EP_4 Receptor (rat) Activation Assay Kit (cAMP)

Item No. 600410

Customer Service 800.364.9897 * **Technical Support** 888.526.5351
www.caymanchem.com

TABLE OF CONTENTS

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	Storage
600351	EP_4 Receptor (rat) Reverse Transfection Strip Plate	1 plate	-20°C
600341	EP Receptor Assay Prostaglandin E_2 Positive Control	1 vial	-20°C
481002	Cyclic AMP EIA Antiserum	1 vial	-20°C
481000	Cyclic AMP AChE Tracer	1 vial	-20°C
481004	Cyclic AMP EIA Standard	1 vial	-20°C
400060	EIA Buffer Concentrate (10X)	1 vial	-20°C
400062	Wash Buffer Concentrate (400X)	1 vial	-20°C
400035	Polysorbate 20	1 vial	Room temperature
400004	Precoated (Mouse Anti-Rabbit IgG) EIA 96-Well Strip Plate	1 plate	4°C
10008978	Cell-Based Assay IBMX Solution (1,000X)	1 vial	-20°C
400050	Ellman's Reagent	3 vials	4°C
400012	96-Well Cover Sheet	1 cover	Room temperature

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 laboratory research use only: not for administration to humans. Not for human or veterinary diagnostic or therapeutic use.

Precautions

Please read these instructions carefully before beginning this assay.

For research use only. Not for human or diagnostic use.

If You Have Problems

Technical Service Contact Information

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

Fax: 734-971-3641

Email: techserv@caymanchem.com

Hours: M-F 8:00 AM to 5:30 PM EST

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

Remove the EP Receptor Assay Prostaglandin E_2 Positive Control from the kit and store at -20°C (be careful to avoid repeated freeze/thaw cycles). Store the EP_4 Receptor (rat) Reverse Transfection Strip Plate at -20°C. If you are planning to use the plate within one or two months, the plate can be stored at 4°C. The Precoated (Mouse Anti-Rabbit IgG) EIA 96-Well Strip Plate should be stored at 4°C and will be stable for at least one year. All other components should be stored at -20°C. The kit will perform as specified if used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. HEK293T/17 cells. The cell lines can be obtained from ATCC.
2. Culture medium used for maintenance of the cells (DMEM).
3. Reduced Serum Medium such as UltraMEM (Lonza 12-743F) for plating cells on the Reverse Transfection Plate.
4. Fetal bovine serum (FBS).
5. Adjustable pipettes and a repeating pipettor.
6. A source of 'UltraPure' water. Water used to prepare all EIA reagents and buffers must be deionized and free of trace organic contaminants ('UltraPure'). Use activated carbon filter cartridges or other organic scavengers. Glass distilled water (even if double distilled), HPLC-grade water, and sterile water (for injections) are not adequate for EIA. *NOTE: UltraPure water is available for purchase from Cayman (Item No. 400000).*

INTRODUCTION

Background

Prostaglandin E_2 (PGE_2), one of the most important biologically active prostanoids, exerts its actions through binding to four distinct G protein-coupled receptors (GPCRs). These PGE_2 receptor subtypes, EP_1, EP_2, EP_3, and EP_4, exhibit differences in signal transduction mechanisms, tissue localization, and regulation of expression.[1] EP_4 receptors are highly expressed in the intestine, but are found in lower levels in the lung, kidney, thymus, uterus, and brain.[2,3] The receptors are coupled to Gs to stimulate the cAMP second messenger signal transduction pathway. In addition, EP_4 couples to phosphatidylinositol 3-kinase, probably *via* G_i to mediate cell survival.[4] EP_4 receptors play important roles in relaxation of smooth muscle, gastric acid secretion, intestinal epithelial transportation, adrenal aldosterone secretion, and uterine functions.[5,6] PGE_2 regulates immunity and inflammation mainly through its receptor subtypes EP_2 and EP_4.[7,8] EP_4 receptors are predominantly expressed in human colon cancers, suggesting a role for EP_4 receptors in colorectal carcinogenesis.[9] *In vivo* studies demonstrated that activation of the EP_4 receptor was neuroprotective in excitotoxic brain injury.[10] The diverse effects of PGE_2 *via* EP_4 receptors point to the need to identify novel agonists and antagonists, both to further elucidate the function of this receptor subtype and for use as therapeutics for various diseases.

About This Assay

Cayman's Reverse Transfection 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 applied and processed 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_4 Receptor (rat) Activation Assay Kit (cAMP) consists of a 96-well plate coated with a transfection complex containing a DNA construct for rat EP_4 Receptor (EP_4 Reverse Transfection Strip Plate). Cells grown on the transfection complex will express EP_4 at the cell surface. Binding of agonists to EP_4 stimulates cAMP generation and increases intracellular cAMP levels, which can be measured by a competitive EIA using the Mouse Anti-Rabbit IgG Coated Plate and the reagents included in the kit. An EP_4 agonist, PGE_2, is included in the kit as a positive control.

Description of ACE™ Competitive EIAs

This assay is based on the competition between free cAMP and a cAMP-acetylcholinesterase (AChE) conjugate (cAMP Tracer) for a limited number of cAMP-specific rabbit antibody binding sites. Because the concentration of the cAMP Tracer is held constant while the concentration of cAMP varies, the amount of cAMP Tracer that is able to bind to the rabbit antibody will be inversely proportional to the concentration of cAMP in the well. This rabbit antibody-cAMP (either free or tracer) complex binds to the mouse monoclonal anti-rabbit IgG that has been previously attached to the well. The plate is washed to remove any unbound reagents and then Ellman's Reagent (which contains the substrate to AChE) is added to the well. The product of this enzymatic reaction has a distinct yellow color and absorbs strongly at 412 nm. The intensity of this color, determined spectrophotometrically, is proportional to the amount of cAMP Tracer bound to the well, which is inversely proportional to the amount of free cAMP present in the well during the incubation; or

$$\text{Absorbance} \propto [\text{Bound cAMP Tracer}] \propto 1/[\text{cAMP}]$$

A schematic of this process is shown in Figure 1 below.

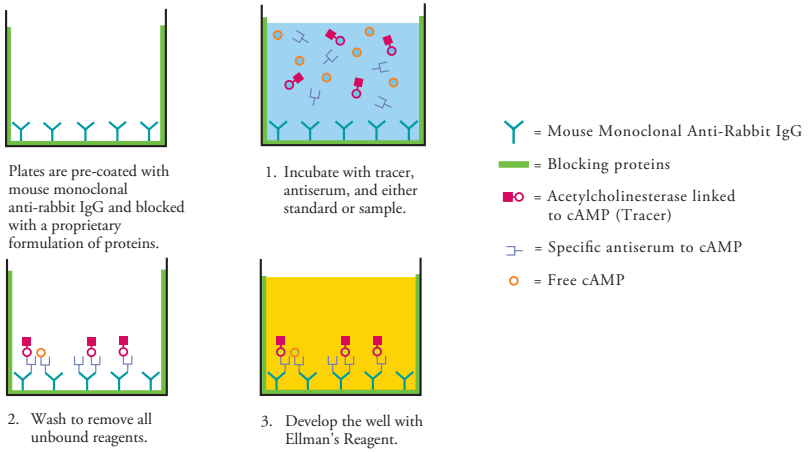


Figure 1. Schematic of the ACE™ EIA

Definition of Key Terms

Blank: background absorbance caused by Ellman's Reagent. The blank absorbance should be subtracted from the absorbance readings of all the other wells, including NSB wells.

Total Activity: total enzymatic activity of the AChE-linked tracer. This is analogous to the specific activity of a radioactive tracer.

NSB (Non-Specific Binding): non-immunological binding of the tracer to the well. Even in the absence of specific antibody a very small amount of tracer still binds to the well; the NSB is a measure of this low binding. Do not forget to subtract the Blank absorbance values.

B_0 (Maximum Binding): maximum amount of the tracer that the antibody can bind in the absence of free analyte.

%B/B_0 (%Bound/Maximum Bound): ratio of the absorbance of a particular sample or standard well to that of the maximum binding (B_0) well.

Standard Curve: a plot of the %B/B_0 values *versus* concentration of a series of wells containing various known amounts of analyte.

Dtn: determination, where one dtn is the amount of reagent used per well.

STIMULATION OF CELLS

IMPORTANT: Please read both Stimulation of Cells Procedure and Performing the Assay Procedure sections carefully before initiating your experiments!

EP_4 Plate Set Up

There is no specific pattern for using the wells on the reverse transfection plate. A typical experimental plate 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. We recommend that each treatment be performed at least in triplicate. If you are running a test compound curve to determine an EC_{50} value, several serial dilutions of the test compound should be included in the assay. The kit contains enough PGE_2 to run a control dose-response curve as well. Record the contents of each well on the template sheet provided on page 27.

Addition of Cells to the EP_4 Reverse Transfection Plate

Important

Before starting the experiment, dilute Penicillin-Streptomycin (100X, Gibco 15140-122) 1:100 in reduced serum medium used for your cells. This will be the culture medium for your experiment.

1. Remove the EP_4 Receptor (rat) Reverse Transfection Strip Plate (Item No. 600351) from the refrigerator or freezer and allow the plate to equilibrate to room temperature. Clean the bag with 70% alcohol before opening the bag. Place the plate in the hood and remove from the bag.

   NOTE: If you are not using the whole plate at one time for your 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, you can vacuum seal the bag and store the remaining strips at -20°C for up to two months.

2. Seed each well of the plate at a density of 50,000-80,000 cells/well in 200 µl of reduced serum medium containing 0.5% FBS. Place the plate in a 37°C incubator with 5% CO_2 and incubate overnight or up to 24 hours.

Cell Stimulation

1. After 16-24 hours of incubation, aspirate the culture media from each well.
2. Add 100 µl of reduced serum media containing IBMX (add 20 µl of the Cell-Based Assay IBMX Solution (1,000X), Item No. 10008978 to 10 ml of the culture media used for your cells) to each well.
3. Prepare test compounds at 2X the desired final concentration in serum-free media and pipette 100 µl to the assigned wells. Wells containing untreated cells receive 100 µl of serum-free media only. For positive controls using the provided PGE_2, dilute the EP Receptor Assay Prostaglandin E_2 Positive Control (Item No. 600341) 1:500 in the serum-free culture media and add 100 µl to corresponding wells. At this concentration, PGE_2 induces a 25-50 fold increase in cAMP levels, depending on the cell type and stimulation time used.
4. Incubate the cells in a cell culture incubator for 30 minutes.
5. Centrifuge the plate at 1,000 x rpm for 10 minutes.
6. Aspirate the supernatant.
7. Add 100 µl of Assay Buffer to each well and put the plate with the lid in a -80°C freezer. Freeze the sample in the -80°C for one to two hours.
8. Take the plate out from the -80°C freezer and leave it at room temperature to thaw completely (30-60 minutes).
9. Centrifuge the plate at 1,000 x rpm for 10 minutes. The supernatant is now ready for cAMP measurement following the protocol below.

PERFORMING cAMP ASSAY

NOTE: Water used to prepare all EIA reagents and buffers must be deionized and free of trace organic contaminants ('UltraPure'). Use activated carbon filter cartridges or other organic scavengers. Glass distilled water (even if double distilled), HPLC-grade water, and sterile water (for injections) are not adequate for EIA. UltraPure water may be purchased from Cayman (Item No. 400000).

Buffer Preparation

Store all diluted buffers at 4°C; they will be stable for about two months.

1. EIA Buffer Preparation

Dilute the contents of one vial of EIA Buffer Concentrate (10X) (Item No. 400060) with 90 ml of UltraPure water. Be certain to rinse the vial to remove any salts that may have precipitated. *NOTE: It is normal for the concentrated buffer to contain crystalline salts after thawing. These will completely dissolve upon dilution with water.*

2. Wash Buffer Preparation

Dilute the contents of the vial of Wash Buffer Concentrate (400X) (Item No. 400062) to a total volume of 2 liters with UltraPure water and add 1 ml of Polysorbate 20 (Item No. 400035). A smaller volume of Wash Buffer can be prepared by diluting the Wash Buffer Concentrate 1:400 and adding Polysorbate 20 (0.5 ml/L of Wash Buffer). The diluted buffer will be stable for two months at 4°C.

NOTE: Polysorbate 20 is a viscous liquid and cannot be measured by a pipette. A positive displacement device such as a syringe should be used to deliver small quantities accurately.

Preparation of Assay-Specific Reagents

cAMP EIA Standard

Reconstitute the cAMP EIA Standard (Item No. 481004) with 1 ml of EIA Buffer. The concentration of this solution will be 7,500 pmol/ml. Store this solution at 4°C; it will be stable for approximately six weeks. We have included enough cAMP to run ten standard curves. This surplus should accomodate any experimental design.

To prepare the standard for use in EIA: Obtain eight clean test tubes and number them #1 through #8. Aliquot 900 µl EIA Buffer to tube #1 and 600 µl EIA Buffer to tubes #2-8. Transfer 100 µl of the bulk standard (7,500 pmol/ml) to tube #1 and mix thoroughly. The concentration of this standard, the first point on the standard curve, is 750 pmol/ml. Serially dilute the standard by removing 300 µl from tube #1 and placing in tube #2; mix thoroughly. Next, remove 300 µl from tube #2 and place it into tube #3; mix thoroughly. Repeat this process for tubes #4-8. These diluted standards should not be stored for more than 24 hours.

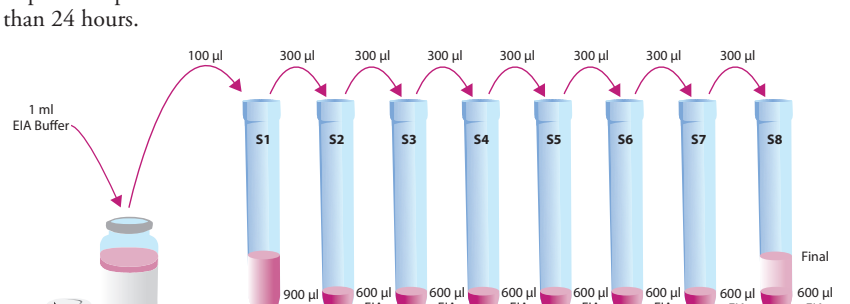


Figure 3. