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# **Product Sheet**

# **H\_FGF21 Reporter HEK-293 Cell Line**

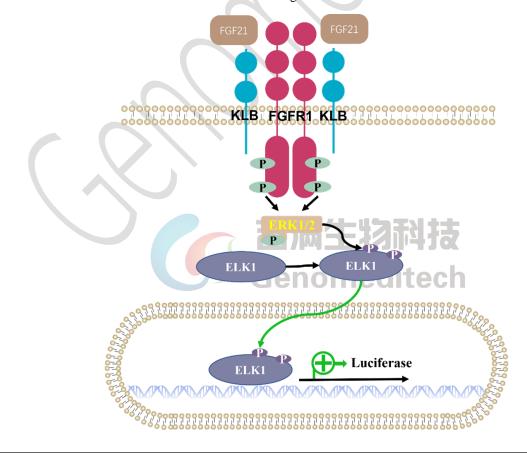
Catalog number: GM-C19834

Version 3.3.1.241217

FGF21 (Fibroblast Growth Factor 21) is a protein secreted by the liver, adipose tissue, and muscle, belonging to the FGF family. It is essential for energy metabolism, glucose homeostasis, and fatty acid oxidation, especially during hunger and metabolic stress. FGF21 regulates these processes by binding to its receptor FGFR and activating downstream signaling pathways, with secretion influenced by nutritional status, insulin levels, and inflammation.

The FGF21 signaling pathway is mediated by FGFR and the co-receptor  $\beta$ -klotho. Binding to FGFR activates downstream pathways like MAPK and PI3K/Akt, promoting fatty acid oxidation, glucose uptake, and insulin sensitivity. FGF21 also regulates gene expression in the liver and adipose tissue, impacting energy and lipid metabolism. Thus, FGF21 is a potential therapeutic target for metabolic syndrome and diabetes.

H\_FGF21 Reporter HEK-293 Cell Line is a clonal stable HEK-293 cell line constructed using lentiviral technology, constitutive expression of the Klothoβ and FGFR1c gene, along with signal-dependent expression of a luciferase reporter gene. When FGF21 binds to FGF21 Receptor Signaling Complex, 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 FGF21.





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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

Hygromycin+0.75 μg/mL Puromycin

Note None

Freezing Medium 90% FBS+10% DMSO

**Growth properties** Adherent

**Growth Conditions** 37°C, 5% CO<sub>2</sub>

**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
G418	Genomeditech/GM-040402
Hygromycin	Genomeditech/GM-040403
Puromycin	Genomeditech/GM-040401
Recombinant Human FGF-21	R&D SYSTEMS/2539-FG/CF
Human FGFa (140AA)	Novoprotein/C049
Human FGFb (157AA)	Novoprotein/C046
Anti-Human CD331/FGFR1 Antibody (A08)	atagenix/FHC88110
GMOne-Step Luciferase Reporter Gene Assay Kit	Genomeditech/GM-040503



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#### **Figures**

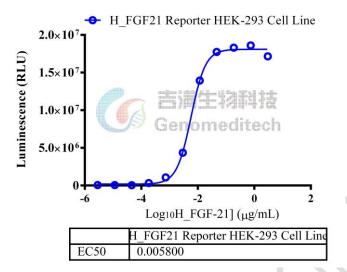


Figure 1 | Response to Recombinant Human FGF-21. The H\_FGF21 Reporter HEK-293 Cell Line (Cat. GM-C19834) at a concentration of 1.3E4 cells/well (96-well format) was stimulated with serial dilutions of Recombinant Human FGF-21 (R&D SYSTEMS/2539-FG/CF) in assay buffer (DMEM + 1% FBS + 1% P.S) for 16 hours. The firefly luciferase activity was measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). The maximum induction fold was approximately [379.4]. Data are shown by drug mass concentration.

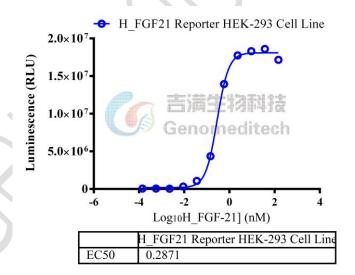
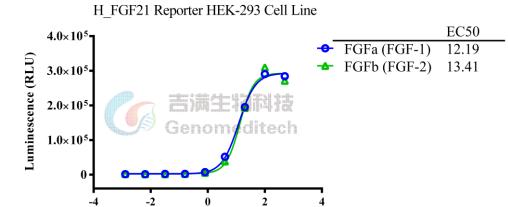


Figure 2 | Response to Recombinant Human FGF-21. The H\_FGF21 Reporter HEK-293 Cell Line (Cat. GM-C19834) at a concentration of 1.3E4 cells/well (96-well format) was stimulated with serial dilutions of Recombinant Human FGF-21 (R&D SYSTEMS/2539-FG/CF) in assay buffer (DMEM + 1% FBS + 1% P.S) for 16 hours. The firefly luciferase activity was measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). The maximum induction fold was approximately [379.4]. Data are shown by drug molar concentration.



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Log<sub>10</sub>[Sample] (ng/mL)

Figure 3 | Response to Human FGFa (140AA) and Human FGFb (157AA). The H\_FGF21 Reporter HEK-293 Cell Line (Cat. GM-C19834) at a concentration of 1.3E4 cells/well (96-well format) was stimulated with serial dilutions of Human FGFa (140AA) (Novoprotein/C049) and Human FGFb (157AA) (Novoprotein/C046) in assay buffer (DMEM + 1% FBS + 1% P.S) for 6 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 [125.1] and [165.0], respectively. Data are shown by drug mass concentration.

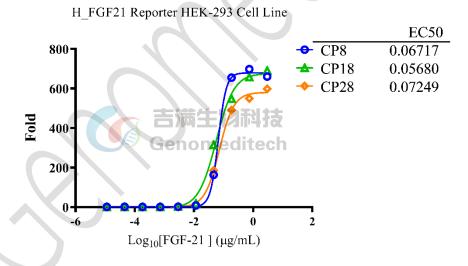
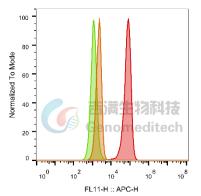


Figure 4 | The passage stability of response to Recombinant Human FGF-21 Protein. The passage 8, 18 and 28 of H\_FGF21 Reporter HEK-293 Cell Line (Cat. GM-C19834) at a concentration of 1.3E4 cells/well (96-well format) were stimulated with serial dilutions of Recombinant Human FGF-21 Protein (R&D SYSTEMS/2539-FG/CF) 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.



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SampleID	Geometric Mean : FL11-H
HEK-293 Anti-H_FGFR1+APC-2nd Ab	2199
H_FGF21 Reporter M_IgG+APC-2nd Ab	1213
H_FGF21 Reporter Anti-H_FGFR1+APC-2nd Ab	71297

Figure 5 | H\_FGF21 Reporter HEK-293 Cell Line (Cat. GM-C19834) was determined by flow cytometry using Anti-Human CD331/FGFR1 Antibody (A08) (atagenix/FHC88110).

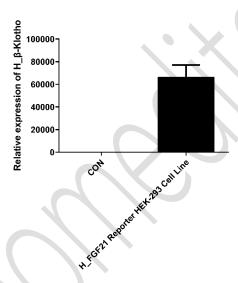


Figure 6 | The mRNA expression levels of  $H_{\beta}$ -Klotho in the  $H_{FGF21}$  Reporter HEK-293 Cell Line (Cat. GM-C19834) were determined by RT-qPCR.

### **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).
- 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.

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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<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

- 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+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

#### 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.



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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)		
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		
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 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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