Description

GLP-1R/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 GLP-1R (Glucagon-like peptide 1 receptor; accession number BC113493). Activation of GLP-1R in these cells can be monitored by measuring luciferase activity.

The functionality of GLP-1R/CRE Luciferase Reporter HEK293 Cell Line was validated in dose-response assays using the peptide agonists Glucagon-like peptide 1 (7-37), Glucagon-like peptide 1 (7-36) amide, Exendin-4, Lixisenatide, GLP-1 moiety from dulaglutide, Semaglutide, Tirzepatide and Retatrutide. This cell line has also been validated in a dose-response assay using a small molecule agonist, Danuglipron. GLP-1R/CRE Luciferase Reporter HEK293 Cell Line responds to agonists by inducing luciferase activity in a dose-dependent manner. Inhibition of agonist induced luciferase activity in this cell line was validated using Exendin (9-39).

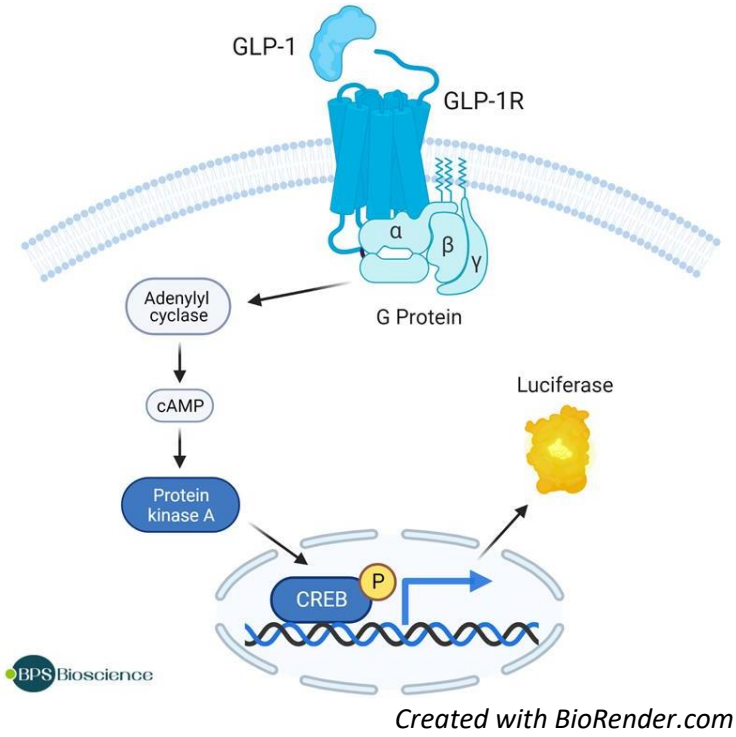

Figure 1. Illustration of mechanism of action in the GLP-1R/CRE Luciferase Reporter HEK293 Cell Line.

Background

GLP-1R, a member of the class B family of G protein-coupled receptors (GPCRs or secretin-like receptors), is a transmembrane protein primarily found in pancreatic β cells and brain neurons. GLP-1R is activated by the peptide hormone glucagon-like peptide 1 (GLP-1), which has two active forms, GLP-1 (7-37) and GLP-1 (7-36) amide. GLP-1R plays an important role in controlling blood sugar level by enhancing glucose-stimulated insulin secretion, glucose, lipid metabolism, and satiety. Its role in the brain seems to be in the control of appetite.

Research efforts have focused on the regulation of the GLP-1R mediated signaling pathway as a therapeutic approach to type 2 diabetes (T2DM) and have resulted in the development of several GLP-1 FDA-approved agonists. In addition to their role in insulin secretion, GLP-1R agonists can also contribute to weight management, decrease the potential for cardiovascular diseases and protect beta cells. A role in tumor development in patients with T2DM is also being investigated but further studies are required to fully understand the functions of GLP-1R and its agonists.

Application
Screen for agonists and inhibitors of human GLP-1R 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 1A	BPS Bioscience #79528

Materials Required for Cellular Assay

Name	Ordering Information
Bovine Serum Albumin (BSA, protease free)	Sigma #A4919
Exendin-4	BPS Bioscience #82640
GIP	BPS Bioscience #82666
GLP-1 (7-37)	BPS Bioscience #82667
GLP-1 (7-36) Amide	BPS Bioscience #82668
GLP-1, Fc-Fusion Recombinant (GLP-1 Agonist)	BPS Bioscience #102006
Lixisenatide	BPS Bioscience #82641
Semaglutide	BPS Bioscience #82647
Tirzepatide	MedChemExpress #HY-P1731B
Retatrutide	BPS Bioscience #82638
Danuglipron	Selleckchem #S9851
Exendin (9-39)	BPS Bioscience #82669
Opti-MEM reduced serum medium (Assay Medium)	Thermo Fisher #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 1A (BPS Bioscience #79528):

MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin, 400 µg/ml of Geneticin and 100 µg/ml of Hygromycin B.

