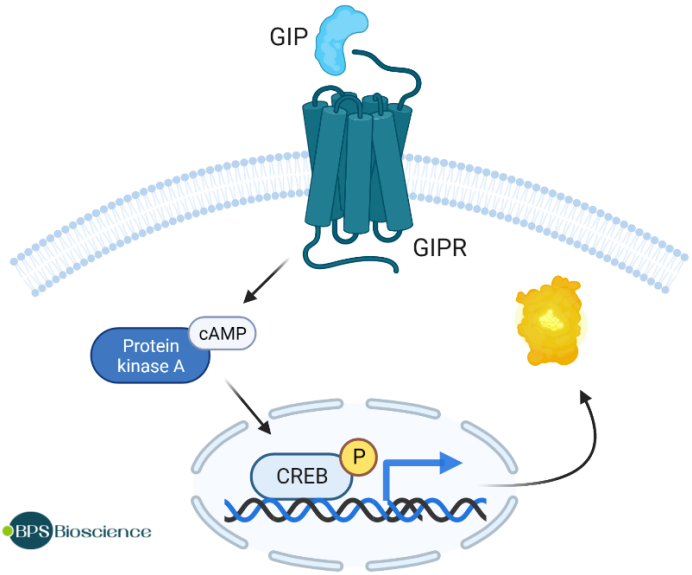
**Description**

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 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 dose-response assays using the agonists gastric inhibitory peptide (GIP), Tirzepatide, and Retatrutide. These agonists induce luciferase activity in a dose-dependent manner as depicted in Figure 1.




*Created with BioRender.com*

*Figure 1. Illustration of the mechanism of action in the 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 human 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 1G | BPS Bioscience #79544 |

*Materials Required for Cellular Assay*

| Name | Ordering Information |
|---|---|
| Gastric Inhibitory Peptide (GIP), human | Genscript #RP10795 |
| Tirzepatide hydrochloride | MedChemExpress #HY-P1731 |
| Retatrutide | MedChemExpress #HY-P3506 |
| 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 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.

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

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.

3. Transfer the resuspended cells to a T25 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 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.

*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 1G 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 weekly 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.

**Validation Data**

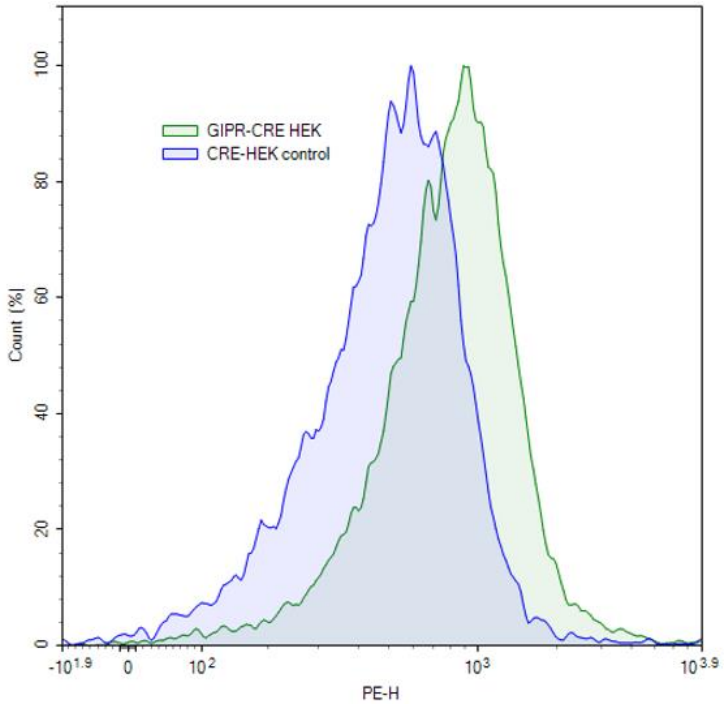



*Figure 2. Cell surface expression of GIPR in the GIPR/CRE Luciferase Reporter HEK293 Cell Line.* GIPR/CRE Luciferase Reporter HEK293 cells (green) or control CRE Luciferase Reporter HEK293 cells (blue) were stained with Human-GIPR PE-conjugated Antibody (R&D Systems #FAB8210P) and analyzed by flow cytometry. Y-axis represents the % cell number. X-axis indicates the intensity of PE.

