Prostaglandin F Receptor Reporter Assay Kit

Item No. 601800

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GENERAL INFORMATION

Materials Supplied

Kit will arrive packaged as a -20°C kit. For best results, remove components and store as stated below.

Item Number	Item	Quantity/Size	Storage
700181	FP Receptor Reverse Transfection Strip Plate	1 plate	-20°C
700182	Bimatoprost (free acid) Positive Control, 10 mM	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 in the Materials Supplied section on page 3 and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. HEK293T/17 cells, available from the American Type Culture Collection (ATCC)
2. Complete culture medium used for maintenance of the cells (DMEM + 10% FBS + 1X penicillin-steptomycin)
3. Serum-free stimulation medium (DMEM + 1X penicillin-streptomycin)
4. A plate reader capable of measuring luminescence
5. Adjustable pipettes and a repeating pipettor
6. An incubator set at 65°C

INTRODUCTION

Background

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is a biologically important prostanoid that exerts its actions through binding to the $PGF_{2\alpha}$ receptor (FP).[1] $PGF_{2\alpha}$ is synthesized in many tissues, including the eye and organs of the female reproductive system. Activation of ocular FP receptors *via* agonists such as bimatoprost and travoprost reduces ocular hypertension.[2,3] Antagonism of the FP receptor inhibits uterine contraction and delays parturition in rodent models, while knockout of the receptor inhibits parturition in mice, an effect that can be restored through ovariectomy during pregnancy.[4,5] FP receptor antagonism also decreases endometriosis-like lesions and reduces cell proliferation and angiogenesis in a nude mouse endometrial xenograft model.[6] In addition, FP receptor knockout increases neurological deficits and lesion volume in mice following intracerebral hemorrhagic stroke.[7] These findings highlight the importance of the FP receptor in reproductive and neurological disorders and emphasize the need for continued research and drug discovery in these areas.

About This Assay

Cayman's FP Receptor Reporter Assay Kit consists of a 96-well plate coated with a transfection complex containing DNA constructs for expressing the FP receptor and a CREB-regulated SEAP reporter (FP Receptor Reverse Transfection Strip Plate).

Cayman's FP Receptor Reporter Assay kit consists of a 96-well plate coated with a transfection complex containing DNA constructs for expressing the FP receptor and a CREB-regulated secreted alkaline phosphatase (SEAP) reporter (FP Receptor Reverse Transfection Strip Plate). Cells grown on the transfection complex will express the FP receptor inside the cells within 24 hours from an engineered plasmid construct. Binding of agonists to the FP receptor triggers a signaling pathway leading to the activation of CREB. The binding of CREB on the promoter region of the CRE-SEAP reporter construct results in the expression of SEAP, which is secreted into the cell culture medium. Aliquots of media are collected 6-24 hours after stimulation, and SEAP activity is measured following addition of a luminescence-based alkaline phosphatase substrate (SEAP Substrate (Luminescence)). The kit is easy to use and can be readily applied to high-throughput screening for agonists or antagonists of FP receptor. A selective agonist, bimatoprost free acid (17-phenyl trinor prostaglandin $F_{2\alpha}$), is included in the kit for use as a positive control.[8] 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 experimental plate will include wells with cells treated with Bimatoprost (free acid) Positive Control (Item No. 700182), 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 amount of Biomatoprost (free acid) Positive Control provided is sufficient to run a full dose-response curve with replicates up to 10 µM.

Addition of Cells to the Reverse Transfection Plate

1. Remove the FP Receptor Reverse Transfection Strip Plate (Item No. 700181) from the freezer and allow it to equilibrate to room temperature within the sealed bag.
2. After plate has reached room temperature and before opening the bag, clean the bag with 70% alcohol and place the plate inside the hood.
3. Seed HEK293T/17 cells at a density of 30,000 - 50,000 cells/well in 200 µl of complete culture medium.
4. Allow the plate to sit inside the hood for 30-45 minutes.
5. Place the plate in a 37°C incubator with 5% CO_2 and incubate for 18-24 hours.

*NOTE: If the whole plate will not be completely used within one experiment, remove the number of strips needed and place the remaining strips back in the bag. Store in a desiccator, **protected from UV light**, at room temperature for up to a week. Alternatively, you can vacuum seal the bag and store the remaining strips at -20°C for up to two months.*

Cell Stimulation

1. After 18-24 hours of incubation, aspirate the culture medium from each well carefully.
2. Replenish the cells 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 media and pipette 50 µl to the assigned wells.
4. For untreated control cells, pipette 50 µl of serum-free stimulation media per well.
5. For positive control wells, dilute the provided bimatoprost (free acid) positive control 1:2,500 in the stimulation medium and add 50 µl per well.
6. Return the cells to the incubator at 37°C with 5% CO_2 and incubate for 6-8 hours.