Media Required for Functional Cellular Assay

Assay Medium:

Opti-MEM reduced serum medium with 0.1% BSA (protease free).

GLP-1R agonists can be unstable in the presence of serum. Replace Thaw Medium 1 with Assay Medium if testing any of the agonists mentioned above. If the test agonist is stable in the presence of 10% Fetal Bovine Serum (FBS), Thaw Medium 1 may be used instead of Assay Medium. *See Figures 12 and 13 below.*

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 1A.

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 1A.

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:5 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 1A 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

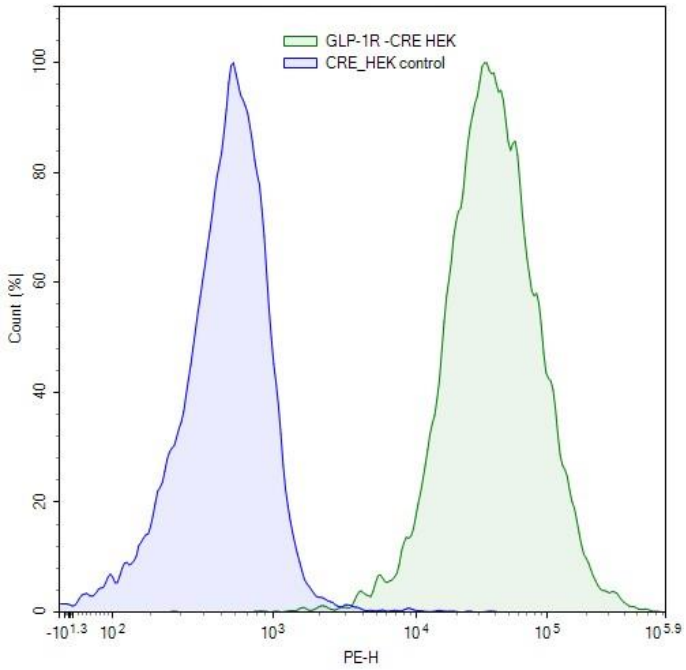

Figure 2. Cell surface expression of GLP-1R in GLP-1R/CRE Luciferase Reporter HEK293 Cell Line by flow cytometry.
GLP-1R/CRE Luciferase Reporter HEK293 cells (green) or control CRE Luciferase Reporter HEK293 cells (blue) were stained with Human-GLP-1R PE-conjugated Antibody (R&D Systems #FAB2814P) and 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 format. To perform the assay in a different format, the cell number and reagent volume should be scaled appropriately.
- The assay conditions should be performed in triplicate.
- Assay A should include "Background Control", "Stimulated", and "Unstimulated Control" conditions.
- Assay B should include "Background Control", "No Inhibitor Control", "No Inhibitor, No Agonist Control" and "Test Inhibitor" conditions.

Assay Medium: Opti-MEM reduced serum medium with 0.1% BSA (protease free).

A. Dose response of GLP-1R/CRE Luciferase Reporter-HEK293 Cell Line to GLP-1R agonists

1. Seed GLP-1R/CRE Luciferase Reporter HEK293 cells into a white clear-bottom 96-well microplate at a density of ~30,000 cells per well in 100 µl of Thaw Medium 1. Leave a few wells empty for use as the cell-free control wells ("Background Control").

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

3. The next day, prepare a serial dilution of a GLP-1R agonists in Assay Medium (100 µl/well). For each peptide agonists it is recommended to use a new pipet tip for each dilution to avoid sample carry over.

3.1 For agonists soluble in water-based buffers, prepare a serial dilution in Assay Medium at the desired concentrations.

Assay Medium is the Diluent Solution.

OR

3.2 For agonists soluble in DMSO, such as Danuglipron, prepare a stock solution in 100% DMSO at a concentration 1,000x higher than the highest desired final concentration, then dilute it 1000-fold with Assay Medium to prepare the highest concentration of the serial dilution. The concentration of DMSO is now 0.1% DMSO.

Prepare a serial dilution at the desired concentrations using Assay Medium containing 0.1% DMSO.

For controls use Assay Medium with 0.1% DMSO (Diluent Solution).

Note: The concentration of DMSO should not exceed 0.1% in the final reaction.

