GLP-2 Receptor (human) Reporter Assay Kit

Item No. 702950

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GENERAL INFORMATION

Materials Supplied

This kit will arrive packaged as a -80°C kit. After opening the kit, store individual components as stated below.

Item Number	Item	100 Tests Quantity/Size	Storage
401125	GLP-2R Reverse Transfection Strip Plate	1 plate	-20°C
401123	Human GLP-2 Positive Control (0.1 mM)	1 vial/25 µl	-80°C
600183	SEAP Substrate (Luminescence)	1 vial/15 ml	4°C
700029	96-Well Solid Plate (white)	3 plates	RT
400012	96-Well Cover Sheet	3 ea	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

The reagents in this kit have been tested and formulated to work exclusively with Cayman Chemical's GLP-2 Receptor (human) Reporter Assay Kit. This kit may not perform as described if any reagent or procedure is replaced or modified.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section (see page 3) and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. HEK293T (or HEK293T/17) cells, available from ATCC
2. Dulbecco's Modified Eagle's Medium (DMEM)
3. Fetal bovine serum (FBS)
4. Penicillin-streptomycin (100X) (ThermoFisher™ Scientific, Catalog No. 15140-122)
5. A plate reader capable of measuring luminescence
6. Adjustable and multichannel pipettes with pipette tips
7. An incubator/oven set at 65°C

INTRODUCTION

Background

Glucagon-like peptide 2 receptor (GLP-2R) is a G protein-coupled receptor (GPCR) and a member of the secretin family of receptors.[1,2] It is primarily expressed in the gastrointestinal system, including the jejunum, duodenum, ileum, colon, and stomach, but has also been found in the dorsomedial nucleus of the hypothalamus.[2,3] Following food intake, the incretin hormone GLP-2 is released from enteroendocrine L-cells and binds to GLP-2R, inducing signaling through the $G\alpha_s$-coupled cAMP/PKA pathway to induce crypt cell proliferation, inhibit epithelial apoptosis, regulate nutrient absorption, maintain the intestinal barrier, and increase intestinal blood flow.[1,2,4,5] GLP-2R can also couple to β-arrestin signaling to varying degrees in response to different agonists, a phenomenon known as biased agonism.[5] Intratumoral levels of GLP-2R are increased in patients with gastrointestinal stromal tumors and myenteric plexus levels of GLP-2R are increased in patients with active Crohn's disease.[6] GLP-2R agonists have intestinotrophic activity and reduce intestinal transit time in animal models of short bowel syndrome, as well as reduce inflammation and increase small intestinal mass in a rat model of inflammatory bowel disease.[7-10] These effects highlight the importance of continued research and development of GLP-2R agonists as potential therapeutics for gastrointestinal tract diseases.

About This Assay

Cayman's reverse transfection reporter assays have overcome many of the disadvantages of other transfection approaches. In this method, a proprietary transfection complex containing DNA and an optimized mixture of lipids and proteins has been evenly applied on the culture surface of multi-well plates. Adherent cells, supplied by the user, are applied directly to the plate and allowed to grow in the coated wells. Using this method, the uptake of the DNA complex by the cell increases dramatically compared to solution-phase transfection, enhancing both the transfection efficiency and co-transfection efficiency for multiple plasmids.

Cayman's GLP-2R (human) Reporter Assay Kit consists of a 96-well plate coated with a transfection complex containing DNA constructs for GLP-2R and a cAMP response element-regulated secreted alkaline phosphatase (SEAP) reporter (GLP-2R Reverse Transfection Strip Plate). Cells grown on the transfection complex will express GLP-2R at the cell surface within 24 hours. Binding of agonists to GLP-2R initiates a signal transduction cascade through the $G\alpha_s$-coupled adenylate cyclase pathway resulting in the expression of SEAP, which is secreted into the cell culture medium. Aliquots of culture medium are collected at 6-8 hours after stimulation and SEAP activity is measured following the addition of a luminescence-based alkaline phosphatase substrate provided in the kit (SEAP Substrate). The kit is easy to use and can be readily applied to high-throughput screening for therapeutic compounds regulating the activation of GLP-2R. A peptide agonist, human GLP-2, is included for use as a positive control. The kit provides a sufficient volume of reagents to measure SEAP activity at three time points using the three included white assay plates.

Principle Of This Assay

The binding of an agonist to GLP-2R activates $G\alpha_s$ and triggers the cAMP/PKA/CREB signaling pathway. Phosphorylated CREB binds to the CREB-responsive element (CRE) in the promoter region of the co-transfected reporter, which induces the expression of SEAP. The activity of SEAP is measured as a luminescence signal produced after incubation with the provided substrate.

ASSAY PROTOCOL

Plate Set Up

There is no specific pattern for using the wells on the plate. A typical experiment will include wells containing cells treated with human GLP-2 as a positive control, cells treated with experimental compounds and untreated cells.. It is recommended that each treatment be performed at least in triplicate. To determine the EC_{50} value of a compound, several concentrations of the compound should be included in the assay. The volume of Human GLP-2 Positive Control (0.1 mM) is sufficient to run a full dose-response curve with replicates or to serve as a control at approximately EC_{80} for testing inhibitors on the whole plate. Record the contents of each well on the template sheet provided on page 17.

