SZABO SCANDIC

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# Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!

## Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

## Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand


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**Description**

Recombinant HEK293 cells expressing firefly luciferase gene under the control of cAMP response element (CRE) with constitutive expression of human GLP-1R (Glucagon-like peptide 1 receptor; accession number BC113493).

GLP-1R, a member of the class B family of G protein-coupled receptors (GPCRs) primarily found in pancreatic β cells, is activated by a peptide hormone, glucagon-like peptide 1 (GLP-1) that is secreted from intestinal L-cells after nutrient ingestion. GLP-1R plays an important role in controlling blood sugar level by enhancing glucose-stimulated insulin secretion, so various research efforts have focused on the regulation of the GLP-1R mediated signaling pathway as a therapeutic approach to diabetes.

**Application**

Screen for agonists of human GLP-1R 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 90% FBS, 10% DMSO |

**Host Cell**

HEK293

**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.

*Materials 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 |
|---|---|
| GLP-1 (7-37) | R&D Systems, #5374/1 |
| Opti-MEM reduced serum medium (Assay Medium) | ThermoFisher, #31985-070 |
| ONE-Step™ Luciferase Assay System | BPS Bioscience #60690 |
| Luminometer | |

**Storage Conditions**

Cells will arrive upon 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. To formulate a comparable but not BPS validated media, formulation components can be found below.

Note: Thaw Media does *not* contain selective antibiotics. However, Growth Media *does* contain selective antibiotics, which are used for maintaining cell lines over many passages. Cells should be grown at 37°C with 5% $CO_2$. BPS 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 (Thermo Fisher, #11095098) supplemented with 10% FBS (Thermo Fisher, #26140079), 1% non-essential amino acids (Corning, #25-025-CI), 1 mM Na pyruvate (Corning, #25-000-CI), 1% Penicillin/Streptomycin (Thermo Fisher, #15140163)

*Growth Medium 1A (BPS Bioscience #79528):*

Thaw Medium 1, 400 µg/ml of Geneticin (Thermo Fisher, #11811031) and 100 µg/ml of Hygromycin B (Thermo Fisher, #10687010)

*Assay Medium*: Opti-MEM reduced serum medium (ThermoFisher, #31985-070)

**Cell Culture Protocol**

*Cell Thawing*

1. To thaw the cells, it is recommended to swirl the frozen cells for 30-40 seconds in a 37°C water-bath, then use 1-2 ml Thaw Medium 1 to completely thaw the cells. Transfer the entire contents of the vial to a tube containing 10 ml of Thaw Medium 1 (**no Geneticin or Hygromycin B**).
2. Spin down the cells, remove supernatant and resuspend 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 split before they are fully confluent. At first passage, switch to Growth Medium 1A (**contains Geneticin and Hygromycin B**).

*Cell Passage*

1. To passage the cells, remove the medium, rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with 0.05% Trypsin/EDTA.
2. After detachment, add Growth Medium 1A (**contains Geneticin and Hygromycin B**) and transfer to a tube, spin down cells, resuspend cells in Growth Medium 1A and seed appropriate aliquots of cell suspension into new culture vessels. Sub cultivation ratio: 1:6 to 1:8 weekly or twice per week.

*Cell Freezing*

1. To cryopreserve the cells, remove the medium, rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with 0.05% Trypsin/EDTA.

2. After detachment, add Thaw Medium 1 (**no Geneticin or Hygromycin B**) and count the cells, then transfer to a tube, spin down cells, and resuspend in 4°C Freezing Medium (BPS Bioscience, #79796 or 10% DMSO + 90% FBS) at ~2 x $10^6$ cells/ml.
3. Dispense 1 ml of cell aliquots into cryogenic vials. Place vials in an insulated container for slow cooling and store at -80°C overnight.
4. Transfer to liquid nitrogen the next day for storage.

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

**Validation Data**

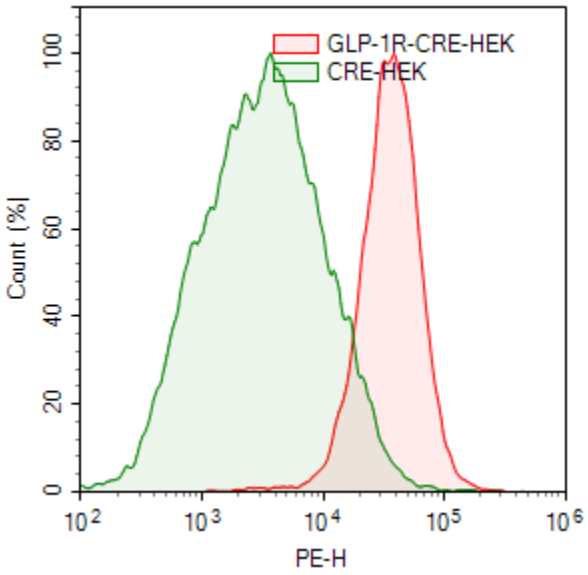


*Figure 1.* GLP-1R/CRE-HEK293 cells (red) or control CRE-HEK293 cells (green) were stained with PE-labeled Anti-GLP-1R Antibody (R&D systems, #FAB2814P) and analyzed by FACS. Y-axis is the % cell number. X-axis is the intensity of PE.