4. Carefully remove the Thaw Medium 1 from the GLP-1R/CRE Luciferase Reporter HEK293 cells.
5. Add 100 µl of GLP-1R agonist dilutions to the "Stimulated" wells.
6. Add 100 µl of Diluent Solution to the "Unstimulated Control" wells.
7. Add 100 µl of Diluent Solution to the "Background Control" wells (for determining background luminescence).
8. Incubate the plate at 37°C in a CO_2 incubator for 5-6 hours.
9. Add 100 µl of the ONE-Step™ Luciferase reagent per well.
10. Rock gently at Room Temperature (RT) for ~15 minutes.
11. Measure luminescence using a luminometer.
12. 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 unstimulated control wells.

    Note: Background subtracted luminescence of unstimulated cells is typically low in this cell line and can vary from assay to assay. This variability can leadto a broad range of fold induction values, as exemplified in the error bars shown in the figures below.

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

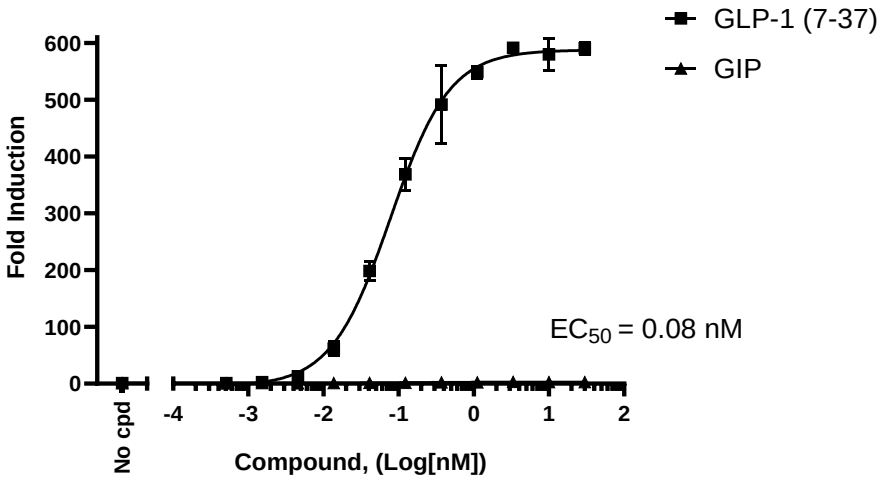

Figure 3. Dose response curve of GLP-1R/CRE Luciferase Reporter HEK293 cells to GLP-1 (7-37) and GIP.

Cells were treated with increasing concentrations of GLP-1 (7-37) and GIP. GLP-1 (7-37) stimulated GLP-1R, inducing luciferase activity. GIP was inactive. Luciferase activity was measured with ONE-Step™ Luciferase Assay System. Results are expressed as fold induction versus the unstimulated control.

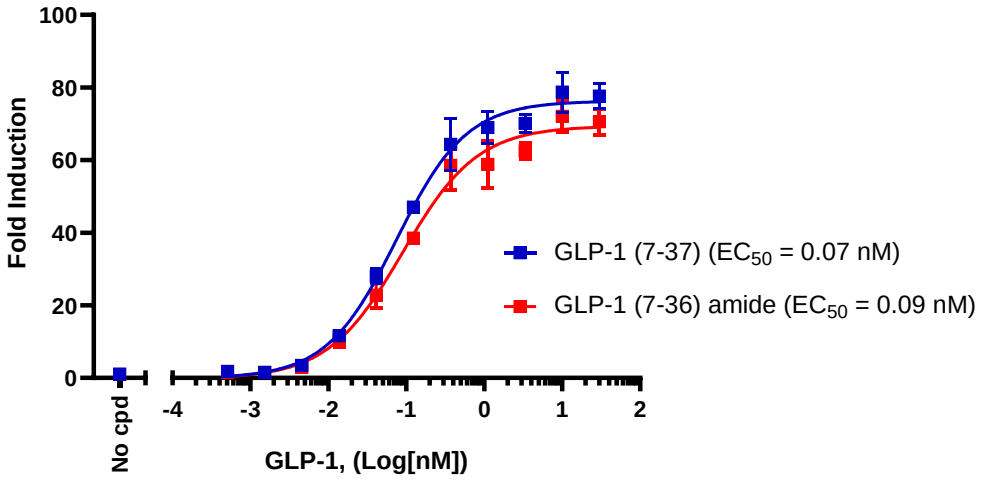

Figure 4. Dose response curve of GLP-1R/CRE Luciferase Reporter HEK293 cells to GLP-1 (7-37) (blue) and GLP-1 (7-36) amide (red).

Cells were treated with increasing concentrations of GLP-1 (7-37) and GLP-1 (7-36) amide. Both peptides stimulated GLP-1R, inducing luciferase activity, with similar EC_{50} values. Luciferase activity was measured with ONE-Step™ Luciferase Assay System. Results are expressed as fold induction versus the unstimulated control.

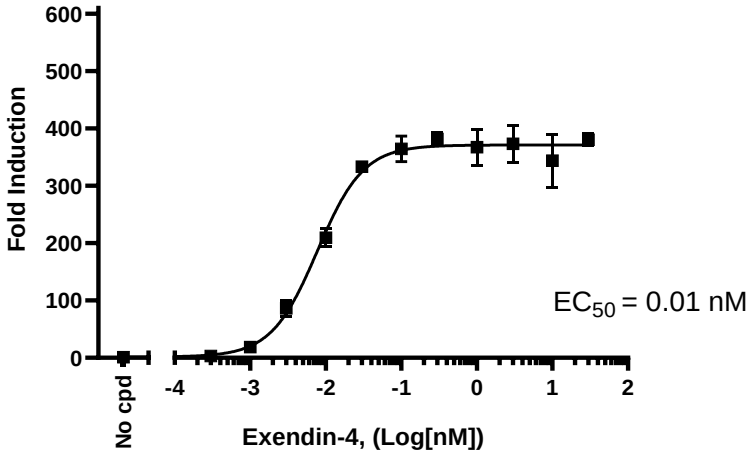

Figure 5. Dose response curve of GLP-1R/CRE Luciferase Reporter HEK293 Cell Line to Exendin-4. Cells were treated with increasing concentrations of Exendin-4. Luciferase activity was measured with ONE-Step™ Luciferase Assay System. Results are expressed as fold induction versus the unstimulated control.

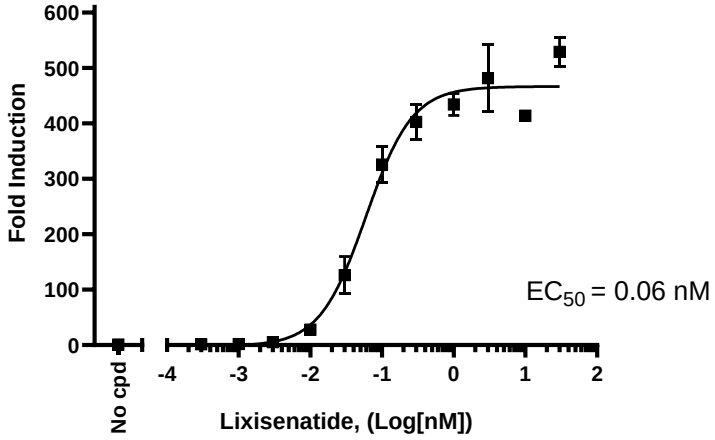

Figure 6. Dose response curve of GLP-1R/CRE Luciferase Reporter HEK293 Cell Line to Lixisenatide. Cells were treated with increasing concentrations of Lixisenatide. Luciferase activity was measured with ONE-Step™ Luciferase Assay System. Results are expressed as fold induction versus the unstimulated control.

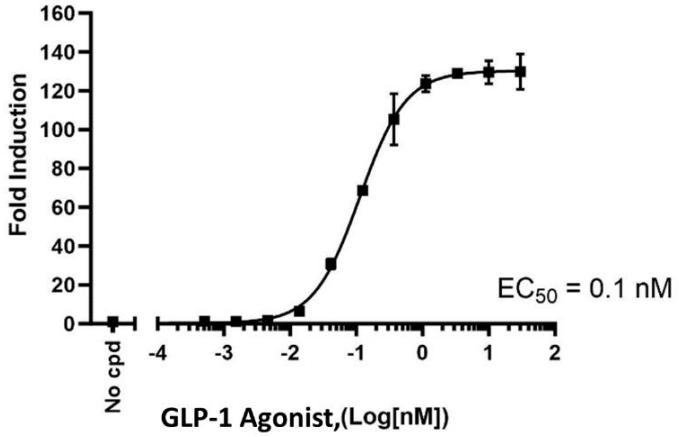

Figure 7. Dose response curve of GLP-1 GLP-1R/CRE Luciferase Reporter HEK293 Cell Line GLP-1, Fc-Fusion Recombinant (GLP-1 Agonist).
Cells were treated with increasing concentrations of GLP-1, Fc-Fusion Recombinant (GLP-1 Agonist). Luciferase activity was measured with ONE-Step™ Luciferase Assay System. Results are expressed as fold induction versus the unstimulated control.

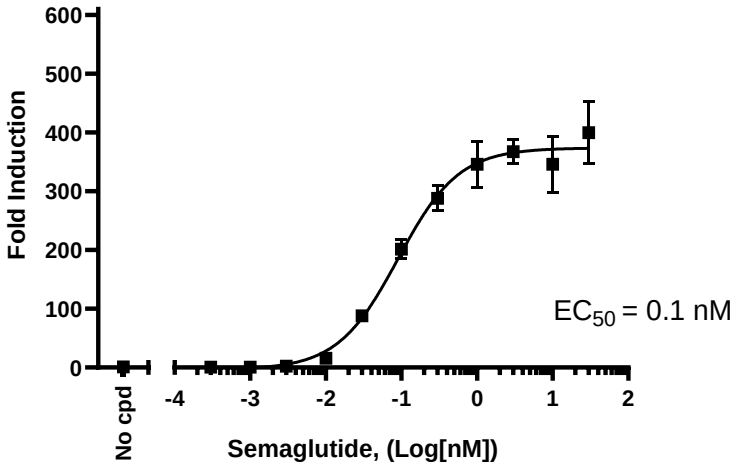

Figure 8. Dose response curve of GLP-1R/CRE Luciferase Reporter HEK293 Cell Line to Semaglutide.
Cells were treated with increasing concentrations of Semaglutide. Luciferase activity was measured with ONE-Step™ Luciferase Assay System. Results are expressed as fold induction versus the unstimulated control.

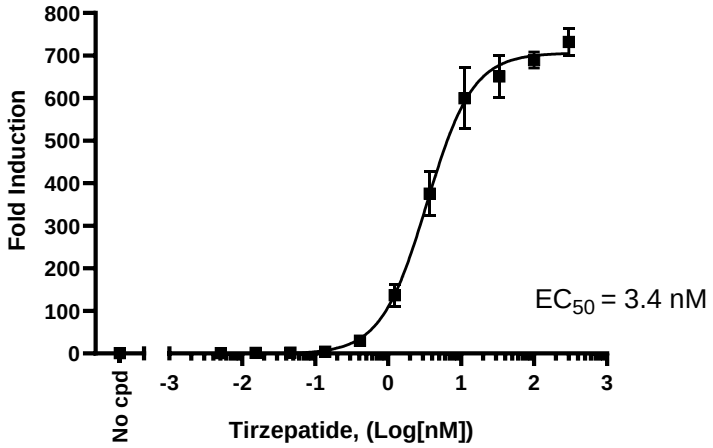

Figure 9. Dose response curve of GLP-1R/CRE Luciferase Reporter HEK293 Cell Line to Tirzepatide, a dual peptide agonist of GLP-1R and GIPR.
Cells were treated with increasing concentrations of Tirzepatide. Luciferase activity was measured with ONE-Step™ Luciferase Assay System. Results are expressed as fold induction versus the unstimulated control.

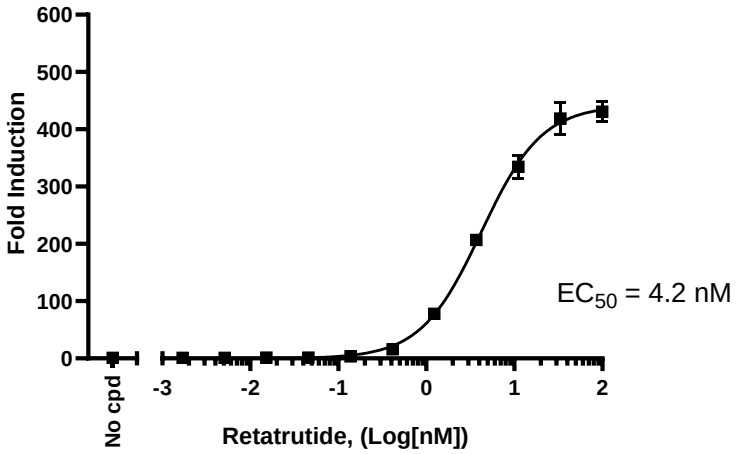

Figure 10. Dose response curve of GLP-1R/CRE Luciferase Reporter HEK293 Cell Line to Retatrutide, a triple agonist peptide of GLP-1R, GIPR, and GCGR.
Cells were treated with increasing concentrations of Retatrutide. Luciferase activity was measured with ONE-Step™ Luciferase Assay System. Results are expressed as fold induction versus the unstimulated control.

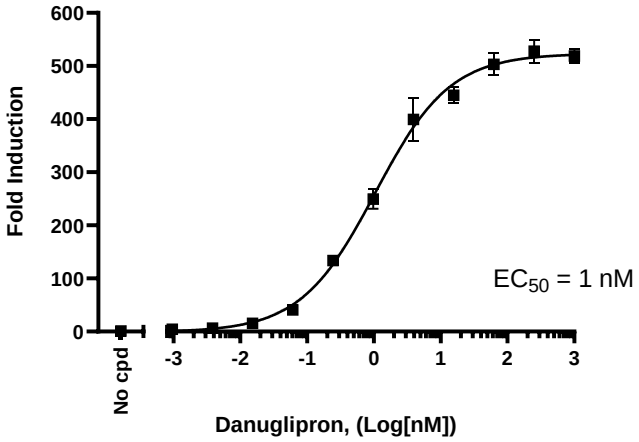

Figure 11. Dose response curve of GLP-1R/CRE Luciferase Reporter HEK293 Cell Line to Danuglipron, a non-peptide agonist of GLP-1R.
Cells were treated with increasing concentrations of Danuglipron. Luciferase activity was measured with ONE-Step™ Luciferase Assay System. Results are expressed as fold induction versus the unstimulated control.

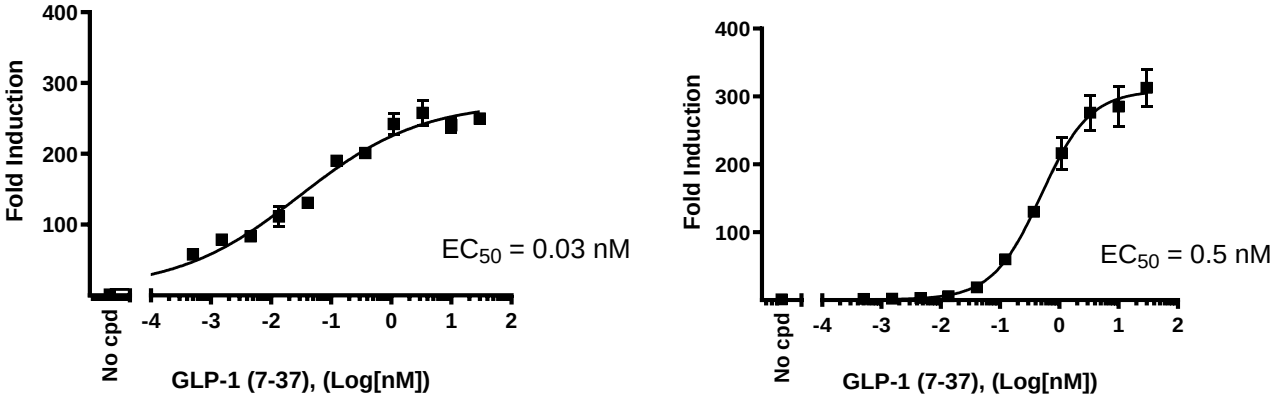

Figure 12. Response of GLP-1R/CRE Luciferase Reporter HEK293 Cell Line to GLP-1 (7-37) shows reduced peptide stability in the presence of Fetal Bovine Serum (FBS).
Left: A serial dilution of GLP-1 (7-37) was prepared as described in the protocol and the assay was performed in serum-free Assay Medium. In the absence of serum, the EC_{50} of GLP-1 (7-37) was 0.03 nM. **Right:** the same serial dilution of GLP-1 (7-37) as shown on the left was prepared and the assay was performed in Thaw Medium 1 containing 10% FBS. In the presence of serum, the EC_{50} of GLP-1 (7-37) increased to 0.5 nM. Luciferase activity was measured with ONE-Step™ Luciferase Assay System. Results are expressed as fold induction versus the unstimulated control.

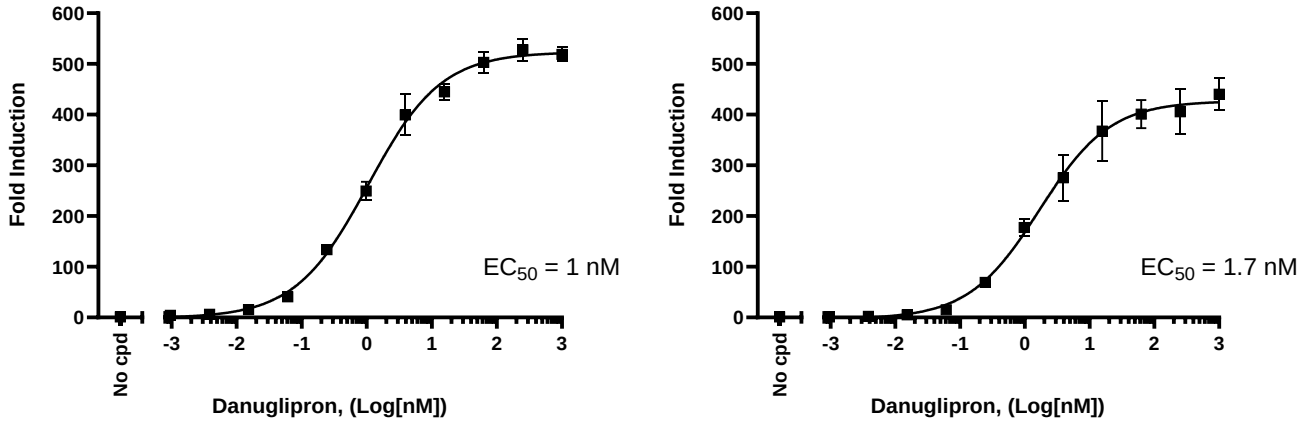

Figure 13. Response of GLP-1R/CRE Luciferase Reporter HEK293 Cell Line to Danuglipron is stable in the presence and absence of FBS.
Left: A serial dilution of Danuglipron was prepared as described in the protocol with the assay being performed in serum-free Assay Medium. In the absence of serum, the EC_{50} of Danuglipron was 1 nM. **Right:** A serial dilution of Danuglipron was prepared and the assay was performed in Thaw Medium 1 containing 10% FBS. Luciferase activity was measured with ONE-Step™ Luciferase Assay System. Results are expressed as fold induction versus the unstimulated control.

B. Inhibition of GLP-1 (7-36) Amide -Induced Reporter Activity in the GLP-1R/CRE Luciferase Reporter HEK293 Cell Line

1. Seed GLP-1R/CRE Luciferase Reporter HEK293 cells into a white clear-bottom 96-well microplate at a density of ~30,000 cells per well in 100 µl of Thaw Medium 1. Leave a few wells empty for use as cell-free control wells ("Background Control").

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

3. The next day, carefully remove Thaw Medium 1 from the GLP-1R/CRE HEK293 cells and add 40 µl of Assay Medium.

4. Prepare a serial dilution of antagonist, Exendin (9-39), in Assay Medium at 2-fold the final desired concentrations (50 µl/well).

5. Add 50 µl of diluted Exendin (9-39) to the "Test Inhibitor" wells.

6. Add 50 µl of Assay Medium to the "No Inhibitor Control" and "No Inhibitor, No Agonist Control" wells.

7. Incubate the plate at 37°C in a CO_2 incubator for 30 minutes.

8. Prepare a dilution of GLP-1 (7-36) amide in Assay Medium at 10-fold the final desired concentration (final [GLP-1 (7-36) amide] on cells = 0.3 nM) (10 µl/well).

9. Add 10 µl of GLP-1 (7-36) amide dilution to the "Test Inhibitor" and "No Inhibitor Control" wells.

10. Add 10 µl of Assay Medium to the "No Inhibitor, No Agonist Control" wells.

11. Add 100 µl Assay Medium to the "Background Control" wells (for determining background luminescence).

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

13. Add 100 µl of the ONE-Step™ Luciferase reagent per well.

14. Rock gently at RT for ~15 minutes.

15. Measure luminescence using a luminometer.

16. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells. The percent luminescence of luciferase reporter expression is the background-subtracted luminescence of the "Test Inhibitor" wells divided by the average background-subtracted luminescence of the "No Inhibitor Control" wells x 100%.

$$Percent\ Luminescence = \left(\frac{Luminescence\ of\ \ Test\ Inhibitor\ Wells - avg.background}{Avg.Luminescence\ of\ No\ Inhibitor\ Wells - avg.background}\right) x100$$

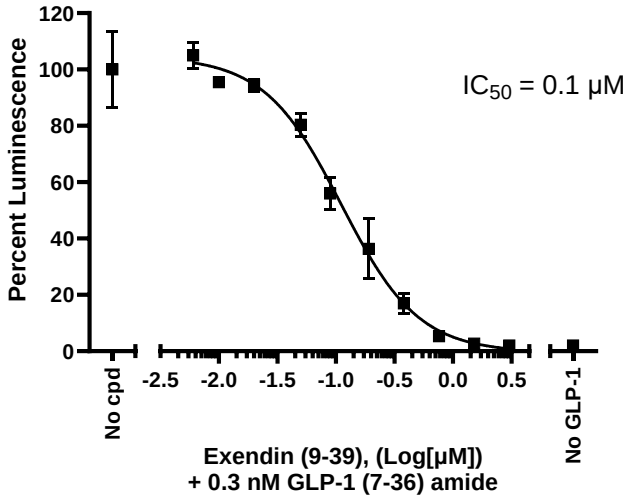

Figure 14. Inhibition of GLP-1 (7-36) amide-induced GLP-1R/CRE activity by the GLP-1R antagonist, Exendin (9-39), in GLP-1R/CRE Luciferase Reporter HEK293 Cell Line.
GLP-1R/CRE Luciferase Reporter HEK293 cells were pre-incubated with increasing doses of Exendin (9-39) prior to stimulation with 0.3 nM GLP-1 (7-36) amide, as described in the protocol above. Luciferase activity was measured with ONE-Step™ Luciferase Assay System. The results are shown as percent luminescence compared to wells without Exendin (9-39) (set at 100%).

