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Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

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See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

GCGR/CRE Luciferase Reporter HEK293 Cell Line is a HEK293 cell line that expresses the firefly luciferase reporter, under the control of the cAMP response element (CRE), and human GCGR (Glucagon receptor; NM_000160.5). Activation of GCGR in these cells can be monitored by measuring luciferase activity.
This cell line was functionally validated with the agonists Glucagon and Retatrutide.

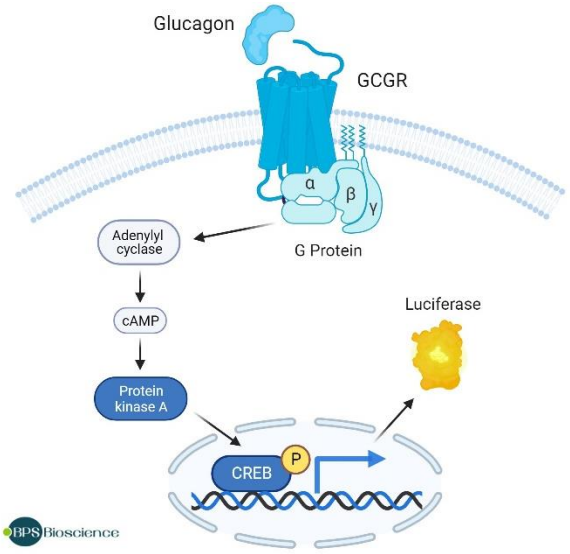

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Figure 1. Example of the mechanism of action of GCGR/CRE Luciferase Reporter HEK293 Cell Line when treated with glucagon.
Binding of glucagon to GCGR triggers the activation of adenylyl cyclase and results in an increase of cAMP in the cells. Protein kinase A is activated, allowing the activation of the CRE promoter and luciferase production. Luciferase activity is thus proportional to GCGR binding to its agonists.

Background

The glucagon receptor (GCGR) belongs to the Class B1 family of G protein-coupled receptors (GPCR family). Along with GLP-1R (glucagon-like peptide 1 receptor) and GIPR (gastric inhibitory polypeptide receptor), it is one of the critical receptors that regulate glucose homeostasis. Glucagon, the ligand of GCGR, increases insulin secretion via GCGR and GLP-1R in pancreatic β cells, although GLP-1R is the main insulin-promoting receptor. In addition, it has been shown that glucagon enhances hepatic lipid metabolism and reduces food intake in rodents and humans. More recently, encouraging clinical results from a triple-hormone-receptor agonist, Retatrutide, and several GLP-1R/GCGR dual agonists, suggest that GCGR could be a promising co-stimulating target for type 2 diabetes and obesity.

Application

Screen for agonists of human GCGR in a cellular model

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains >1 x 10^6 cells in 1 ml of Cell Freezing Medium (BPS Bioscience #79796)

Host Cell
HEK293, Human Embryonic Kidney, epithelial-like cells, adherent

Mycoplasma Testing
The cell line has been screened to confirm the absence of Mycoplasma species.

Materials Required but Not Supplied

These materials are not supplied with this cell line but are necessary for cell culture and cellular assays. BPS Bioscience reagents systems are validated and optimized for use with this cell line and are highly recommended for best results. Media components are provided in the Media Formulations section.

Media Required for Cell Culture

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187
Growth Medium 1G	BPS Bioscience #79544

Materials Required for Cellular Assay

Name	Ordering Information
GCGR agonist such as: Glucagon Retatrutide	 R&D System #6927 MedChemExpress #HY-P3506
Opti-MEM I Reduced Serum Medium	ThermoFisher #31985-070
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
Luminometer	

Storage Conditions

Cells are shipped in dry ice and should immediately be thawed or stored in liquid nitrogen upon receipt. Do not use a -80°C freezer for long term storage. Contact technical support at support@bpsbioscience.com if the cells are not frozen in dry ice upon arrival.

Media Formulations
For best results, it is *highly recommended* to use these validated and optimized media from BPS Bioscience. Other preparations or formulations of media may result in suboptimal performance.

