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Product Sheet

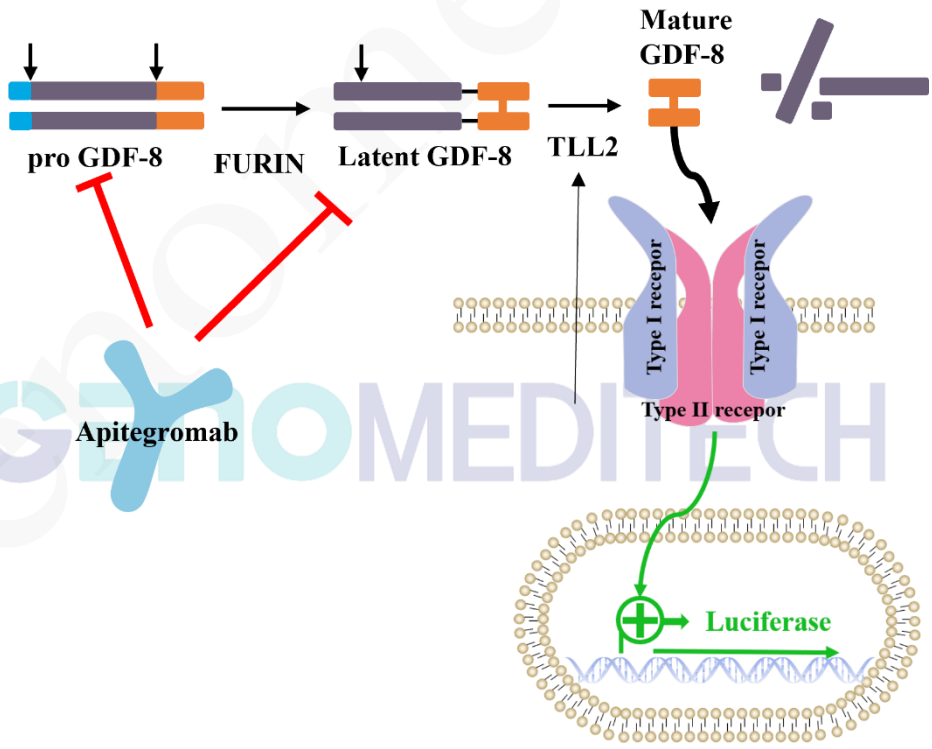
GDF8 Pro-Latent Activation Reporter 293 Cell Line

Catalog number: GM-C42533

Version 4.0.26.1023

GDF-8 (myostatin) is a secreted TGF-β superfamily factor that negatively regulates skeletal muscle mass. It is synthesized as pro-GDF-8, cleaved by furin-like proteases into an N-terminal prodomain and a C-terminal mature domain that form an inactive latent complex. Activation requires a second cleavage by BMP1/Tolloid proteases, with TLL2 being most effective; TLL2 cleaves near D99 in the prodomain, destabilizing the complex and promoting mature GDF-8 release and receptor binding. Thus, GDF-8 function depends on precursor processing and TLL2-mediated activation of the latent complex at this "second cleavage" checkpoint, an important node for muscle homeostasis intervention.

GDF8 Pro-Latent Activation Reporter 293 Cell Line is a clonal stable cell line generated via lentiviral technology. The cells endogenously express both Type I and Type II receptors and constitutively express the TLL2 gene, while a luciferase reporter gene is expressed in a signal-dependent manner. Upon ligand binding to the receptor, downstream signaling pathways are activated, leading to luciferase expression. The luciferase readout reflects the activation level of the signaling pathway and can therefore be used to evaluate the in vitro effects of GDF8-related drugs.



For research use only!

