Description

Recombinant HEK293 cells expressing the firefly luciferase gene under the control of cAMP response element (CRE), and with forced expression of human GIPR (Gastric Inhibitory Polypeptide receptor; NM_000164.4). Activation of GIPR in these cells can be monitored by measuring luciferase activity.

The functionality of the GIPR/CRE Luciferase Reporter HEK293 Cell Line was validated in a dose-response assay using agonists gastric inhibitory peptide (GIP) and tirzepatide hydrochloride. These agonists induce luciferase activity in a dose-dependent manner as depicted in Figure 1.

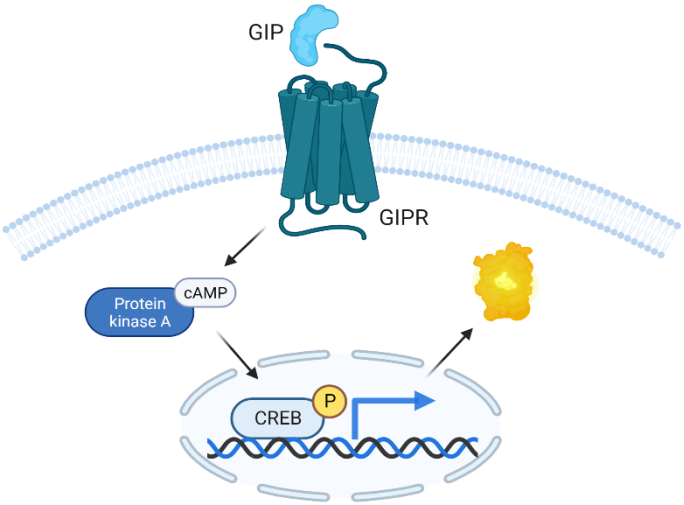

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Figure 1. Illustration of the GIPR/CRE Luciferase Reporter HEK293 Cell line.

Background

The gastric inhibitory polypeptide receptor (GIPR), also known as the 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 has been shown to regulate 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 human GIPR in a cellular context

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains ~2 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
Gastric Inhibitory Peptide (GIP), human*	Genscript #RP10795
Tirzepatide hydrochloride*	MedChemExpress #HY-1731B
Opti-MEM reduced serum medium (Assay Medium)	ThermoFisher #31985-070
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
Luminometer	

**Or agonist o interest*

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 for maintaining the presence of the transfected gene(s) over passages.
Cells should be grown at 37°C with 5% CO_2. BPS Bioscience's cell lines are stable for at least 15 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 reduced serum medium.

Cell Culture Protocol

Cell Thawing

1. Swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. As soon as the cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire contents of the vial to a tube containing 10 ml of pre-warmed Thaw Medium 1 (**no Geneticin or Hygromycin B**).
2. 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 (**no Geneticin or Hygromycin B**).
3. Transfer the resuspended cells to a T25 flask or T75 flask and incubate at 37°C in a 5% CO_2 incubator.
4. After 24 hours of culture, check for cell attachment and viability. Change medium to fresh Thaw Medium 1 (**no Geneticin or Hygromycin B**) and continue growing culture in a 5% CO_2 incubator at 37°C until the cells are ready to be split.
5. Cells should be passaged before they are fully confluent. At first passage and subsequent passages, use Growth Medium 1G (**contains Geneticin and Hygromycin B**).

Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS), 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. Spin down the cells at 300 x g for 5 minutes, remove the medium and resuspend the cells in Growth Medium 1G (**contains Geneticin and Hygromycin B**). Seed into new culture vessels at the desired sub-cultivation ratio of 1:6 to 1:8 weekly or twice per week.

Cell Freezing

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS), 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 Freezing Medium (BPS Bioscience #79796, or 10% DMSO + 90% FBS) at ~2 x 10^6 cells/ml.
4. Dispense 1 ml of cell aliquots into cryogenic vials. 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 storage.

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

Dose-response of a GIPR agonist in GIPR/CRE Luciferase Reporter HEK293 cells

Assay Considerations

- The following assays are designed for 96-well format. To perform the assay in different tissue culture formats, the cell number and reagent volumes should be scaled appropriately.
- The experiments should be performed in triplicate.

1. Seed GIPR/CRE Luciferase Reporter HEK293 cells into a white clear-bottom 96-well microplate at a density of ~45,000 cells per well in 90 µl of Assay Medium Opti-MEM. Leave three wells empty to determine the background luminescence. Incubate cells at 37°C in a CO_2 incubator for 16 to 24 hours.

2. The next day, prepare a serial dilution of GIPR agonists at concentrations 10-fold higher than the desired final concentrations in Assay Medium Opti-MEM in a non-binding 96-well assay plate. Add 10 µl of each dilution to the cells. For example, to test an agonist at 10 ng/ml, prepare it in Assay Medium at 100 ng/ml and add 10 µl to the cells). The total volume is 100 µl.

3. Add 10 µl of Assay Medium to "untreated" cells (negative control).

4. Add 100 µl of Assay Medium to the cell-free control wells (for determining background luminescence).

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

6. Perform the luciferase assay using ONE-Step™ Luciferase Assay buffer, prepared according to the recommended instructions. Add 100 µl of the ONE-Step™ Luciferase reagent per well and rock gently at room temperature for ~15 minutes. Measure luminescence using a luminometer.

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.

$$Fold\ induction = \frac{average\ lumin.of\ treated\ cells - average\ backgound}{average\ lumin.of\ untreated\ cells - average\ background}$$

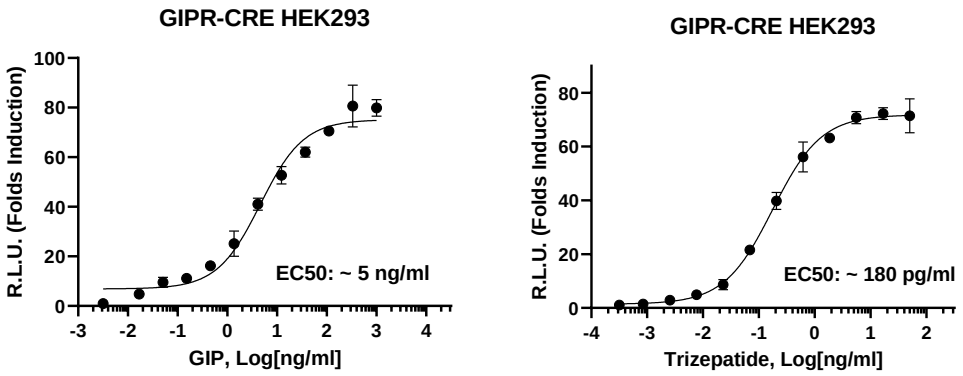

Figure 2. Dose response of GIPR agonists in GIPR/CRE Luciferase Reporter HEK293 cells.
Cells were incubated with increasing concentrations of GIP (left) and Trizepatide (right).

Sequence

Human GIPR sequence (NM_000164.4)

MTTSPILQLLLRLSLCGLLLQRAETGSKGQTAGELYQRWERYRRECQETLAAAEPPSGLACNGSFDMYVCWDYAAPNATARASC
PWYLPWHHHVAAGFVLRQCGSDGQWGLWRDHTQCENPEKNEAFLDQRLILERLQVMYTVGYSLSLATLLLALLILSLFRRLHCT
RNYIHINLFTSFMLRAAAILSRDRLLPRPGPYLGDQALALWNQALAACRTAQIVTQYCVGANYTWLLVEGVYLHSLLVLVGGSEEG
HFRYYLLLGWGAPALFVIPWVIVRYLYENTQCWERNEVKAIWWIIRTPILMTILINFLIFIRILGILLSKLRTRQMRCRDYRLRLARSTL
TLVPLLGVHEVVFAPVTEEQARGALRFAKLGFEIFLSSFQGFLVSVLYCFINKEVQSEIRRGWHHCRLRRSLGEEQRQLPERAFRALP
SGSGPGEVPTSRGLSSGTLPGPGNEASRELESYC

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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
Adenosine A2A Receptor Functional Recombinant Cell Line	79381	2 vials
CGRPR/CRE Luciferase Reporter HEK293 Cell Line	78325	2 vials
CRE/CREB Reporter (Luc) – HEK293 Recombinant Cell Line	60515	2 vials