EP_2 Receptor (human) Reporter Assay Kit

Item No. 601380

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GENERAL INFORMATION

Materials Supplied

This kit will arrive packaged as a -20°C kit. After opening the kit, store individual components as stated below.

Item Number	Item	100 Tests Quantity/Size	Storage
601381	EP_2 Receptor (human) Reverse Transfection Strip Plate	1 plate	-20°C
600341	EP Receptor Assay Prostaglandin E_2 Positive Control	1 vial/20 µl	-20°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 covers	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.

If You Have Problems

Technical Service Contact Information

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

Fax: 734-971-3640

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 and used before the expiration date indicated on the outside of the box. Upon arrival of the kit, store each component at appropriate temperature accordingly, see page 3.

Materials Needed But Not Supplied

1. HEK293T or HEK293T/17 cells, available from ATCC
2. Fetal bovine serum (FBS)
3. Penicillin-streptomycin solution (50X or 100X)
4. Culture medium for maintaining cells (DMEM with 10% FBS and 1X penicillin-streptomycin
5. Reduced serum medium for plating cells on the EP_2 Receptor (human) Reverse Transfection Strip Plateand stimulation, such as UltraMEM™ Reduced Serum Medium (Lonza) or Opti-MEM™ Reduced Serum Medium (Thermo Fisher) supplemented with 1X penicillin-streptomycin
6. A plate reader capable of measuring luminescence
7. Adjustable and multichannel pipettes and pipette tips
8. An incubator/oven set at 65°C

INTRODUCTION

Background

Prostaglandin E_2 (PGE_2) is one of the most important biologically active prostanoids which exerts its actions mainly by binding to four distinct E-type prostanoid receptors: EP_1, EP_2, EP_3, and EP_4. These four G protein-coupled receptors (GPCRs) exhibit differences in signal transduction mechanisms, tissue localization, and regulation of expression.[1,2] EP_2 receptors are expressed in many tissues and cells to mediate various PGE_2 actions.[3] The receptors couple to G_s to stimulate the cAMP second messenger signal transduction pathway.[1,2] EP_2 receptors play important roles in mucosal protection, gastrointestinal secretion, and motility.[4] PGE_2 regulates immunity and inflammation mainly through EP_2 and EP_4 receptors.[5,6] Mice deficient in EP_2 have reduced tumor growth and exhibit cancer-associated immunodeficiency and defective dendritic-cell differentiation.[7] *In vitro* studies demonstrate that activation of the EP_2 receptor was neuroprotective in paradigms of NMDA toxicity and oxygen glucose deprivation.[8] The diverse effects of PGE_2 acting *via* EP_2 receptors make it an interesting drug target. EP_2 subtype selective agonists, antagonists, and modulators have been identified with potential therapeutic applications.[9]

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 is evenly immobilized 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 the co-transfection efficiency for multiple plasmids.

Cayman's EP_2 Receptor (human) Reporter Assay Kit consists of a 96-well plate coated with a transfection complex containing DNA constructs for expressing human EP_2 receptor and a cAMP response element regulated secreted alkaline phosphatase (SEAP) reporter (EP_2 receptor reverse transfection strip plate). Cells grown on the transfection complex will express EP_2 at the cell surface. Binding of agonists to EP_2 initiates a signal transduction cascade through the $G\alpha_s$ and adenylate cyclase pathway resulting in the expression of SEAP which is secreted into the cell culture medium. Aliquots of media are collected between 6-24 hours after stimulation, and SEAP activity is measured following addition of a luminescence-based alkaline phosphatase substrate provided in the kit. The kit is simple to use and can be readily applied to high-throughput screening for therapeutic compounds regulating activation of EP_2. PGE_2 is included in the kit for use as a positive control. The kit provides sufficient reagent to measure SEAP activity at three time points using the three included white assay plates.

ASSAY PROTOCOL

Plate Set Up

There is no specific pattern for using the wells on the plate. A typical experiment will include: wells with cells treated with PGE_2 provided in the kit (positive control), wells with cells treated with experimental compounds, and wells of untreated cells. It is recommended that each treatment be performed at least in triplicate. In order to determine the EC_{50} value of a test compound, serial dilutions of the compound should be included in the assay. The EP Receptor Assay PGE_2 Positive Control (Item No. 600341) provided is sufficient to run a full dose-response curve with replicates up to 10 µM. 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 enough volume of culture medium and make sure sufficient actively growing cells would be available.

