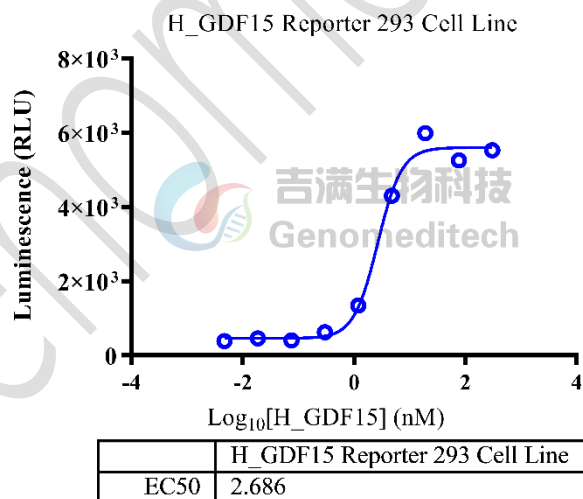


Figure 2 | Response to Human GDF15 Protein; His Tag. The H\_GDF15 Reporter 293 Cell Line (Cat. GM-C06718) at a concentration of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of Human GDF15 Protein; His Tag (Cat. GM-87627RP) in assay buffer (DMEM+1% FBS+1% P.S) for 16 hours. The firefly luciferase activity was measured using the Luciferase Reporter Assay Kit (Genomeditech). The maximum induction fold was approximately [14.0]. Data are shown by drug molar concentration.

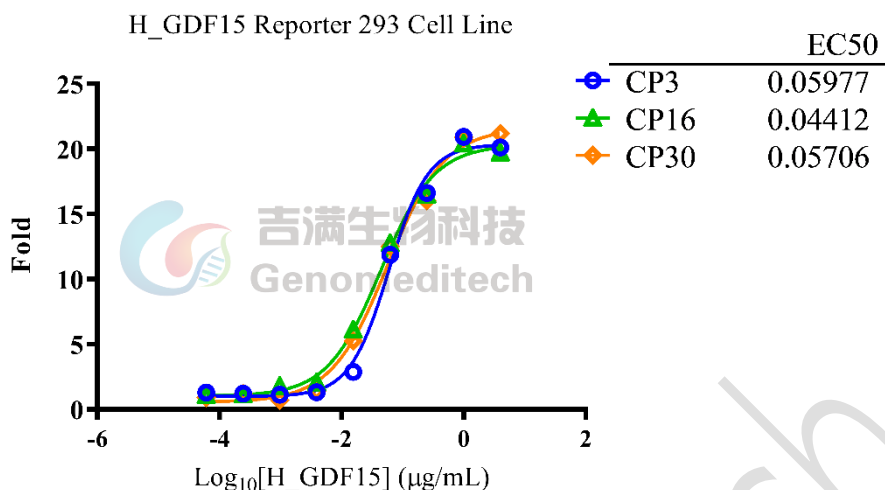


Figure 3 | The passage stability of response to Human GDF15 Protein; His Tag. The passage 3, 16, and 30 of H\_GDF15 Reporter 293 Cell Line (Cat. GM-C06718) at a concentration of 1.5E4 cells/well (96-well format) were stimulated with serial dilutions of Human GDF15 Protein; His Tag (Cat. GM-87627RP) in 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.