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	ExCell/FSP500
Pen/Strep	Thermo/15140-122
Blasticidin	Genomeditech/GM-040404
Puromycin	Genomeditech/GM-040401
96 well round cell culture plate	PakGent/CL-PT096
96-well White Opaque Plate	Thermo/236108
96-well U-bottom Plate	Saining/1014010
Human pro GDF-8 Protein	Kactus/GDF-HM118
Human Latent GDF-8 Protein (pro&latent)	Kactus/GDF-HM108
Anti-GDF8 hIgG4 Reference Antibody (Apitbio)	Genomeditech/GM-87970MAB
GMOne-Step 2.0 Luciferase Reporter Gene Assay Kit	Genomeditech/GM-040513
Countess 3 Automated Cell Counter	ThermoFisher/A49891
SpectraMax® L Microplate Luminometer	Moleculardevices/SpectraMax L

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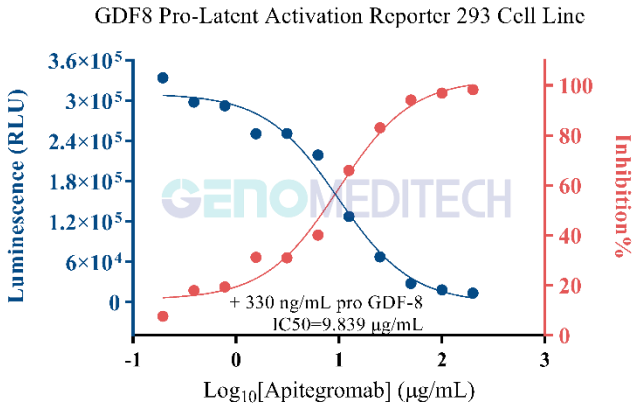
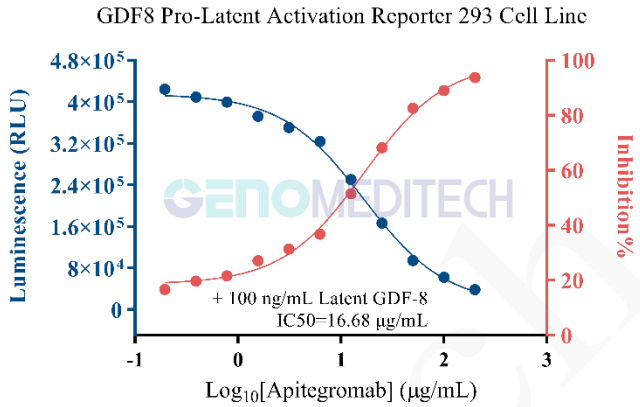
Data

Applications



Inhibition of Human pro GDF-8 Protein-induced reporter activity by Anti-GDF8 hIgG4 Reference Antibody (Apitbio).

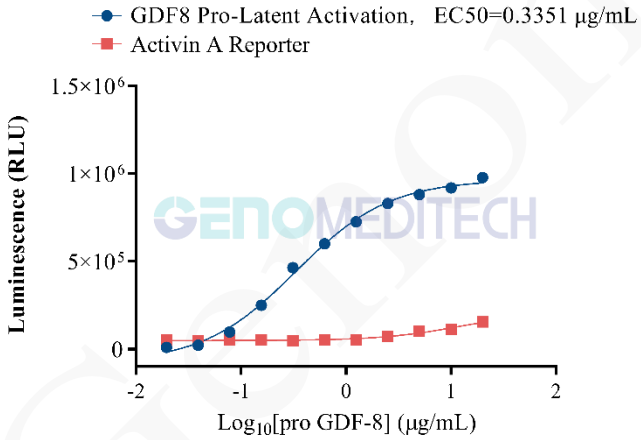
Serial dilutions ofAnti-GDF8 hIgG4 Reference Antibody (Apitbio)(Cat. GM-87970MAB) were incubated with 33 ng/well of Human pro GDF-8 Protein (Kactus/GDF-HM118) for 1 hour in assay buffer (DMEM+1% FBS+1% P.S). After pre-incubation, add the mixture to the GDF8 Pro-Latent Activation Reporter 293 Cell Line (Cat. GM-C42533) at a density of 1.5E4 cells/well in a 96-well format, and incubate for 24 hours. Firefly luciferase activity was measured using the Luciferase Reporter Assay Kit (Genomeditech) (left Y-axis, relative luminescence units, RLU), with inhibition percentages shown on the right Y-axis. Data are shown by drug mass concentration.