Note: Thaw Media do *not* contain selective antibiotics. However, Growth Media *do* contain selective antibiotics, which are used to maintain selective pressure on the cell population expressing the gene of interest.
Cells should be grown at 37°C with 5% CO_2. BPS Bioscience's cell lines are stable for at least 10 passages when grown under proper conditions.

Media Required for Cell Culture

Thaw Medium 1 (BPS Bioscience #60187):
MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin.

Growth Medium 1G (BPS Bioscience #79544):
MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin, 400 µg/ml of Geneticin and 50 µg/ml of Hygromycin B.

Assay Medium: Opti-MEM I Reduced Serum Medium.

Cell Culture Protocol
Note: HEK293 cells are derived from human material and thus the use of adequate safety precautions is recommended.

Cell Thawing

1. Retrieve a cell vial from liquid nitrogen storage. Keep on dry ice until ready to thaw.

2. When ready to thaw, swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. Once cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire content of the vial to an empty 50 ml conical tube.

   Note: Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.

3. Using a 10 ml serological pipette, slowly add 10 ml of pre-warmed Thaw Medium 1 to the conical tube containing the cells. Thaw Medium 1 should be added dropwise while gently rocking the conical tube to permit gentle mixing and avoid osmotic shock.

4. Immediately spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 5 ml of pre-warmed Thaw Medium 1.

5. Transfer the resuspended cells to a T25 flask and incubate at 37°C in a 5% CO_2 incubator.

6. After 24 hours of culture, check for cell attachment and viability. Change medium to fresh Thaw Medium 1 and continue growing culture in a 5% CO2 incubator at 37°C until the cells are ready to be split.

7. Cells should be passaged before they are fully confluent. At first passage and subsequent passages, use Growth Medium 1G.

Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS) without Ca^{2+}/Mg^{2+}, and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 1A and transfer to a tube.

3. Spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in Growth Medium 1G.

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:6 to 1:8 once or twice per week.

Cell Freezing

1. Aspirate the medium, wash the cells with PBS) without Ca^{2+}/Mg^{2+}, and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 1G and count the cells.

3. Spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 4°C Cell Freezing Medium (BPS Bioscience #79796) at ~2 x 10^6 cells/ml.

4. Dispense 1 ml of cell suspension into each cryogenic vial. Place the vials in an insulated container for slow cooling and store at -80°C overnight.

5. Transfer the vials to liquid nitrogen the next day for long term storage.

Note: It is recommended to expand the cells and freeze at least 10 vials at an early passage for future use.

Functional Validation

A. Dose response of GCGR/CRE Luciferase Reporter HEK293 Cell Line to GCGR agonists

- The following assay was designed for a 96-well format. To perform the assay in different tissue culture formats, the cell number and reagent volume should be scaled appropriately.
- All conditions should be performed in triplicate.
- The Assay should include “Cell-Free Control”, “Untreated Control” and “Treated” conditions.

1. Seed GCGR/CRE Luciferase Reporter HEK293 cells into a white clear-bottom 96-well microplate at a density of 30,000-40,000 cells per well in 90 µl of Assay Medium. Leave a few wells empty to use as the “Cell-Free Control” (Background Signal).

2. Incubate cells at 37°C in a CO_2 incubator for 16 to 24 hours.

3. Prepare a serial dilution of the GCGR agonists at concentrations 10-fold higher than the desired final concentration in Assay Medium (10 µl/well).

4. Add 10 µl of the agonist serial dilution to the “Treated” wells.

5. Add 10 µl of Assay Medium to the “Untreated Control” wells.

6. Add 100 µl of Assay Medium to the “Cell-Free Control” wells (for determining background luminescence).

7. Incubate the plate at 37°C in a CO_2 incubator for 5-6 hours.

8. Add 100 µl of the ONE-Step™ Luciferase reagent per well and rock gently at room temperature for ~15 minutes.

9. Measure luminescence using a luminometer.

10. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells. The fold induction of CRE luciferase reporter expression is the average background-subtracted luminescence of treated well divided by the average background-subtracted luminescence of untreated control wells.

$$Fold\ induction = \frac{luminescence\ of\ treated\ cells - avg\ background}{avg\ luminescence\ of\ untreated\ cells - avg\ cbackground}$$