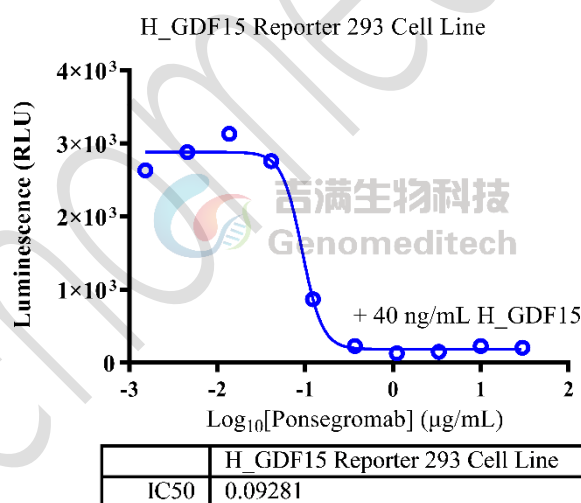


Figure 4 | Response to Anti-GDF15 hIgG1 Reference Antibody(Ponsbio). Serial dilutions of Anti-GDF15 hIgG1 Reference Antibody(Ponsbio) (Cat. GM-87500MAB) was incubated with 4 ng/well of Human GDF15 Protein (GM-87627RP) for 1 hour in assay buffer (DMEM+1% FBS+1% P.S). After pre-incubation, add the mixture to the H\_GDF15 Reporter 293 Cell Line (Cat. GM-C06718) 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). The results indicated maximum blocking folds of approximately [9.4]. Data are shown by drug mass concentration.

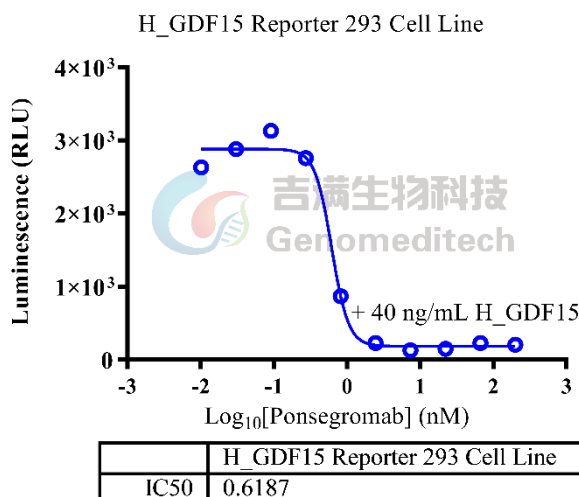


Figure 5 | Response to Anti-GDF15 hIgG1 Reference Antibody(Ponsbio). Serial dilutions of Anti-GDF15 hIgG1 Reference Antibody(Ponsbio) (Cat. [GM-87500MAB](#)) was incubated with 4 ng/well of Human GDF15 Protein ([GM-87627RP](#)) for 1 hour in assay buffer (DMEM+1% FBS+1% P.S). After pre-incubation, add the mixture to the H\_GDF15 Reporter 293 Cell Line (Cat. GM-C06718) 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). The results indicated maximum blocking folds of approximately [9.4]. Data are shown by drug molar concentration.

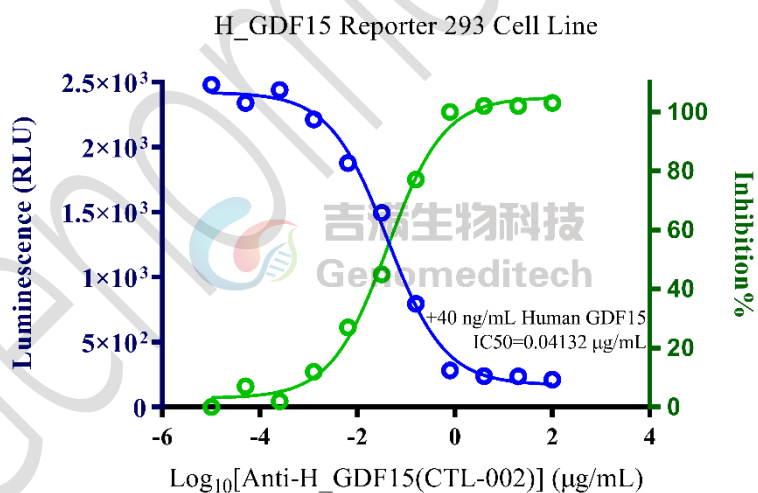


Figure 6 | Inhibition of Human GDF15 protein-induced reporter activity by Anti-H\_GDF15 hIgG4 Antibody(CTL-002). Serial dilutions of Anti-H\_GDF15 hIgG4 Antibody(CTL-002) (Cat. [GM-71274AB](#)) was incubated with 40 ng/mL of Human GDF15 Protein (Cat. [GM-87627RP](#)) for 1 hour in assay buffer (DMEM+1% FBS+1% P.S). After pre-incubation, add the mixture to the H\_GDF15 Reporter 293 Cell Line (Cat. GM-C06718) 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.