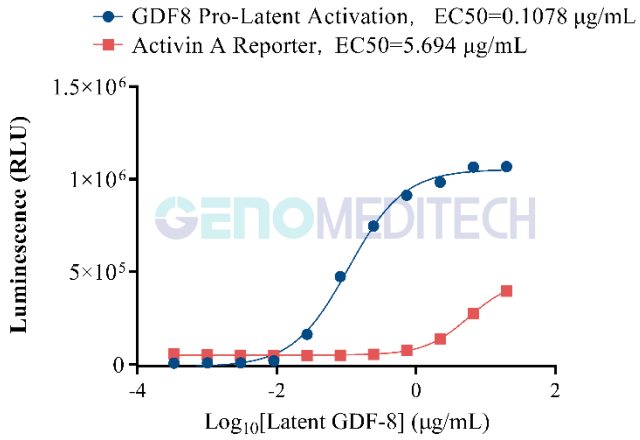
Inhibition of Human Latent GDF-8 Protein-induced reporter activity by Anti-GDF8 hIgG4 Reference Antibody (Apitbio).

Serial dilutions of Anti-GDF8 hIgG4 Reference Antibody (Apitbio)(Cat. GM-87970MAB) were incubated with 10 ng/well of Human Latent GDF-8 Protein (Kactus/GDF-HM108) for 1 hour in assay buffer (DMEM+1% FBS+1% P.S). After pre-incubation, add the mixture to the GDF8 Pro-Latent Activation Reporter 293 Cell Line (Cat. GM-C42533) at a density of 1.5E4 cells/well in a 96-well format, and incubate for 24 hours. Firefly luciferase activity was measured using the Luciferase Reporter Assay Kit (Genomeditech) (left Y-axis, relative luminescence units, RLU), with inhibition percentages shown on the right Y-axis. Data are shown by drug mass concentration.

Signaling Pathway

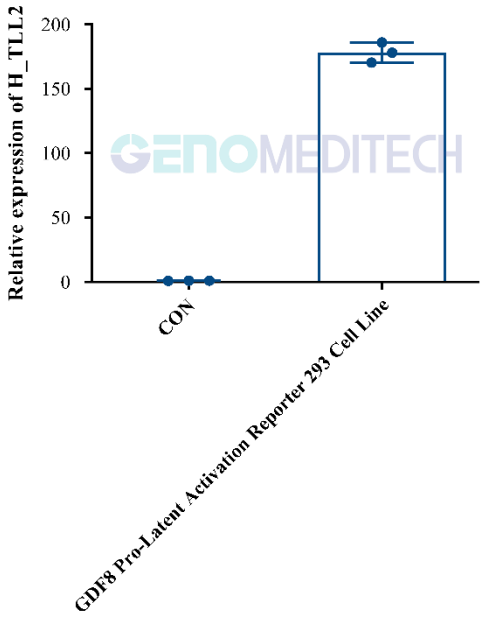


Response to Human pro GDF-8 Protein. The GDF8 Pro-Latent Activation Reporter 293 Cell Line (Cat. GM-C42533) and Activin A Reporter Cell Line (Cat. GM-C26082) at a density of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of Human pro GDF-8 Protein (Kactus/GDF-HM118) in assay buffer (DMEM+1% FBS+1% P.S) for 24 hours. The firefly luciferase activity was measured using the Luciferase Reporter Assay Kit (Genomeditech). Data are shown by drug mass concentration.



Response to Human Latent GDF-8 Protein. The GDF8 Pro-Latent Activation Reporter 293 Cell Line (Cat. GM-C42533) and Activin A Reporter Cell Line (Cat. GM-C26082) at a density of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of Human Latent GDF-8 Protein(Kactus/GDF-HM108) in assay buffer (DMEM+1% FBS+1% P.S) for 24 hours. The firefly luciferase activity was measured using the Luciferase Reporter Assay Kit (Genomeditech). Data are shown by drug mass concentration.

Expression



The mRNA expression levels of H_TLL2 in the GDF8 Pro-Latent Activation Reporter 293 Cell Line (Cat. GM-C42533) were determined by RT-qPCR.