C. "Thaw-and-Use" Agonist Testing with GLP-1R/CRE Luciferase Reporter HEK293 Cell Line

"Thaw-and-Use" Functional Validation

- The "thaw-and-use" protocol is designed for scientists that wish to perform the assay with cells just thawed, with any cell culture being performed.
- The following assay is designed for a 96-well format. To perform the assay in a different format, the cell number and reagent volume should be scaled appropriately.
- The assay conditions should be performed in triplicate.

- Assay should include “Background Control”, “Stimulated”, and “Unstimulated Control” conditions.

1. Prior to the assay, make sure to have enough vials of GLP-1R/CRE Luciferase Reporter HEK293 cells for any future assay requirements.

2. Thaw the required number of vials of cells needed for the assay, considering some cell loss will occur during thawing.

3. Follow steps 1 and 2 described in the *Cell Thawing* protocol.

4. After resuspending the cells in fresh Thaw Medium 1, seed cells into a white clear-bottom 96-well microplate at a density of ~30,000 cells per well in 90 µl of Thaw Medium 1. Leave a few wells empty to use as the cell-free control wells (“Background Control”).

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

6. The next day, prepare a serial dilution of a GLP-1R agonists in Thaw Medium 1 at 10-fold the final desired concentrations (10 µl/well). For peptide agonists it is recommended to use a new pipet tip for each dilution to avoid sample carry over.

7. Add 10 µl GLP-1R agonist to the “Stimulated” wells.

8. Add 10 µl Thaw Medium 1 to the “Unstimulated Control” wells.

9. Add 100 µl Thaw Medium 1 to the “Background Control” wells (for determining background luminescence).

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

11. Add 100 µl of the ONE-Step™ Luciferase reagent per well.

12. Rock gently at room temperature for ~15 minutes.

13. Measure luminescence using a luminometer.

14. 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 stimulated well divided by the average background-subtracted luminescence unstimulated control wells.

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

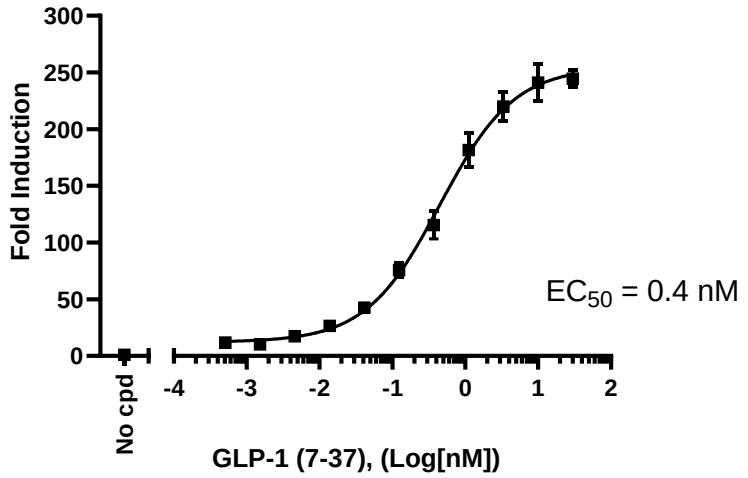

Figure 15. Dose response curve of GLP-1R/CRE Luciferase Reporter HEK293 cells to GLP-1 (7-37) in cells thawed right before the assay was performed.
Cells were thawed directly into a white clear-bottom 96-well microplate in Thaw Medium 1 at 30,000 cells/well. After an overnight incubation, cells were treated with increasing concentrations of GLP-1 (7-37) in Thaw Medium 1. 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 GLP-1R sequence (accession number BC113493)
MAGAPGPLRLALLLLGMVGRAGPRPQGATVSLWETVQKWREYRRQCQRSLTEDPPPATDLFCNRTFDEYACWPDGEPGSFVN
VSCPWYLPWASSVPQGHVYRFCTAEGLWLQKDNSSLPWRDLSECEESKRGERSSPEEQLLFLYIIYTVGYALSFSALVIASAILLGFR
HLHCTRNYIHLNLFASFILRALSVFIKDAALKWMYSTAAQQHQWDGLLSYQDSLSCRLVFLLMQYCVAANYYWLLVEGVYLYTLL
AFSVFSEQWIFRLYVSIGWGVPLLFVVPWGIVKYLYEDEGCWTRNSNMNYWLIIRLPILFAIGVNFLIFVRVICIVVSKLKANLMCK
TDIKCRLAKSTLTLIPLLGTHEVIFAFVMDEHARGTLRFIKLFTELSFTSFQGLMVAILYCFVNNEVQLEFRKSWERWRLEHLHIQRD
SSMKPLKCPTSSLSSGATAGSSMYTATCQASCS

References
Zhao X., *et al.*, 2021 *Front. Endocrinol.* 12:721135.

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Troubleshooting Guide
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Products	*Catalog #*	*Size*
Adenosine A2A Receptor Functional Recombinant Cell Line	79381	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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