Description

The GLP-2R/CRE Luciferase Reporter HEK293 Cell Line is a HEK293 cell line engineered to express Glucagon-like peptide 2 receptor (GLP-2R) (NM_004246.3). The construct was delivered by lentiviral transduction of CRE/CREB Luciferase Reporter HEK293 Cell Line (#60515), which express a firefly luciferase reporter driven by cAMP response elements (CRE). After activation by GLP-2, the endogenous transcription factor CREB binds to the response elements, inducing transcription of the luciferase reporter gene.

This cell line has been validated to respond to GLP-2, Tirzepatide, Dapiglutide, and Teduglutide.

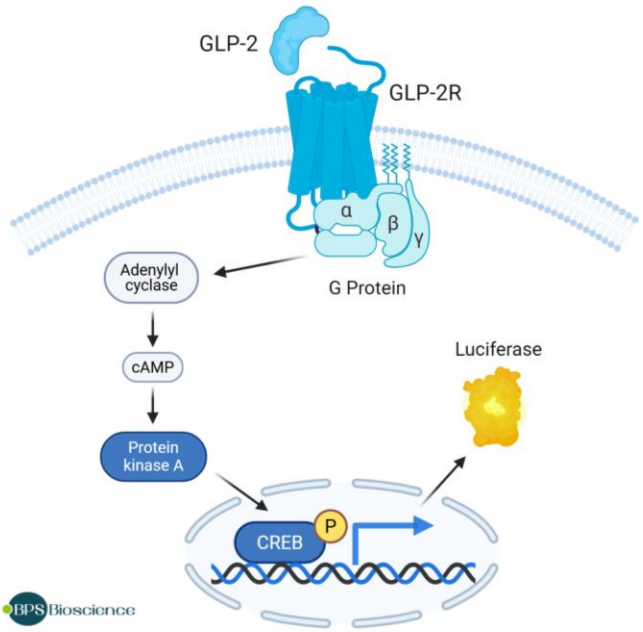

Figure 1: Illustration of mechanism of action in the GLP-2R/CRE Luciferase Reporter HEK293 Cell Line.

Background

Glucagon-Like Peptide-2 Receptor (GLP-2R), a member of the class B G protein-coupled receptors (GPCRs), is a transmembrane protein predominately expressed in the pancreas, gut, and brain. GLP-2R is activated by the peptide hormone glucagon-like peptide 2 (GLP-2), which is rapidly secreted from endocrine L cells in response to nutrient absorption. GLP-2R plays an important role in gut health by triggering epithelial cell proliferation in the small and large intestines and suppressing apoptosis. As a result, both intestinal crypts and villi increase in size, leading to an overall expansion of the absorbative surface area in the intestines. Therapeutic strategies targeting the GLP-2 receptor are under investigation, predominantly centered on the development of long-acting receptor agonists. GLP-2 analog Teduglutide is an FDA approved injectable for the treatment of short bowel syndrome (SBS). Teduglutide reduces the need for intravenous feeding in patients with SBS by promoting intestinal cell growth and aiding in the absorption of nutrients and fluids. Other therapies include Dapiglutide, a unique dual GLP-1/GLP-2 receptor agonist that is currently in clinical trials for the treatment of obesity and associated intestinal inflammation.

Application

Screen for GLP-2R agonists

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)

Parental Cell Line

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 the cell line but are necessary for cell culture and cellular assays. BPS Bioscience's reagents 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 below.

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
Teduglutide	BPS Bioscience #83763
Dapiglutide	BPS Bioscience #83764
Glucagon-Like Peptide (GLP) II, human	Genscript #RP10769-0.5
Semaglutide	BPS Bioscience #82647
Tirzepatide	BPS Bioscience #82639
GLP-1 (7-37)	BPS Bioscience #82667
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, to the use of these 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 plus 0.5 µg/ml Puromycin and 100 µg/ml Hygromycin B

Media Required for Functional Cellular Assay

Thaw Medium 1 (BPS Bioscience #60187):

MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin.