   NOTE: At 1 µM, bimatoprost (free acid) typically induces a >5-fold increase in SEAP activity in 6-8 hours over untreated control. Prepare aliquots of bimatoprost (free acid) positive control 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 Bimatoprost (free acid) near the EC_{80} (~30 nM). Antagonists should be added to the cells 30 minutes before the addition of Bimatoprost free acid.

Performing the SEAP Assay

Pipetting Hints

- Use different tips to pipette each reagent.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

Before performing the assay, allow the SEAP Substrate (Luminescence) (Item No. 600183) to equilibrate to room temperature.

1. After stimulation with test compounds and controls, use a multi-channel pipette to gently pipette up and down a few times, collect 10 µl of media from each well, and pipette it into the corresponding well of the 96-well Solid Plate (white) (Item No. 700029).

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

   NOTE: The sealed sample plate may be stored at -20°C if not assaying immediately.
3. Incubate the plate in an incubator 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 to room temperature.
5. Add 50 µl SEAP Substrate to each well, shake/tap briefly to mix, and incubate at room temperature for 5-15 minutes.
6. Scan the plate for luminescence in a microplate reader.

   NOTE: The plate should be read immediately following 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 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 13, for a typical Bimatoprost (free acid) 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) using the following formula:

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

Graph % response *versus* log drug concentration. In the resulting sigmodial 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

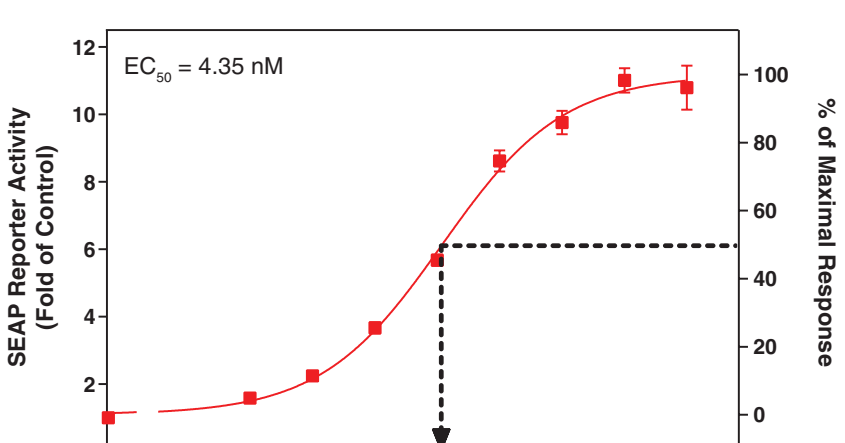

Figure 1. SEAP activity in HEK293T/17 cells transiently transfected with the FP receptor in response to bimatoprost (free acid) stimulation. HEK293T/17 cells were plated on an FP Receptor Reverse Transfection Strip Plate at a density of 50,000 cells/well and incubated overnight. The next day, cells were replenished with fresh medium and treated with serial dilutions of bimatoprost (free acid) up to 1 µM in serum-free medium. After 6.5 hours of stimulation, 10 µl of culture media was collected from each well and the SEAP activity of each sample was measured as described. The calculated EC_{50} value from the fitted curve is 4.35 nM and the Z' value is >0.6. *NOTE: The fold of stimulation, Z' value, and calculated EC_{50} may vary with cell lines, cell passages, and culture conditions*