Addition of Cells to the Reverse Transfection Plate

IMPORTANT

Before starting the experiment, pre-warm the required volume of culture medium and make sure a sufficient number of actively growing cells are available.

1. Remove the GLP-2R Reverse Transfection Strip Plate (Item No. 401125) from the freezer and allow it to equilibrate to room temperature inside the sealed bag. After it has reached room temperature, clean the bag with 70% alcohol before opening it and place the plate inside a cell culture hood.

   NOTE: If not using the whole plate within one experiment, remove the number of strips needed, place the remaining strips back in the bag, and store them in a desiccator ***protected from UV light*** *at room temperature for up to a week. Alternatively, the remaining strips can be sealed in the bag with the desiccant pack and stored at -20°C for up to two months.*

2. Seed HEK293T cells at a density of 40,000-60,000 cells/well in 200 µl of DMEM containing 10% FBS and 1X penicillin/streptomycin. Leave the plate in the hood for 30-45 minutes.
3. Place the plate in a 37°C CO_2 incubator and incubate for 16-20 hours.

Cell Stimulation

1. After 16-20 hours of incubation, carefully aspirate the culture medium from each well.
2. Replenish the cells with 150 µl of pre-warmed stimulation medium (DMEM with 0.5% FBS) per well.
3. Prepare test compounds at 4X the desired final concentration in DMEM and pipette 50 µl into the assigned wells. Pipette 50 µl of DMEM into wells designated for untreated cells. For positive control wells, dilute the Human GLP-2 Positive Control (0.1 mM) (Item No. 401123) 1:500 in serum-free DMEM and add 50 µl to the positive control wells. At this concentration (50 nM), human GLP-2 induces a >5-fold increase in SEAP activity in 6-8 hours compared to the untreated control.

Performing the SEAP Assay

Pipetting Hints

- Use different tips to pipette each reagent.
- Avoid introducing bubbles to the well.
- Do not expose the pipette tip to the reagent(s) already in the well.

Before performing the assay, remove the SEAP Substrate (Luminescence) (Item No. 600183) from the refrigerator and allow it to equilibrate to room temperature.

1. After 6-8 hours of stimulation with test compounds and controls, transfer the plate from the incubator to a cell culture hood.
2. Inside the culture hood, use a multi-channel pipette to gently pipette up and down a few times. Collect 10 µl of medium from each well and transfer it to the corresponding well of a 96-Well Solid Plate (white) (Item No. 700029).

   NOTE: Avoid contact of the pipette tip with the plate bottom to minimize disruption of the cell layer. Perform this step inside the cell culture hood and return the plate to the incubator if sampling at later time point(s) is needed.
3. Cover the assay plate with the 96-Well Cover Sheet (Item No. 400012) and incubate the plate in a 65°C incubator for 30 minutes to heat inactivate endogenous alkaline phosphatase. The SEAP expressed in this assay is stable under this condition.
4. Remove the plate from the 65°C incubator, remove the cover sheet, and allow the plate to cool to room temperature.
5. Add 50 µl SEAP Substrate to each well, shake/tap briefly to mix, and incubate the plate at room temperature for 5-15 minutes.
6. Scan the plate for luminescence in a microplate reader.

NOTE: The plate should be read immediately after 5-15 minutes of incubation with SEAP Substrate. When multiple plates are processed at the same time, the time interval between plates for the addition of substrate and plate reading should be consistent.

PERFORMANCE CHARACTERISTICS

Calculations

Determination of EC_{50}

The term half-maximal effective concentration (EC_{50}) refers to the concentration of a drug that induces a response halfway between the baseline and maximum after a specific exposure time. The dose-response curve of a typical agonist follows a sigmoidal curve with a bottom plateau (untreated cells) and a top plateau (drug saturation). See Figure 1 on page 14 for a typical GLP-2R receptor agonist dose-response curve.

For each compound, normalize the relative luminescent unit (RLU) results to run from 0% (no drug added) to 100% (saturating dose) by using the following formula:

% Response at X Concentration =

$$\left[\frac{\text{(RLU at X concentration) - (RLU of untreated cells)}}{\text{Maximal RLU (saturation) - (RLU of untreated cells)}}\right] \times 100$$

Graph the percent response versus log drug concentration. In the resulting sigmoidal dose-response curve, find the best-fit value for the log EC_{50} (the concentration that gives a 50% response; the middle of the curve).