**Functional Validation**

- The following assays are designed for 96-well (protocol A) and 384-well format (protocol B). 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.
- The assay should include “Cell-Free Control”, “Unstimulated Control” and “Stimulated” conditions.

*Assay Medium: Opti-MEM Reduced Serum Medium*

**A. 96-Well Assay Format: Dose-response of GIPR/CRE Luciferase Reporter HEK293 Cell Line to GIPR agonists**

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 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 GIPR agonists at concentrations 10-fold higher than the desired final concentrations in Assay Medium.
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.

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

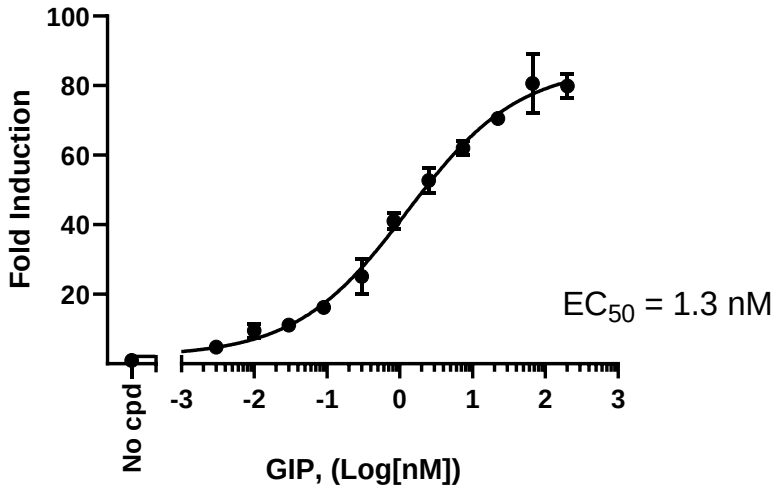



*Figure 3. Dose response curve of GIPR/CRE Luciferase Reporter HEK293 Cell Line to GIP in a 96-well assay format.*

Cells were treated with increasing concentrations of GIP in a 96-well plate. Luciferase activity was measured with ONE-Step™ Luciferase Assay System. Results are expressed as fold induction versus the unstimulated control.

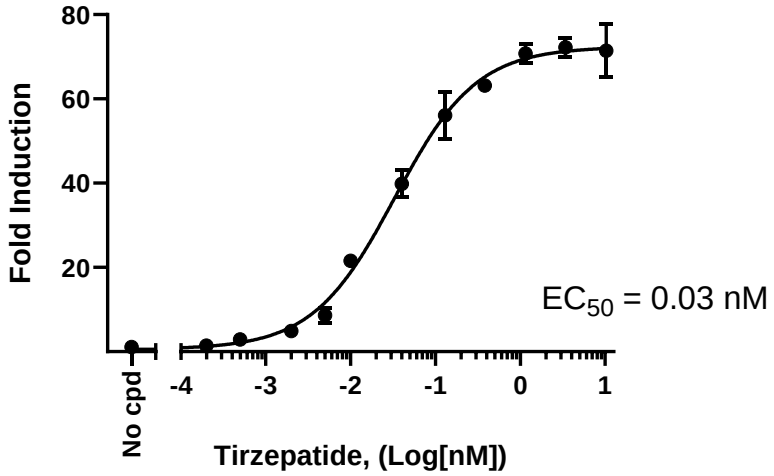



*Figure 4. Dose response curve of GIPR/CRE Luciferase Reporter HEK293 Cell Line to Tirzepatide, a dual peptide agonist of GIPR and GLP-1R, in a 96-well assay format.*
Cells were treated with increasing concentrations of Tirzepatide in a 96-well plate. Luciferase activity was measured with ONE-Step™ Luciferase Assay System. Results are expressed as fold induction versus the unstimulated control.

