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Product Sheet

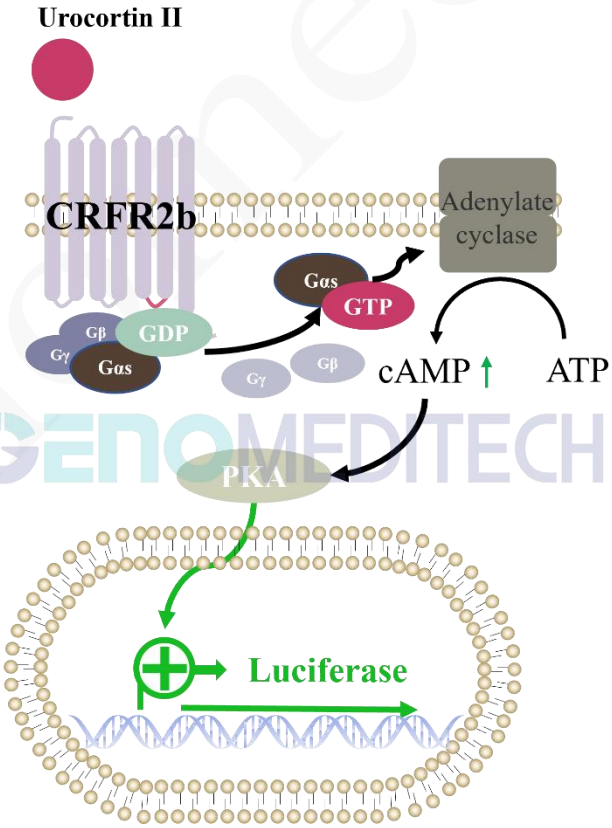
H_CRFR2b Reporter CHO-K1 Cell Line

Catalog number: GM-C50131

Version 4.0.26.1120

Corticotropin-releasing factor receptor 2 beta (CRFR2b) is a protein encoded by the CRHR2 gene in the human body, activated by corticotropin-releasing factor (CRF) family peptides such as CRF and urocortins, and belongs to a family of G protein-coupled receptors. CRFR2b is mainly found in the central nervous system, cardiovascular system, gastrointestinal tract, and skeletal muscle. When CRF family peptides activate CRFR2b, it binds to the heterotrimeric Gs (αβγ), inducing the activation of adenylate cyclase, which increases the levels of cAMP in the cytoplasm. The rise in cAMP activates PKA, leading to the phosphorylation of proteins that regulate gene transcription, such as CREB, causing them to relocate to the nucleus.

H_CRFR2b Reporter CHO-K1 Cell Line is a clonal stable CHO-K1 Cell Line constitutively expressing the human CRFR2b, along with signal-dependent expression of a luciferase reporter gene. The binding of CRF family peptides to CRFR2b activates downstream reporter genes, leading to luciferase expression. The luciferase readout represents the activation level of the signaling pathway and can thus be used for evaluating the in vitro effects of related drugs of CRFR2b.



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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+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.

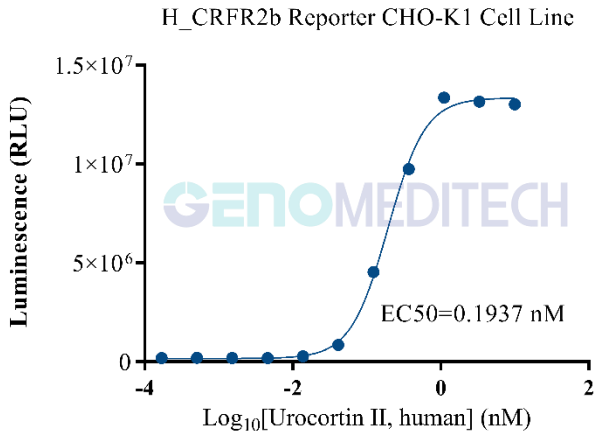
Materials

Reagent	Manufacturer/Catalogue No.
F12K	BOSTER/PYG0036
Fetal Bovine Serum	ExCell/FSP500
Pen/Strep	Thermo/15140-122
Blasticidin	Genomeditech/GM-040404
Puromycin	Genomeditech/GM-040401
Countess 3 Automated Cell Counter	ThermoFisher/A49891
SpectraMax® L Microplate Luminometer	Moleculardevices/SpectraMax L
GMOne-Step 2.0 Luciferase Reporter Gene Assay Kit	Genomeditech/GM-040513
96 well round cell culture plate	PakGent/CL-PT096
96-well White Opaque Plate	Thermo/236108
96-well U-bottom Plate	Saining/1014010
Urocortin II, human	MCE/HY-P1752

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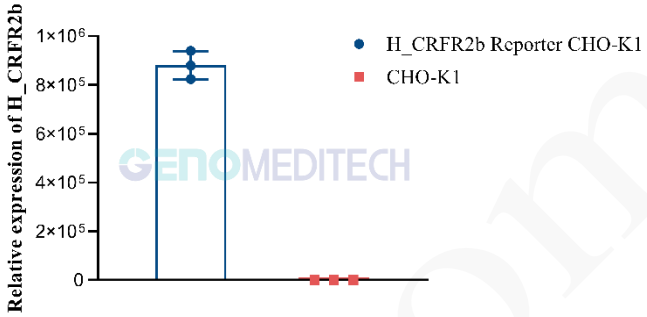
Data

Signaling Pathway



Response to Urocortin II, human. H_CRFR2b Reporter CHO-K1 Cell Line (Cat. GM-C50131) was seeded at a density of 1E4 cells per well in a 96-well plate and incubated overnight. The next day, serial dilutions of the Urocortin II, human (MCE/HY-P1752) were added to the pre-seeded cells. The mixture was incubated for an additional 6 hours. The firefly luciferase activity was measured using the Luciferase Reporter Assay Kit (Genomeditech). The maximum induction fold was approximately [63.6]. Data are shown by drug molar concentration.

Expression



The mRNA expression levels of H_CRFR2b in the H_CRFR2b Reporter CHO-K1 Cell Line (Cat. GM-C50131) were determined by RT-qPCR.

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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+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.

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.
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