Description

Rat GIPR/CRE Luciferase Reporter HEK293 Cell Line is an engineered HEK293 cell line expressing firefly luciferase under the control of cAMP response element (CRE), and rat GIPR (*Rattus norvegicus* Gastric Inhibitory Polypeptide receptor; NM_012714.2). Activation of GIPR in these cells can be monitored by measuring luciferase activity.

The functionality of the Rat GIPR/CRE Luciferase Reporter HEK293 Cell Line was validated in dose-response assays using the agonist rat gastric inhibitory peptide (rat GIP).

Rat GIPR/CRE Luciferase Reporter HEK293 Cell Line is a *Rattus norvegicus* version of GIPR/CRE Luciferase Reporter HEK293 Cell Line (BPS Bioscience #78589).

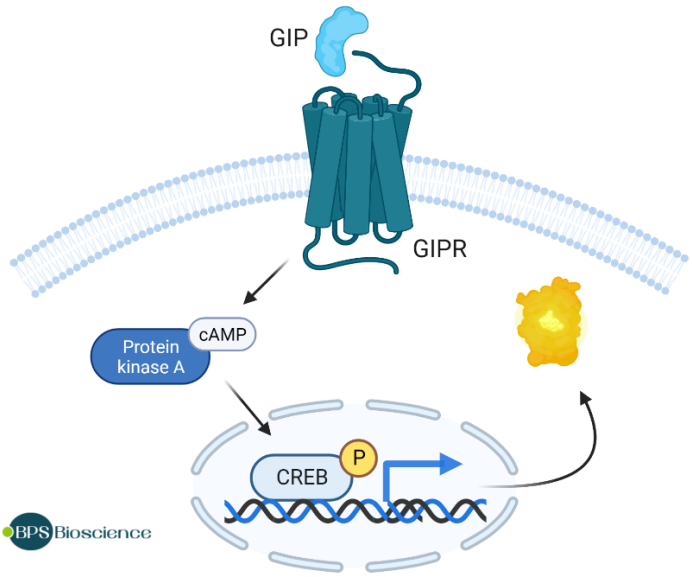

Figure 1. Illustration of the mechanism of action of Rat GIPR/CRE Luciferase Reporter HEK293 Cell Line.

Background

The gastric inhibitory polypeptide receptor (GIPR), also known as glucose-dependent insulinotropic polypeptide receptor, belongs to the Class B1 G protein-coupled receptor (GPCR) family. GIPR is primarily found in the β-cells of the pancreas and serves as a receptor for the gastric inhibitory polypeptide (GIP) hormone. As one of the incretin hormones, GIP modulates glucose metabolism by stimulating the pancreatic β-cells to release insulin. Since GIPR/GLP-1R heterodimerization regulates GLP-1R signaling, dual agonists that bind both GIPR and GLP-1R have shown promising clinical efficacy for treating type II diabetes mellitus (T2DM) and obesity.

Application(s)

Screen and characterize agonists of rat GIPR 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 1U	BPS Bioscience #78548

Materials Required for Cellular Assay

Name	Ordering Information
Gastric Inhibitory Peptide (GIP) (Rat)	BPS Bioscience #83923
Opti-MEM Reduced Serum Medium (Assay 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, the use of validated and optimized media from BPS Bioscience is *highly recommended*. 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 1U (BPS Bioscience #78548):
MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin, 0.5 µg/ml of Puromycin and 100 µg/ml of Hygromycin B.

Media Required for Functional Cellular Assay

Assay Medium:
Opti-MEM 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. 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 or T75 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 in a 5% CO_2 incubator at 37°C until the cells are ready to passage.

   Note: Recovery of the frozen cells can take longer than a week. Change medium to fresh Thaw Medium 1 after a week.

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

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 1U 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 1U.

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:5 to 1:8 once or twice a 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 1U 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

- The following assays are designed for 96-well. To perform the assay in different tissue culture formats, the cell number and reagent volumes should be scaled appropriately.
- The conditions should be performed in triplicate.
- The assays should include "Cell-Free Control", "Unstimulated Control" and "Stimulated" conditions.