1. Remove the EP_2 Receptor (human) Reverse Transfection Strip Plate (Item No. 601381) from the freezer and allow to equilibrate to room temperature within sealed bag.
2. After plate has reached room temperature, clean the bag with 70% alcohol before opening the bag and put the plate inside the hood.
3. Seed HEK293T or HEK293T/17 cells at a density of 50,000-70,000 cells/well in 200 µl of reduced serum medium with 1X penicillin-streptomycin.
4. Allow the plate to sit inside the hood for 30-45 minutes.
5. Place the plate in a 37°C CO_2 incubator with 5% CO_2 and incubate for 20-24 hours.

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

NOTE: HEK293T cells should be maintained in complete medium with 10% FBS. Since serum may contain agonist for EP_2 receptor, caution should be taken to avoid carry-over of maintenance medium when plating cells on the reverse transfection plate in reduced serum medium.

Cell Stimulation

1. After 20-24 hours of incubation, aspirate the culture medium from each well carefully.
2. Replenish the cell with 150 µl pre-warmed serum-free stimulation medium per well.
3. Prepare test compounds at 4X the desired final concentration in serum-free stimulation medium and pipette 50 µl to the assigned wells.
4. Untreated cells receive 50 µl of stimulation medium per well.
5. For positive control wells, dilute the provided 6 mM EP Receptor Assay PGE_2 Positive Control (Item No. 600341) 1:300 in the serum-free stimulation medium and add 50 µl per well.

NOTE: At 5 µM, the EP Receptor Assay PGE_2 Positive Control typically induces a >5-fold increase in SEAP activity in 6-8 hours over untreated control. Prepare aliquots of EP Receptor Assay PGE_2 Positive Control if needed to minimize freeze-thaw cycles. This kit could be used to characterize antagonists by co-incubation of the experimental compound with a fixed dose of PGE_2 between the EC_{50} and EC_{80}.

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 to equilibrate to room temperature.

1. After 6-8 hours of stimulation with test compounds and controls, use a multichannel pipette to gently pipette up and down a few times and collect 10 µl media from each well onto corresponding well of a 96-Well Solid Plate (white) (Item No. 700029).

   *NOTE: Avoid contact of pipette tip with plate bottom to minimize disruption of cell layer. Perform inside cell culture hood and return the plate into incubator **if** sampling at later time point(s) is needed.*
2. Cover the sample plate with provided 96-Well Cover Sheet (Item No. 400012).

   NOTE: Sealed sample plate may be stored at -20°C if not assaying immediately.
3. Incubate the plate in an oven set at 65°C for 30 minutes to heat inactivate endogenous alkaline phosphatase.
4. Remove the plate from the 65°C incubator, discard the cover sheet, and allow the plate to cool down 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 addition of substrate and for plate reading should be consistent.

ANALYSIS

Calculations

Determination of EC_{50}

The term half maximal effective concentration (EC_{50}) refers to the concentration of a drug which induces a response halfway between the baseline and maximum after some 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 EP Receptor Assay PGE_2 Positive Control 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 % response *versus* the 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

