Genomeditech (Shanghai) Co.,Ltd.
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Product Sheet

H_CALCR RAMP3(AMY3) Reporter CHO-K1 Cell Line

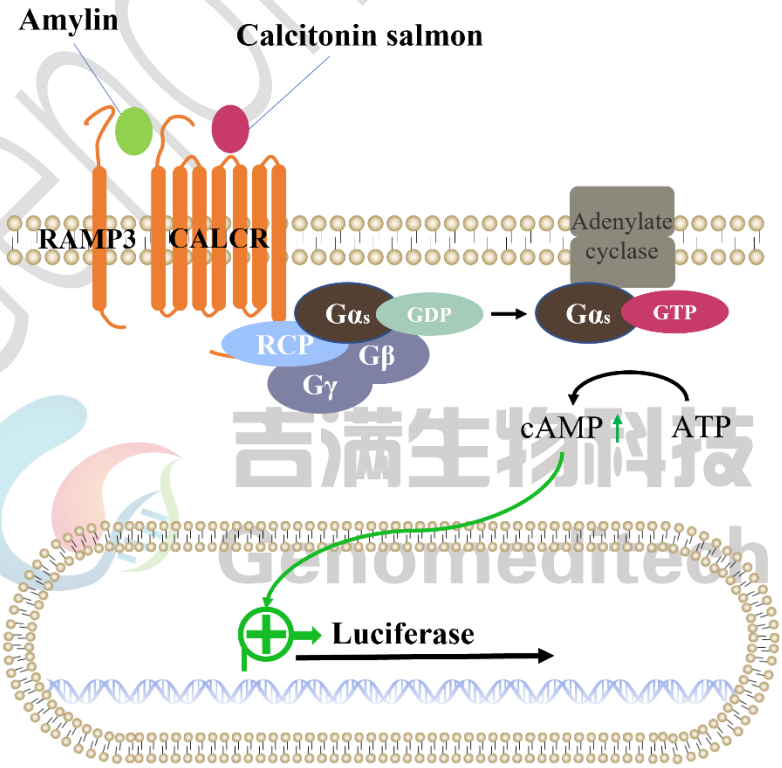
Catalog number: GM-C26533

Version 3.3.1.241129

The Calcitonin Gene Related Peptide (CGRP) family includes several important peptides: Calcitonin (CT), Amylin, and Adrenomedullin (AM). Amylin is produced by the pancreas and is a hormone that regulates nutrient intake. The binding of Amylin to its receptors involves three classes of potential receptors, which are complexes of the Calcitonin Receptor (CALCR) and Receptor Activity Modifying Proteins (RAMPs). RAMPs are a series of type I single-transmembrane proteins that function by forming heterodimers with G protein-coupled receptors. There are three RAMP subtypes: RAMP1, RAMP2, and RAMP3. Although these RAMPs are structurally similar, their amino acid sequences are only about30% identical.

After binding to the receptor, Amylin can form different complexes: CALCR combined with RAMP1 forms the Amy1 complex, CALCR combined with RAMP2 forms the Amy2 complex, and CALCR combined with RAMP3 forms the Amy3 complex.

The H_CALCR RAMP3 (AMY3) Reporter CHO-K1 Cell Line is a clonal, stable cell line that constitutively expresses human CALCR and human RAMP3, along with a signal-dependent expression of a luciferase reporter gene. The binding of Amylin to the receptor complex activates downstream reporter genes, leading to luciferase expression. The luciferase readout indicates the activation level of the signaling pathway and can thus be used to evaluate the in vitro effects of drugs related to the CALCR RAMP3 (AMY3) complex.



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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	F12K +10% FBS+1% P.S
Growth medium	F12K+10% FBS+1% P.S+4 μg/mL Blasticidin+200 μg/mL G418+4 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.
F12K	BOSTER/PYG0036
Fetal Bovine Serum	Cegrogen biotech/A0500-3010
Pen/Strep	Thermo/15140-122
Blasticidin	Genomeditech/GM-040404
G418	Genomeditech/GM-040402
Puromycin	Genomeditech/GM-040401
Calcitonin salmon (Salmon calcitonin)	GlpBio/GC32851
Amylin, human, amide	GenScript/RP11278CN
GMOne-Step Luciferase Reporter Gene Assay Kit	Genomeditech/GM-040503

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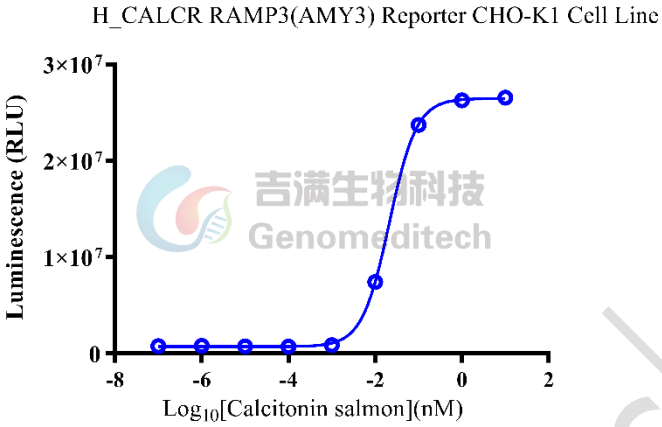
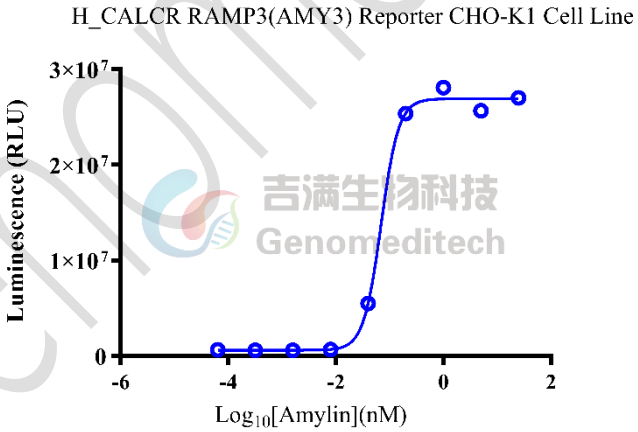
Figures



	H_CALCR RAMP3(AMY3) Reporter
EC50	0.02134

Figure 1 | Response to Calcitonin salmon. H_CALCR RAMP3(AMY3) Reporter CHO-K1 Cell Line (Cat. GM-C26533) at a concentration of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of Calcitonin salmon(Glpbio/GC32851) in assay buffer (F12K +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 [31.21]. Data are shown by drug molar concentration.



	H_CALCR RAMP3(AMY3) Reporter CHO
EC50	0.06974

Figure 2 | Response to Amylin. H_CALCR RAMP3(AMY3) Reporter CHO-K1 Cell Line (Cat. GM-C26533) at a concentration of 1E4 cells/well (96-well format) was stimulated with serial dilutions of Amylin (Genscript/RP11278CN) in assay buffer (F12K +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 [43.5]. Data are shown by drug molar concentration.