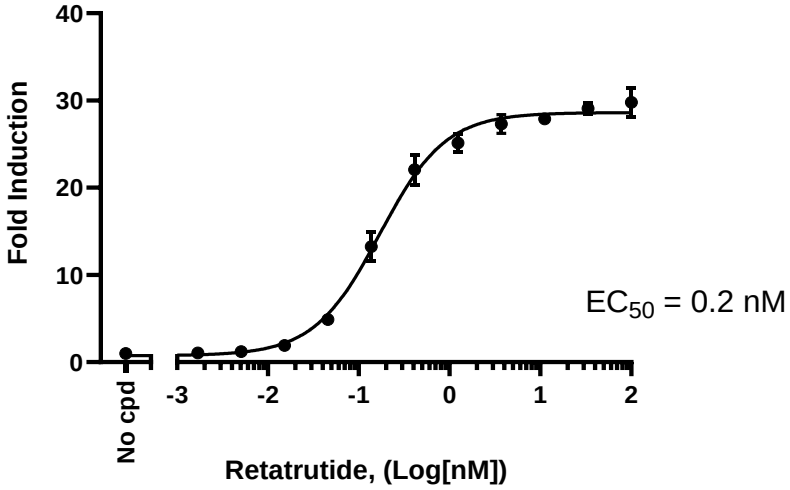



*Figure 5. Dose response curve of GIPR/CRE Luciferase Reporter HEK293 Cell Line to Retatrutide, a triple agonist peptide of GIPR, GLP-1R and GCGR, in a 96-well assay format.*
Cells were treated with increasing concentrations of Retatrutide in a 96-well plate. Luciferase activity was measured with ONE-Step™ Luciferase Assay System. Results are expressed as fold induction versus the unstimulated control.

**B. 384-Well Assay Format: Dose-response of GIPR/CRE Luciferase Reporter HEK293 Cell Line to GIPR agonists**

1. Seed GIPR/CRE Luciferase Reporter HEK293 cells into a white opaque, tissue culture treated 384-well microplate at a density of ~20,000 cells per well in 30 µl of Assay Medium (Opti-MEM). Leave 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 GIPR agonists at concentrations 4-fold higher than the desired final concentrations in Assay Medium.
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 40 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 40 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.

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

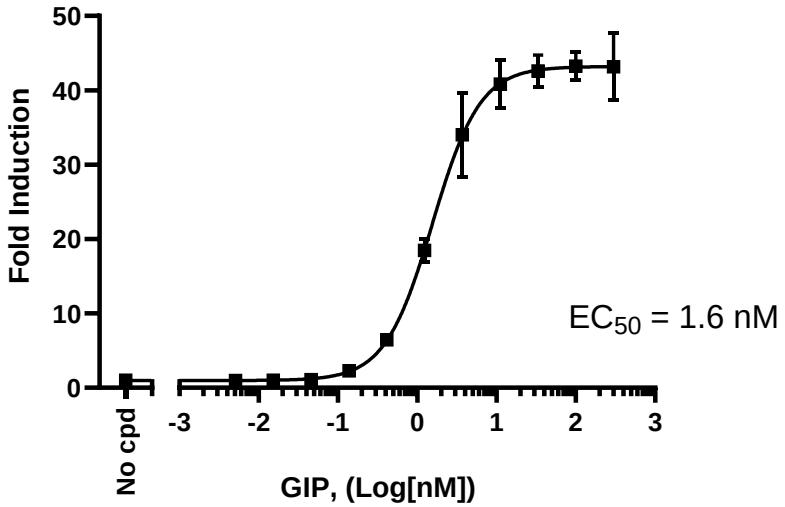



*Figure 6. Dose response curve of GIPR/CRE Luciferase Reporter HEK293 Cell Line to GIP in a 384-well assay format.*

Cells were treated with increasing concentrations of GIP in a 384-well plate. Luciferase activity was measured with ONE-Step™ Luciferase Assay System. Results are expressed as fold induction versus the unstimulated control.