Performance Characteristics

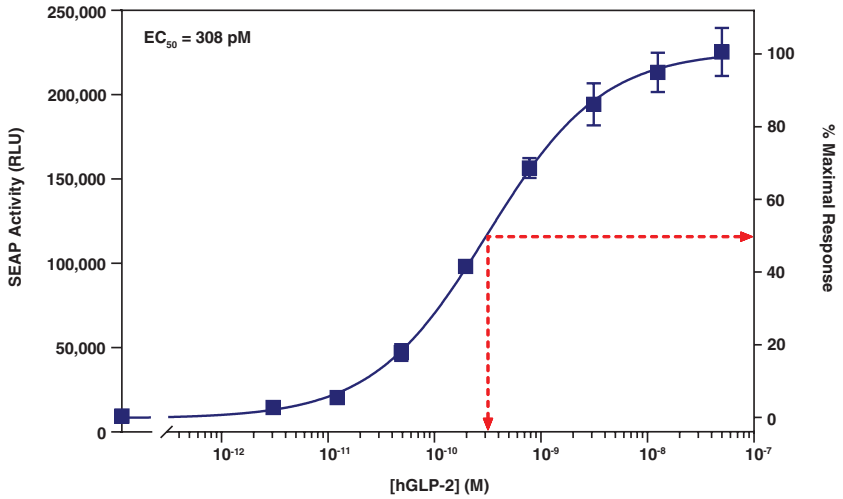

Figure 1. SEAP activity in HEK293T cells transiently transfected with GLP-2R receptor in response to human GLP-2 stimulation. HEK293T cells were seeded on a GLP-2R Reverse Transfection Strip Plate at a density of 40,000 cells/well and incubated overnight. The next day, the cells were treated with different doses of human GLP-2 (hGLP-2) up to 50 nM in serum-free medium. After six hours of stimulation, 10 µl of culture medium was collected from each well and the SEAP activity of each sample was measured according to the **Performing the SEAP Assay** section (see page 12). The calculated EC_{50} value from the fitted curve is 308 pM and the Z' value is >0.75.
NOTE: The fold of stimulation, Z' value, and calculated EC_{50} may vary with cell lines, cell passage, and culture conditions.

RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Dispersion of replicates or erratic response curve of test compounds	A. Uneven cell distribution B. Poor pipetting C. Not well mixed at the sampling step D. Bubble in assay wells	A. Make sure cells are in a homogenous suspension at the time of plating and allow the cells to sit for 30-45 minutes before putting into the incubator B. Pipette carefully C. Pipette up and down a few times before collecting samples D. Carefully tap the side of the plate to remove bubbles
Low reading in wells	A. Reading time is too short B. Samples overheated/dried C. The SEAP substrate was too cold	A. Increase the integration time B. Do not heat-inactivate the sample plate above 65°C or longer than 30 minutes C. Warm up the SEAP substrate to room temperature before use
Sample signal is too strong	A. Cell density was too high B. Insufficient heat inactivation of endogenous alkaline phosphatase activity	A. Reduce cell plating density B. Correct the duration or temperature of heat inactivation step
Poor control curve/signal	A. Control compound degraded B. Pipetting error C. Splashing of sample D. Volume carry-over during dilution	A. Avoid free-thaw of positive control B. Check pipette volume C. Dispense carefully D. Use new tip for each pipetting

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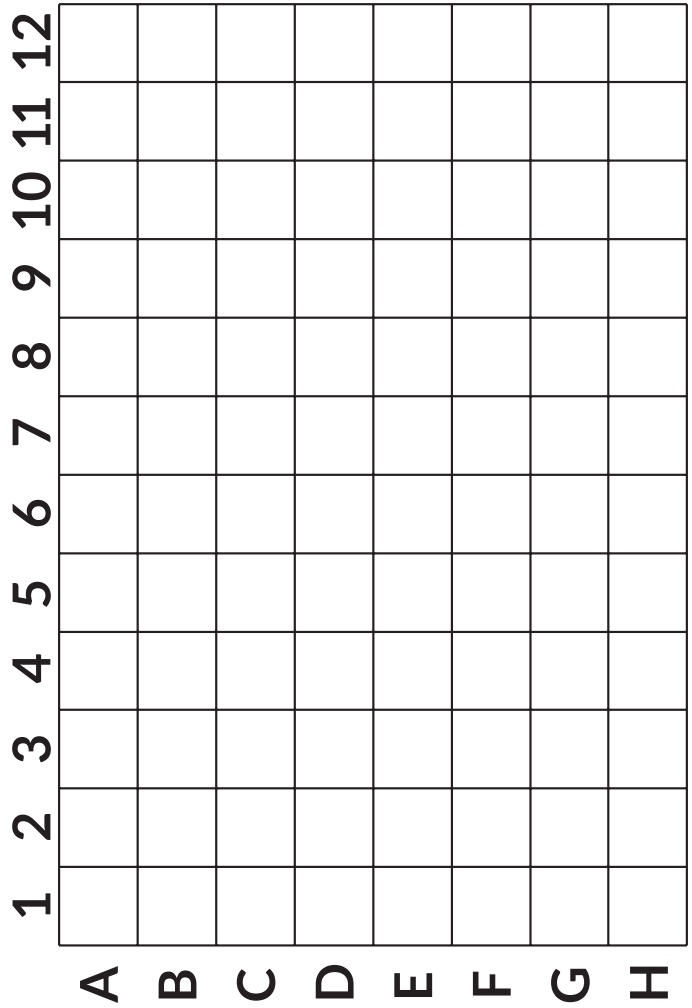

NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.