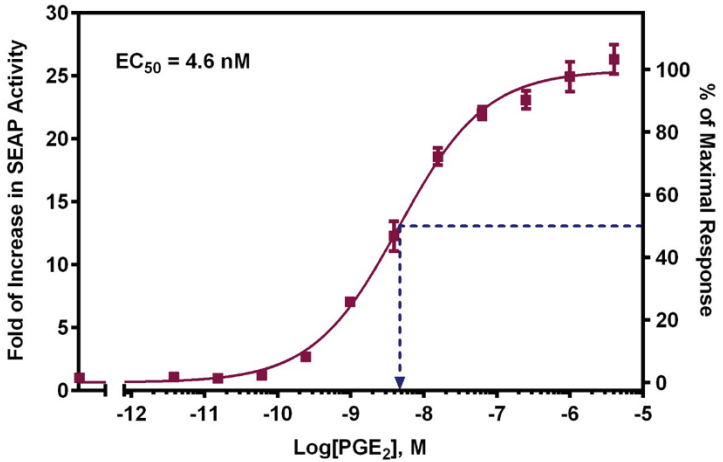

Figure 1. SEAP activity in HEK293T cells transiently-transfected with human EP_2 receptor in response to EP Receptor Assay PGE_2 Positive Control stimulation. HEK293T cells were plated on an EP_2 Receptor (human) Reverse Transfection Strip Plate in serum-free medium at a density of 65,000 cells/well and incubated overnight. The next day, cells were replenished with fresh medium and treated with serial dilutions of PGE_2. After seven hours of stimulation, 10 µl of culture media was collected from each well and the SEAP activity from each sample was measured according to the protocol described on page 11. The calculated EC_{50} value from the fitted curve is 4.6 nM and the Z´ value is >0.8. *NOTE: The fold of stimulation, Z´ value, and calculated EC_{50} may vary with cell lines, cell passages, 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 when sampling D. Bubble in assay well(s)	A. Make sure cells are in homogenous suspension at plating and allow the cells to sit for 30-45 min before putting into incubator B. Pipette carefully C. Pipette up and down a few times before collecting sample D. Carefully tap the side of the plate to remove bubbles
Low reading in well(s)	A. Reading time is too short B. Samples overheated/dried C. The substrate is too cold	A. Increase the integration time B. Keep the plate away from heat source C. Warm up the 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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4. Dey, I., Lejeune, M., and Chadee, K. Prostaglandin E_2 receptor distribution and function in the gastrointestinal tract. *Br. J. Pharmacol.* **149**, 611-623 (2006).
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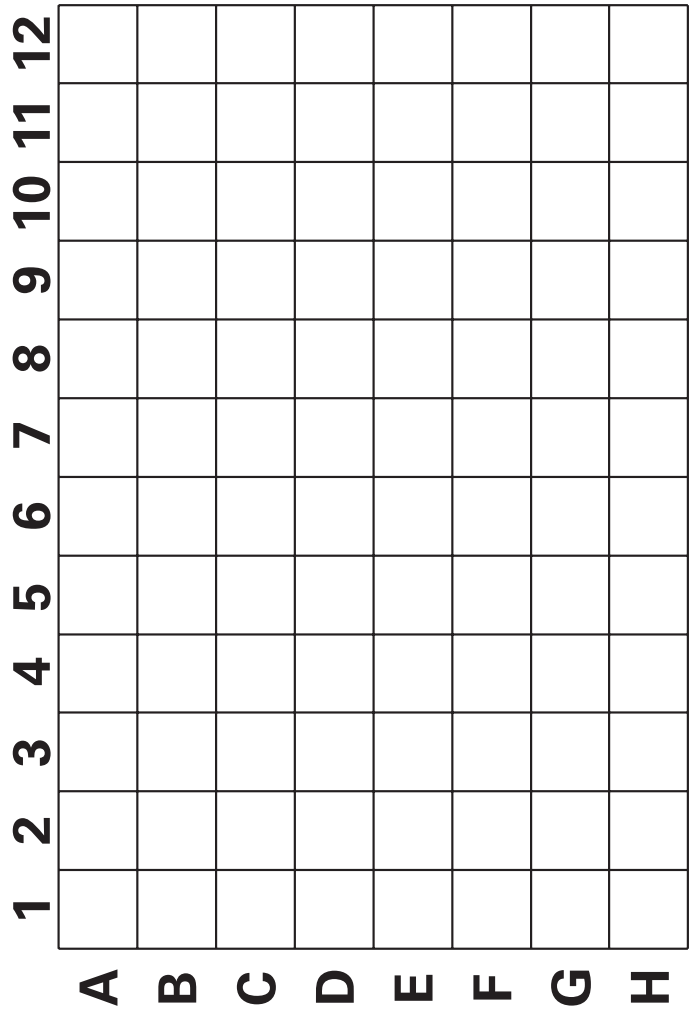

NOTES

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