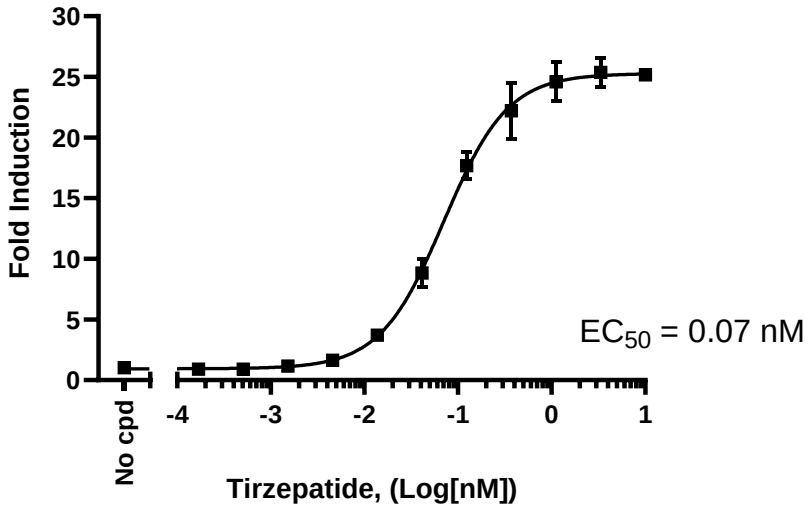



*Figure 7. Dose response curve of GIPR/CRE Luciferase Reporter HEK293 Cell Line to Tirzepatide, a dual peptide agonist of GIPR and GLP-1R, in a 384-well assay format.*
Cells were treated with increasing concentrations of Tirzepatide in a 384-well plate. Luciferase activity was measured with ONE-Step™ Luciferase Assay System. Results are expressed as fold induction versus the unstimulated control.

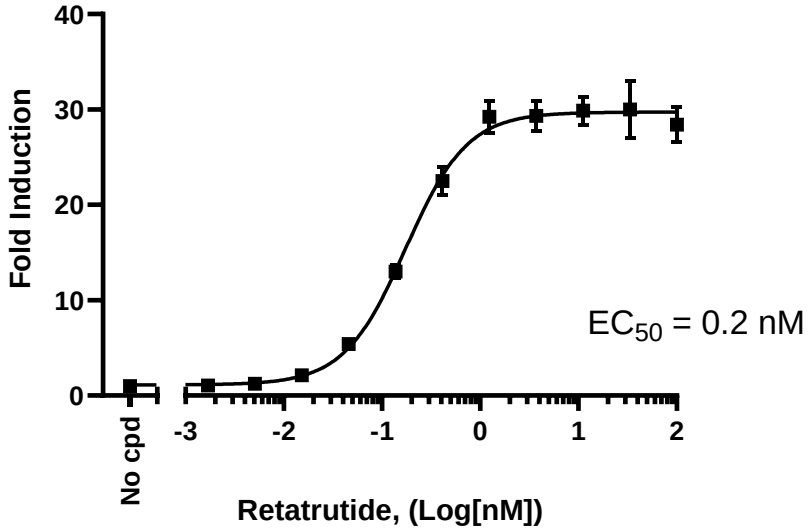



*Figure 8. 384-well format: Dose response curve of GIPR/CRE Luciferase Reporter HEK293 Cell Line to Retatrutide, a triple agonist peptide of GIPR, GLP-1R and GCGR, in a 384-well assay format.*
Cells were treated with increasing concentrations of Retatrutide in a 384-well plate. Luciferase activity was measured with ONE-Step™ Luciferase Assay System. Results are expressed as fold induction versus the unstimulated control.

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

**Sequence**
Human GIPR sequence (NM_000164.4)

MTTSPILQLLLRLSLCGLLLQRAETGSKGQTAGELYQRWERYRRECQETLAAAEPPSGLACNGSFDMYVCWDYAAPNATARASC
PWYLPWHHHVAAGFVLRQCGSDGQWGLWRDHTQCENPEKNEAFLDQRLILERLQVMYTVGYSLSLATLLLALLILSLFRRLHCT
RNYIHINLFTSFMLRAAAILSRDRLLPRPGPYLGDQALALWNQALAACRTAQIVTQYCVGANYTWLLVEGVYLHSLLVLVGGSEEG
HFRYYLLLGWGAPALFVIPWVIVRYLYENTQCWERNEVKAIWWIIRTPILMTILINFLIFIRILGILLSKLRTRQMRCRDYRLRLARSTL
TLVPLLGVHEVVFAPVTEEQARGALRFAKLGFEIFLSSFQGFLVSVLYCFINKEVQSEIRRGWHHCRLRRSLGEEQRQLPERAFRALP
SGSGPGEVPTSRGLSSGTLPGPGNEASRELESYC

**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 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 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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