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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 Reporter HEK-293 DDX35TM Cell Line	Cynomolgus_GCGR 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)	
Human GCGR(26-136) Protein; hFc Tag	Human GCGR(26-136) Protein; His Tag
GLP1R	
H_GLP1R GIPR GCGR Triple Reporter 293 Cell Line	H_GLP1R GIPR Reporter CHO-K1 Cell Line
H_GLP1R Reporter CHO-K1 Cell Line	H_GLP1R Reporter HEK-293 Cell Line
H_GLP1R Reporter HEK-293 DDX35TM Cell Line	H_GLP1R β-Arrestin Reporter CHO-K1 Cell Line
Cynomolgus_GLP1R GIPR CHO-K1 Cell Line	Cynomolgus_GLP1R HEK-293 Cell Line
H_GLP1R CHO-K1 Cell Line	H_GLP1R GIPR CHO-K1 Cell Line
H_GLP1R HEK-293 Cell Line	Mouse_GLP1R GIPR CHO-K1 Cell Line
Mouse_GLP1R HEK-293 Cell Line	Rat_GLP1R HEK-293 Cell Line
Anti-GLP1R hIgG1 Antibody(mAb-36986)	Anti-H_GLP1R hIgG1 Antibody(glutazumab)
FGFR1	
H_FGF21 Reporter HEK-293 Cell Line	Mouse_FGF21 Reporter HEK-293 Cell Line
Human FGF-21 Protein; His Tag	
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)	
GPR75	
H_GPR75 HEK-293 Cell Line	
GIPR	
H_GIPR Reporter CHO-K1 Cell Line	H_GIPR Reporter HEK-293 Cell Line
H_GIPR Reporter HEK-293 DDX35TM Cell Line	H_GIPR β-Arrestin2 Reporter CHO-K1 Cell Line
Cynomolgus_GIPR CHO-K1 Cell Line	Cynomolgus_GIPR HEK-293 Cell Line
H_GIPR CHO-K1 Cell Line	H_GIPR HEK-293 Cell Line
Mouse_GIPR CHO-K1 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	ACVR2B KO HEK-293 Cell Line
Activin A Reporter Cell Line	BRE Reporter 293 Cell Line
H_ACVR2A Reporter Cell Line	H_ACVR2B Reporter Cell Line
H_ACVR2B Reporter DDX35TM 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-ACVR2A hIgG1 Antibody(LAE-102)	Anti-ACVR2B hIgG1 Antibody(Bimagrumab)
Anti-ACVR2B hIgG1 Antibody(Fab-17G05)	Anti-ACVR2B mIgG2a Antibody(Bimagrumab)
Anti-GDF8 hIgG4 Reference Antibody (Apitbio)	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 A Protein; His Tag (CHO)
Human Activin B Protein; His Tag	Human ACVR2A Protein; hFc Tag
Human ACVR2A Protein; hFc Tag (Sotatercept)	Human ACVR2A Protein; His Tag
Human ACVR2B Protein; hFc Tag	Human ACVR2B Protein; His Tag
Human latent GDF-8 Protein; His Tag	Mouse ACVR2A Protein; His Tag
Mouse ACVR2B Protein; His Tag	
AMY:CALCR RAMP	
H_CALCR RAMP1(AMY1) Reporter CHO-K1 Cell Line	H_CALCR RAMP2(AMY2) Reporter CHO-K1 Cell Line
H_CALCR RAMP3(AMY3) Reporter CHO-K1 Cell Line	H_CALCR RAMP3(AMY3) β-Arrestin Reporter CHO-K1 Cell Line
H_CALCR Reporter CHO-K1 Cell Line	H_CALCR β-Arrestin Reporter CHO-K1 Cell Line
H_GLP1R CALCR RAMP3(AMY3) Reporter CHO-K1 Cell Line	Rat_CALCR RAMP1(AMY1) Reporter COS-7 Cell Line
Rat_CALCR RAMP3(AMY3) Reporter COS-7 Cell Line	Rat_CALCR Reporter COS-7 Cell Line
THRB	
H_THRβ Reporter HEK-293 Cell Line	
MC4R	
H_MC4R Reporter HEK-293 Cell Line	
GLP2R	
H_GLP2R Reporter 293 Cell Line	H_GLP2R HEK-293 Cell Line
ASGR1	
Cynomolgus_ASGR1 CHO-K1 Cell Line	H_ASGR1 CHO-K1 Cell Line
H_ASGR1 HEK-293 Cell Line	
Anti-ASGR1 hIgG1 Antibody(4A2.001)	
Cynomolgus ASGR1 Protein; hFc Tag	Cynomolgus ASGR1 Protein; His Tag
Human ASGR1 Protein; hFc Tag	Human ASGR1 Protein; His Tag
Mouse ASGR1 Protein; His Tag	

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