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 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 and continue growing in a 5% CO_2 incubator at 37°C until the cells are ready to passage.

5. 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 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 desired sub-cultivation ratio of 1:8 to 1:15 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 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.

Validation Data

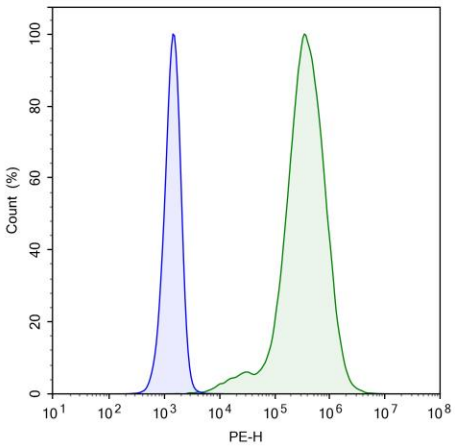

Figure 2. Cell surface expression of GLP-2R in GLP-2R/CRE Luciferase Reporter HEK293 Cell Line by flow cytometry.
GLP-2R/CRE Luciferase Reporter HEK293 cells (green) or control CRE Luciferase Reporter HEK293 cells (blue) were stained with Human-GLP-2R Antibody (R&D Systems #MAB4285) followed by staining with PE Goat anti-mouse IgG (BioLegend # 405307) 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 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 A should include "Background Control", "Stimulated", and "Unstimulated Control" conditions.

A. Dose response of GLP-2R/CRE Luciferase Reporter-HEK293 Cell Line to GLP-2R agonists

1. Seed GLP-2R/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 "Background Control" (cell free control wells).

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

3. The next day, prepare a serial dilution, at the final desired concentration, of GLP-2R agonists in Assay Medium (100 µl/well).

Note: For each peptide agonist it is recommended to use a new pipet tip for each dilution to avoid sample carryover.

4. Carefully remove the media from the GLP-2R/CRE Luciferase Reporter HEK293 cells.

5. Add 100 µl of GLP-2R agonist dilutions to the "Stimulated" wells.

6. Add 100 µl of Assay Medium to the "Unstimulated Control" wells.

7. Add 100 µl of Assay Medium 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.

$$Fold\ induction = \frac{Luminescence\ of\ Stimulated\ Wells\ -\ avg.background}{Avg.Luminescence\ of\ Unstimulated\ Wells\ -\ avg.background}$$

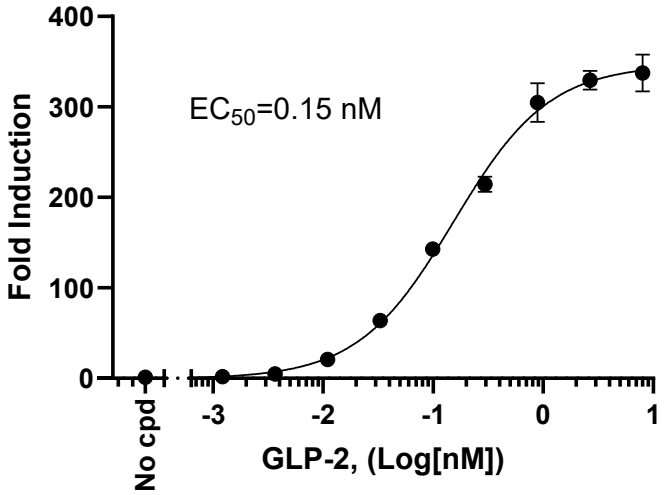

Figure 3. Dose response curve of GLP-2R/CRE Luciferase Reporter HEK293 Cell Line to GLP-2.
Cells were treated with increasing concentrations of GLP-2. Luciferase activity was measured with ONE-Step™ Luciferase Assay System. Results are expressed as fold induction versus unstimulated control.