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.

**A. Dose response of GLP-1R/CRE Reporter-HEK293 cells to GLP-1 (7-37) – GLP-1R agonist assay**

1. Harvest GLP-1R/CRE Reporter-HEK293 cells from culture in the Growth Medium 1A and seed cells at a density of ~30,000 cells per well into a white clear-bottom 96-well microplate in 100 µl of the Growth Medium 1A. Leave a couple of wells empty for use as the cell-free control.
2. Incubate cells at 37°C in a $CO_2$ incubator for 16 to 24 hours.
3. Prepare two-fold serially diluted GLP-1 (7-37) (R&D Systems, #5374/1) or other GLP-1R agonists in 100 µl Assay Medium (see above) in a separate 96-well plate (non-binding plate).

4. Take out the plate containing the GLP-1R/CRE Reporter-HEK293 cells from the incubator and carefully remove the Growth Medium 1A and replace it with 100 µl Assay Medium containing GLP-1 or other agonists prepared in step 3.

HEK293 cells are easily detached during the medium change. Use a pipette and slowly remove the medium. Do not use an aspirator. Some degrees of detachment should not affect the results as long as well-to-well variations are not significant.

GLP-1 peptide can be unstable in the presence of serum and degrade over time. If using GLP-1, replace Growth Medium 1A with Assay Medium. If your agonist is stable in the presence of 10% FBS, it can be diluted in Growth Medium 1A at 10X testing concentrations. Then, add 10 µl of the diluted agonist to the wells without medium replacement (see below).

5. Incubate the plate at 37°C in a $CO_2$ incubator for 6 hours.
6. Perform luciferase assay using ONE-Step Luciferase Assay buffer, prepared according to the recommended instructions. Add 100 µl of the final ONE-Step™ Luciferase reagent per well and rock at room temperature for ~15 to 30 minutes. Measure luminescence using a luminometer.
7. Data Analysis: Subtract average background luminescence (cell-free control wells) from the luminescence reading of all wells.

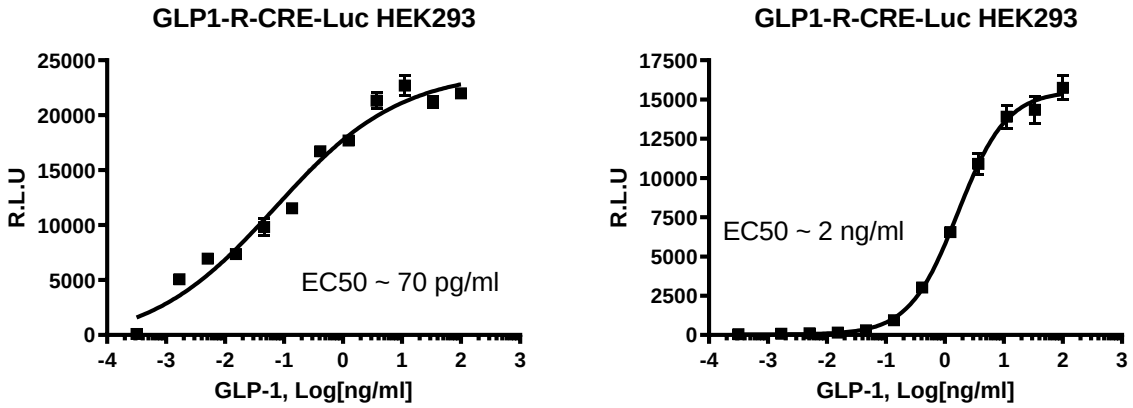


**Figure 2. Dose response of GLP-1R/CRE Reporter -HEK293 cells to GLP-1 (7-37).** *Left - Growth Medium 1A was replaced with Assay Medium containing GLP-1 (7-37); Right - GLP-1 (7-37) was diluted in the Growth Medium 1A at 10X testing concentration and 10 µl was added to the wells*

**Sequence**
Human GLP-1R sequence

MAGAPGPLRLALLLLGMVGRAGPRPQGATVSLWETVQKWREYRRQCQRSLTEDPPPATDLFCNRTFDEYACWPDGEPGSFVN
VSCPWYLPWASSVPQGHVYRFCTAEGLWLQKDNSSLPWRDLSECEESKRGERSSPEEQLLFLYIIYTVGYALSFSALVIASAILLGFR
HLHCTRNYIHLNLFASFILRALSVFIKDAALKWMYSTAAQQHQWDGLLSYQDSLSCRLVFLLMQYCVAANYYWLLVEGVYLYTLL
AFSVFSEQWIFRLYVSIGWGVPLLFVVPWGIVKYLYEDEGCWTRNSNMNYWLIIRLPILFAIGVNFLIFVRVICIVVSKLKANLMCK
TDIKCRLAKSTLTLIPLLGTHEVIFAFVMDEHARGTLRFIKLFTELSFTSFQGLMVAILYCFVNNEVQLEFRKSWERWRLEHLHIQRD
SSMKPLKCPTSSLSSGATAGSSMYTATCQASCS

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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* |
|---|---|---|
| Thaw Medium 1 | 60187 | 100 ml |
| Growth Medium 1A | 79528 | 500 ml |
| ONE-Step™ Luciferase Assay System | 60690 | Various Sizes |