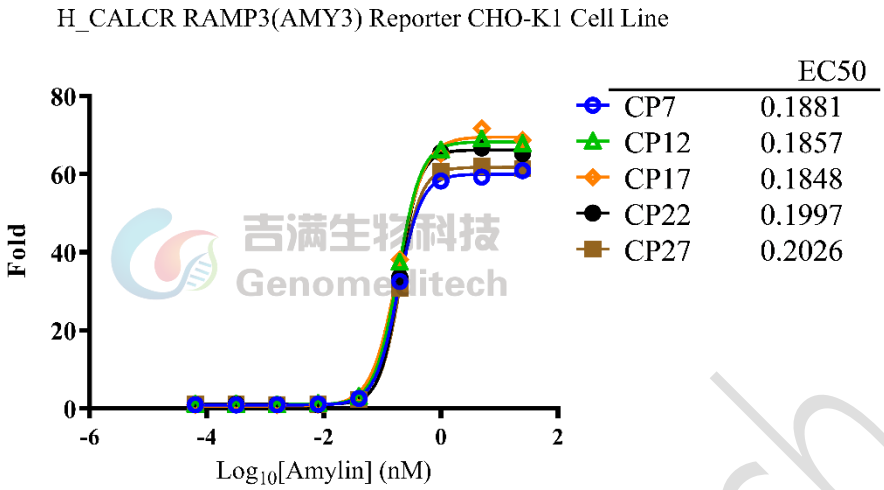


Figure 3 | The passage stability of response to Amylin. The passage 7, 12, 17, 22, and 27 of H_CALCR RAMP3(AMY3) Reporter CHO-K1 Cell Line (Cat. GM-C26533) at a concentration of 1E4 cells/well (96-well format) was stimulated with serial dilutions of Amylin (Genscript/RP11278CN) in assay buffer (F12K +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). Data are shown by drug molar concentration.

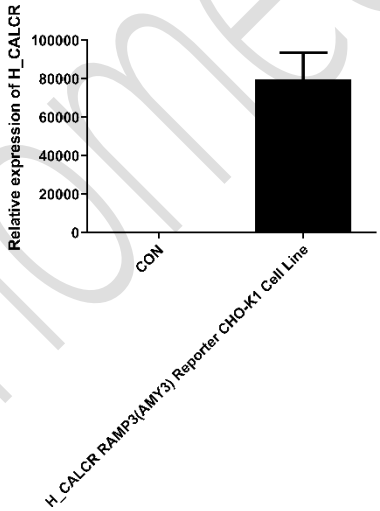


Figure 4 | The mRNA expression levels of H_CALCR in the H_CALCR RAMP3(AMY3) Reporter CHO-K1 Cell Line(Cat. GM-C26533) were determined by RT-qPCR.

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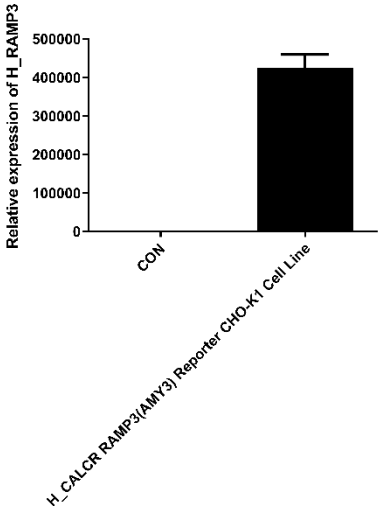

Figure 5 | The mRNA expression levels of H_RAMP3 in the H_CALCR RAMP3(AMY3) Reporter CHO-K1 Cell Line(Cat. GM-C26533) were determined by RT-qPCR.

Cell Recovery

Recovery Medium: F12K +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: F12K+10% FBS+1% P.S+4 μg/mL Blasticidin+200 μg/mL G418+4 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) Remove and discard culture medium.

b) Briefly rinse the cell layer with PBS to remove all traces of serum that contains trypsin inhibitor.

c) 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 2 to 3 minutes at 37°C).

d) 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.

e) Add 2.0 mL of growth medium to mix well and aspirate cells by gently pipetting.

f) After centrifugation, resuspend the pellet and add appropriate aliquots of the cell suspension to new culture vessels.

g) Incubate cultures at 37°C.

Subcultivation Ratio: A subcultivation ratio of 1:4 - 1:5 is recommended

Medium Renewal: Every 2 to 3 days

Notes

a) After the stabilization of the cell condition, there will be fewer dead cells post-passage, the cell growth rate will tend to stabilize, cell morphology will become uniform, and the cells will appear robust.

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)
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 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
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 Reporter CHO-K1 Cell Line	

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