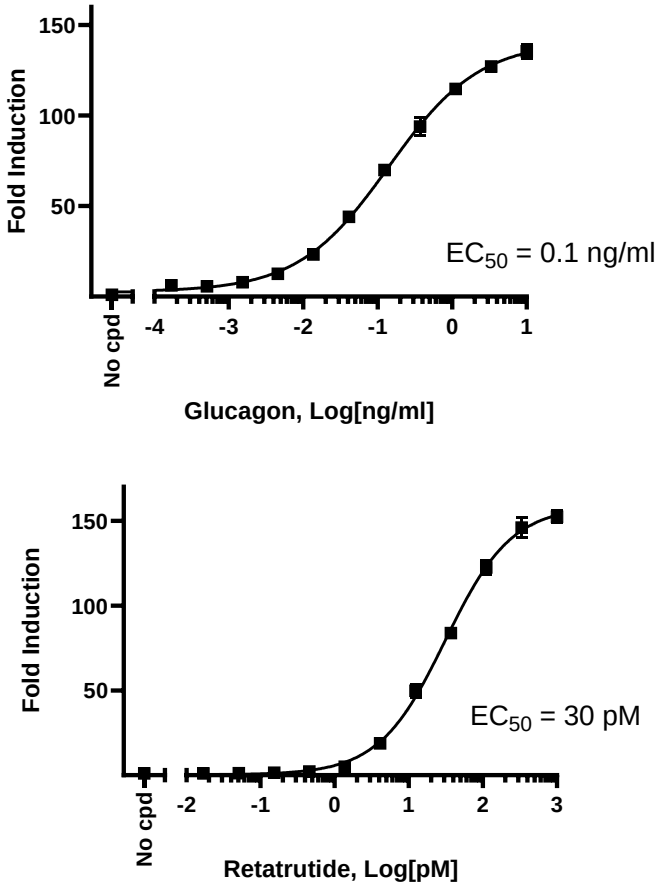

Figure 2. Dose response curves of GCGR/CRE Luciferase Reporter HEK293 Cell Line to the GCGR agonists Glucagon and Retatrutide.
Cells were treated with increasing concentrations of Glucagon (top panel) and Retatrutide (bottom panel). Luminescence was measured using ONE-Step™ Luciferase Assay System.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

Sequence
Human GCGR sequence (NM_000160.5)

MPPCQPQRPLLLLLLLLACQPQVPSAQVMDFLFEKWKLYGDQCHHNLSLLPPPTELVCNRTFDKYSCWPDTPANTTANISCPWY
LPWHHKVQHRFVFKRCGPDGQWVRGPRGQPWRDASQCQMDGEEIEVQKEVAKMYSSFQVMYTVGYSLSLGALLLALAILGG
LSKLHCTRNAIHANLFASFVLKASSVLVIDGLLRTRYSQKIGDDLSVSTWLSDGAVAGCRVAAVFMQYGIVANYCWLLVEGLYLHN
LLGLATLPERSFFSLYLGIGWGAPMLFVVPWAVVKCLFENVQCWTSNDNMGFWWILRFPVFLAILINFFIFVRIVQLLVAKLRARQ
MHHTDYKFRLAKSTLTLIPLLGVHEVVFAFVTDEHAQGTLRSAKLFFDLFLSSFQGLLVAVLYCFLNKEVQSELRRRWHRWRLGKV
LWEERNTSNHRASSSPGHGPPSKELQFGRGGGSQDSSAETPLAGGLPRLAESPF

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Troubleshooting Guide
Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

Related Products

Products	*Catalog #*	*Size*
GLP-1R/CRE Luciferase Reporter HEK293 Cell Line	78176	2 vials
GIPR/CRE Luciferase Reporter HEK293 Cell Line	78589	2 vials
CGRPR/CRE Luciferase Reporter HEK293 Cell Line	78325	2 vials
GPRC5D CHO Cell Line	78337	2 vials
GPRC5D HEK293 Cell Line	78345	2 vials
GPRC5D (Cynomolgus) CHO Cell Line	78338	2 vials
GPRC5D (Cynomolgus) HEK293 Cell Line	78346	2 vials

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