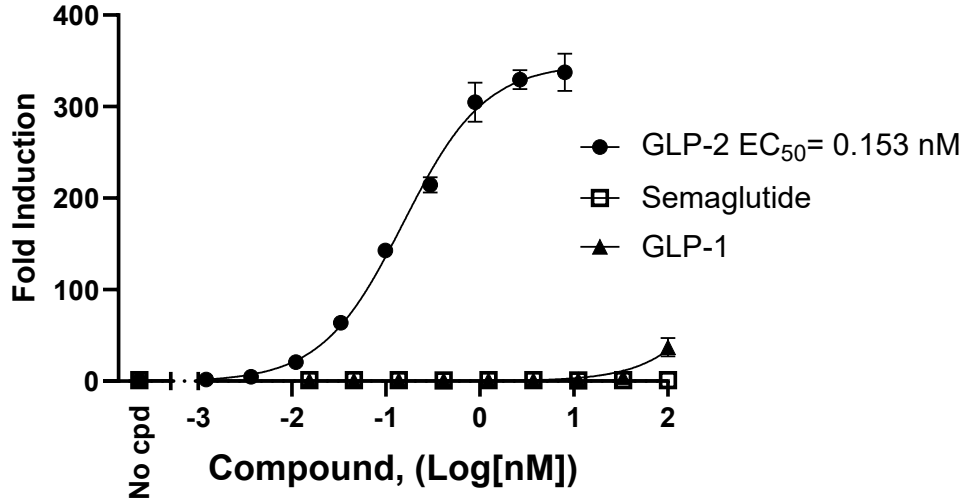

Figure 4. Dose response curve of GLP-2R/CRE Luciferase Reporter HEK293 Cell Line to GLP-2, Semaglutide and GLP-1.
Cells were treated with increasing concentrations of GLP-2, Semaglutide, and GLP-1. Luciferase activity was measured with ONE-Step™ Luciferase Assay System. Results are expressed as fold induction versus unstimulated control.

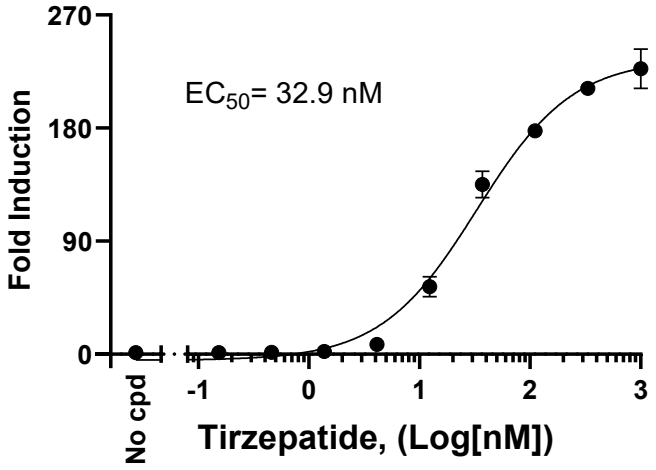

Figure 5. Dose response curve of GLP-2R/CRE Luciferase Reporter HEK293 Cell Line to Tirzepatide. Cells were treated with increasing concentrations of Tirzepatide. Luciferase activity was measured with ONE-Step™ Luciferase Assay System. Results are expressed as fold induction versus unstimulated control.

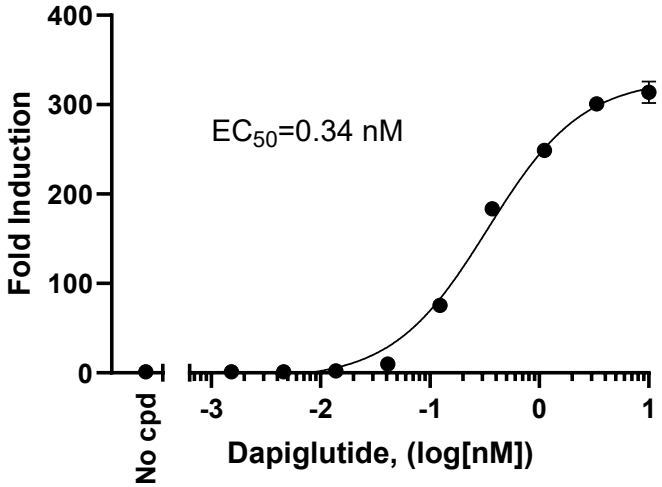

Figure 6. Dose response curve of GLP-2R/CRE Luciferase Reporter HEK293 Cell Line to Dapiglutide. Cells were treated with increasing concentrations of Dapiglutide. Luciferase activity was measured with ONE-Step™ Luciferase Assay System. Results are expressed as fold induction versus unstimulated control.