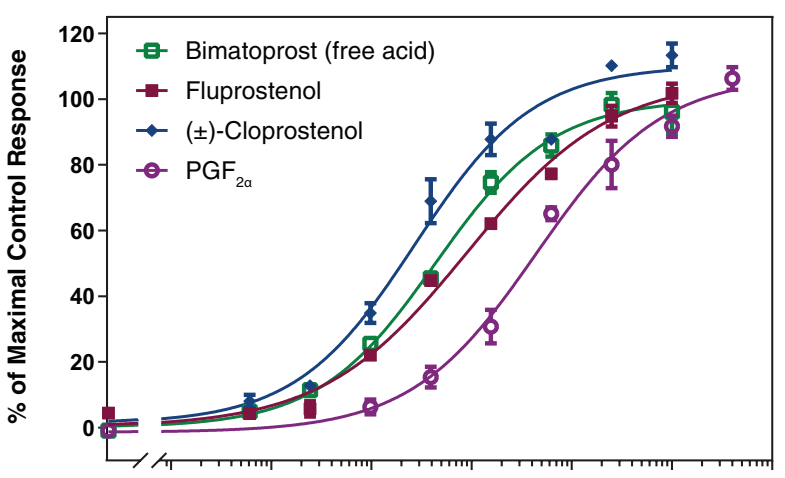

Figure 2. Validation of the Prostaglandin F Receptor Reporter Assay Kit with agonists. In addition Bimatoprost (free acid) Positive Control, three additional known FP receptor agonists were examined in the FP receptor reporter assay. HEK293T/17 cells transfected on strip well plates were treated with serial dilutions of bimatoprost (free acid), fluprostenol, (±)-cloprostenol, and the native agonist $PGF_{2\alpha}$. Media samples were collected and analyzed as described above. The reporter activities were normalized to the responses by Bimatoprost (free acid) Positive Control on the same plate. All examined compounds appeared to be full agonists. The calculated EC_{50} values for bimatoprost (free acid), fluprostenol, (±)-cloprostenol, and $PGF_{2\alpha}$ are 4.35, 8.96, 2.71, and 43.96 nM, respectively.

RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Dispersion of replicates or erratic response curve of test compounds	A. Uneaven cell distribution B. Poor pipetting C. Not well mixed when sampling D. Bubble in assay wells	A. Make sure cells are in homogenous suspension at plating and allow the cells to sit for 30-45 min before placing 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 wells	A. Reading time was too short B. Samples overheated/dried C. The substrate was too cold	A. Increase the integration time B. Keep the plate away from heat sources C. Warm 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 the 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 freeze-thaw of positive control B. Check pipette volume C. Dispense carefully D. Use new tip for pipetting into each well

References

1. Hata, A.N. and Breyer, R.M. Pharmacology and signaling of prostaglandin receptors: Multiple roles in inflammation and immune modulation. *Pharmacol. Ther.* **103(2)**, 147-166 (2004).
2. Maxey, K.M., Johnson, J., Camras, C.B., *et al.* The hydrolysis of bimatoprost in corneal tissue generates a potent prostanoid FP receptor agonist. *Surv. Ophthalmol.* **47(4)**, 34-40 (2002).
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4. Pohl, O., Chollet, A., Kim, S.H., *et al.* OBE022, an oral and selective prostaglandin $F_{2\alpha}$ receptor antagonist as an effective and safe modality for the treatment of preterm labor. *J. Pharm. Exp. Ther.* **366(2)**, 349-364 (2018).
5. Sugimoto, Y., Yamasaki, A., Segi, E., *et al.* Failure of parturition in mice lacking the prostaglandin F receptor. *Science* **277(5326)**, 681-683 (1997).
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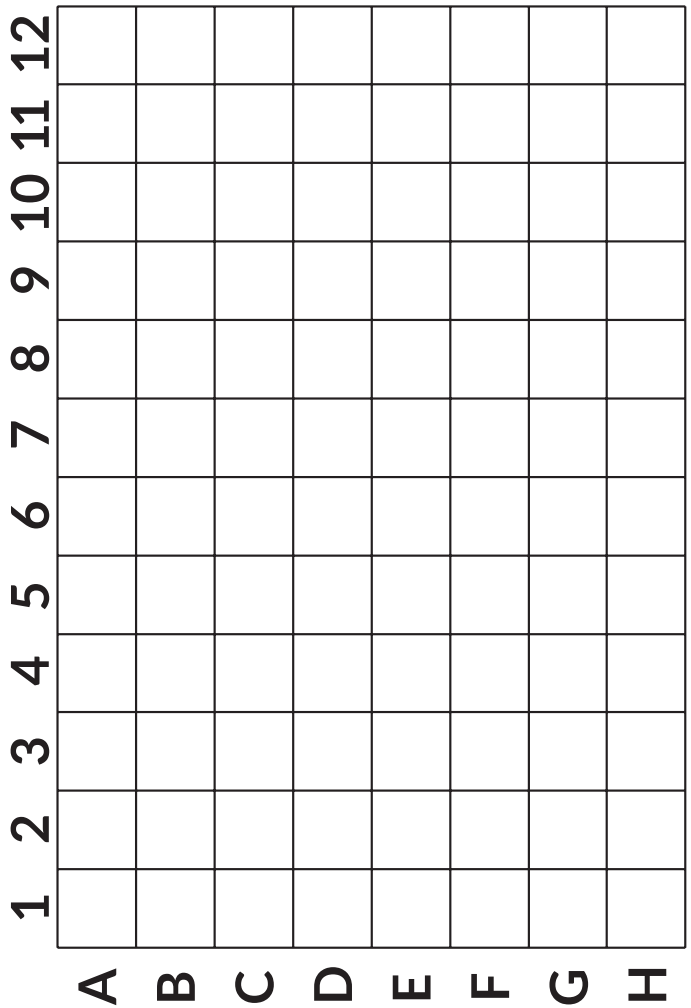

NOTES

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.