Assay Medium: Opti-MEM Reduced Serum Medium

A. Dose-response of Rat GIPR/CRE Luciferase Reporter HEK293 Cell Line to rat GIPR agonists

1. Seed Rat GIPR/CRE Luciferase Reporter HEK293 cells into a white clear-bottom 96-well culture plate at a density of ~45,000 cells per well in 90 µl of Assay Medium (Opti-MEM). Leave a few empty wells to determine the background luminescence.

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

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

4. Add 10 µl of each dilution to the "Stimulated" wells.

5. Add 10 µl of Assay Medium to the "Unstimulated Control" (negative 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 hours.

8. Add 100 µl of the ONE-Step™ Luciferase reagent to each well.

9. Rock gently at Room Temperature (RT) for ~15 minutes.

10. Measure luminescence using a luminometer.

Data Analysis

Subtract the average background luminescence (cell-free wells) from the luminescence reading of all wells. The fold induction of CRE luciferase reporter expression is the average background-subtracted luminescence of the stimulated wells divided by the average background-subtracted luminescence of the unstimulated control wells.

$$Fold\ induction = \frac{(luminescence\ of\ stimulated\ cells - average\ background)}{(average\ luminescence\ of\ unstimulated\ cells - average\ background)}$$

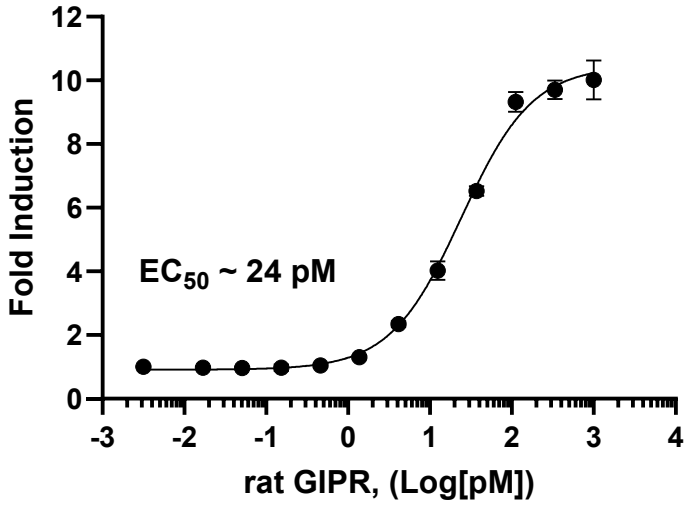

Figure 2. Dose response curve of rat GIPR/CRE Luciferase Reporter HEK293 Cell Line to rat GIP. Cells were treated with increasing concentrations of rat GIP in a 96-well plate. Luciferase activity was measured with ONE-Step™ Luciferase Assay System. Results are expressed as fold induction versus unstimulated control.

Data shown is representative.

Sequence

Rattus norvegicus GIPR sequence (NM_012714.2)

MPLRLLLLLLWLWGLSLQRAETDSEGQTTGELYQRWERYGWECQNTLEATEPPSGLACNGSFDMYACWNYTAANTTARVSCP
WYLPWYRQVAAGFVFRQCGSDGQWGSWRDHTQCENPEKNGAFQDQKLILERLQVVYTVGYSLSLATLLLALLILSLFRRLHCTR
NYIHMNLFTSFMLRAGAILTRDQLLPPLGPYTGNQTPTLWNQALAACRTAQILTQYCVGANYTWLLVEGVYLHHLLVVVRRSEK
GHFRCYLLLGWGAPALFVIPWVIVRYLYENTQCWERNEVKAIWWIIRTPILITILINFLIFIRILGILVSKLRTRQMRCPDYRLRLARST
LTLMPLLGVHEVVFAPVTEEQAEGSLRFAKLAFEIFLSSFQGFLVSVLYCFINKEVQSEIRRLRLSLQEQCPRPHLGQAPRAVPLSSAP
QEAAIRNALPSGMLHVPGDEVLESYC

References

Yang B., *et al.*, 2022 *Molecular Metabolism* 66: 101638.

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Troubleshooting Guide
Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit https://bpsbioscience.com/contact.

Related Products

Products	*Catalog #*	*Size*
GLP-1R/CRE Luciferase Reporter HEK293 Cell Line	78176	2 vials
Adenosine A2A Receptor Functional Cell Line	79381	2 vials
CGRPR/CRE Luciferase Reporter HEK293 Cell Line	78325	2 vials
CRE/CREB Luciferase Reporter HEK293 Cell Line	60515	2 vials

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