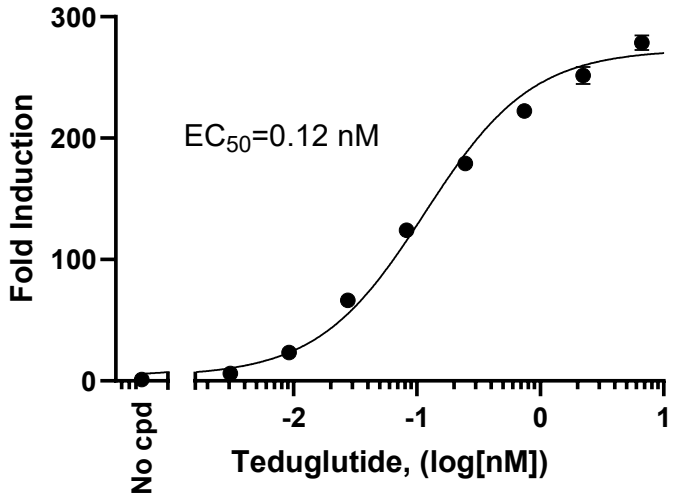

Figure 7. Dose response curve of GLP-2R/CRE Luciferase Reporter HEK293 Cell Line to Teduglutide. Cells were treated with increasing concentrations of Teduglutide. Luciferase activity was measured with ONE-Step™ Luciferase Assay System. Results are expressed as fold induction versus unstimulated control.

Data shown is representative.

Sequence

Human GLP-2R sequence (accession number NM_004246.3)

MKLGSSRAGPGRGSAGLLPGVHELPMGIPAPWGTSPLSFHRKCSLWAPGRPFLTLVLLVSIKQVTGSLLEETTRKWAQYKQACLR
DLLKEPSGIFCNGTFDQYVCWPHSSPGNVSVPCPSYLPWWSEESSGRAYRHCLAQGTWQTIENATDIWQDDSECSENHSFKQN
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STSCRSVQVLLHYFVGANYLWLLVEGLYLHTLLEPTVLPERRLWPRYLLLGWAFPVLFVVPWGFARAHLENTGCWTTNGNKKIW
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VALQYGFANGEVKAELRKYWVRFLLARHSGCRACVLGKDFRFLGKCPKKLSEGDGAEKLRKLQPSLNSGRLLHAMRGLGELGAQ
PQQDHARWPRGSSLSECSEGDVTMANTMEEILEESEI

References

Abdalqadir N., *et al*., 2022 *Microorganisms*. 10(10):2061
Drucker D., *et al*., 2014 *Annu Rev Physiol.* 76:561-83.
Dubé P., *et al*., 2007 *Am J Physiol Endocrinol Metab.* 293(2):E460-5.
Sun W., *et al*., 2020 *Cell Res.* 30(12):1098-1108.

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Troubleshooting Guide

Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For all further questions, please visit https://bpsbioscience.com/contact .

Related Products

Products	*Catalog #*	*Size*
Glucagon Receptor (GCGR)/CRE Luciferase Reporter HEK293 Cell Line	82187	2 vials
GLP-1R/CRE Luciferase Reporter HEK293 Cell Line	78176	2 vials
GIPR/CRE Luciferase Reporter HEK293 Cell Line	78589	2 vials
Amylin Receptor 3 (AMY3R)/CRE Luciferase Reporter HEK293 Cell Line	83544	2 vials

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