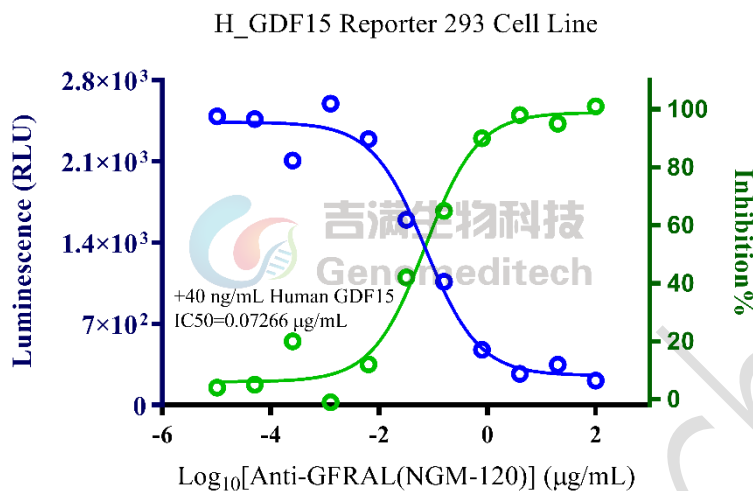


Figure 7 | Inhibition of Human GDF15 protein-induced reporter activity by Anti-GFRAL hIgG1 Antibody(NGM-120). Serial dilutions of the Anti-GFRAL hIgG1 Antibody(NGM-120) (Cat. [GM-77399AB](#)) were incubated with 1.5E4 cells/well of the H\_GDF15 Reporter 293 Cell Line (Cat. GM-C06718) in a 96-well plate for 1 hour in assay buffer (DMEM + 1% FBS + 1% P.S). Subsequently, the Human GDF15 Protein; His Tag (Cat. [GM-87627RP](#)) at a density of 40 ng/mL was added, and the coculture proceeded for an additional 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.

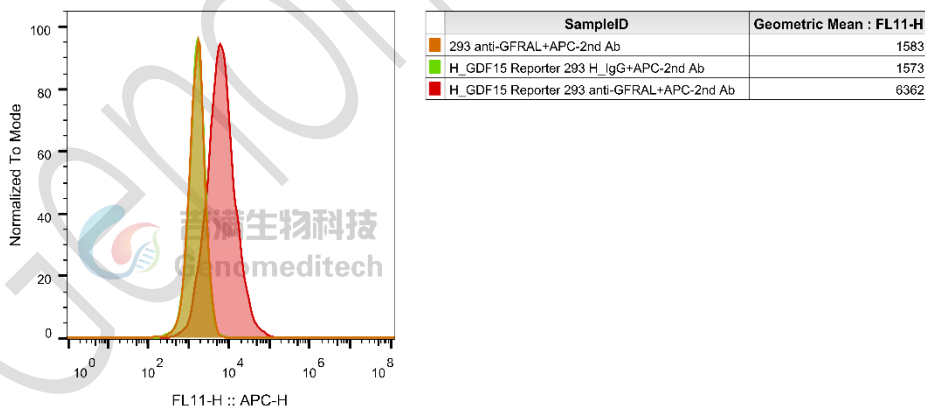


Figure 8 | H\_GDF15 Reporter 293 Cell Line (Cat. GM-C06718) was determined by flow cytometry using Anti-H\_GFRAL hIgG4 Antibody(QUEL-0301) (Cat. [GM-71276AB](#)).

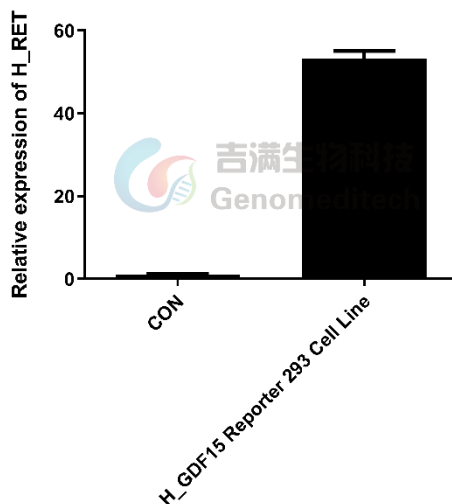


Figure 9 | The mRNA expression levels of H\_RET in the H\_GDF15 Reporter 293 Cell Line (Cat. GM-C06718) were determined by RT-qPCR.

## 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  $-70^{\circ}\text{C}$ . Storage at  $-70^{\circ}\text{C}$  will result in loss of viability.

- Thaw the vial by gentle agitation in a  $37^{\circ}\text{C}$  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  $176 \times g$  for 5 minutes. Discard supernatant.
- Resuspend cell pellet with the recommended recovery medium. And dispense into appropriate culture dishes.
- Incubate the culture at  $37^{\circ}\text{C}$  in a suitable incubator. A 5%  $\text{CO}_2$  in air atmosphere is recommended if using the medium described on this product sheet.

## Cell Freezing

Freezing Medium: 90% FBS+10%DMSO

- Centrifuge at  $176 \times g$  for 3 minutes to collect cells.
- Resuspend the cells in pre-cooled freezing medium and adjust the cell density to  $5 \times 10^6$  cells/mL.
- Aliquot 1 mL into each vial.

- d) Place the vial in a controlled-rate freezing container and store at  $-80^{\circ}\text{C}$  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  $\mu\text{g}/\text{mL}$  Blasticidin+400  $\mu\text{g}/\text{mL}$  G418+125  $\mu\text{g}/\text{mL}$  Hygromycin+0.75  $\mu\text{g}/\text{mL}$  Puromycin

For the first 1 to 2 passages post-resuscitation, use the recovery medium. Once the cells have stabilized, switch to a growth medium.

- 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.
- Remove and discard culture medium.
- Briefly rinse the cell layer with PBS to remove all traces of serum that contains trypsin inhibitor.
- 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  $37^{\circ}\text{C}$ ).
- 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  $37^{\circ}\text{C}$  to facilitate dispersal.
- Add 2.0 mL of growth medium to mix well and aspirate cells by gently pipetting.
- After centrifugation, resuspend the pellet and add appropriate aliquots of the cell suspension to new culture vessels.
- Incubate cultures at  $37^{\circ}\text{C}$ .

**Subcultivation Ratio: A subcultivation ratio of 1:3 - 1:4 is recommended**

**Medium Renewal: Every 2 to 3 days**

## Notes

- After initial thawing, a higher proportion of dead cells is normal. The cell culture generally improves noticeably after approximately one week of adaptation. Once the culture stabilizes, the percentage of dead cells decreases with subsequent passages, and the cell proliferation rate becomes more consistent.
- It is important to maintain the cell density below 80%, as exceeding this threshold can lead to decreased cell viability and metabolic activity due to overcrowding.

## Related Products

GDF15:GFRAL	
<a href="#">H_GDF15 Reporter 293 Cell Line (High Basal)</a>	<a href="#">H_GDF15 Reporter 293 DDX35TM Cell Line</a>
<a href="#">Mouse_GDF15 Reporter 293 Cell Line</a>	<a href="#">H_GDF15 MC38 Cell Line</a>
<a href="#">H_GFRAL CHO-K1 Cell Line</a>	<a href="#">H_GFRAL HEK-293 Cell Line</a>
<a href="#">Membrane Bound H_GDF15 CHO-K1 Cell Line</a>	
<a href="#">Anti-GDF15 hIgG1 Reference Antibody(Ponsbio)</a>	<a href="#">Anti-GDF15 hIgG4 Reference Antibody (Visubio)</a>

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

## 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.
- Users and their contractors engaged for their benefit may use this material and its derivatives only within the agreed research scope; modification of the material is not permitted, nor may it be distributed, sold, transferred, or otherwise provided to any other entity (including affiliates).
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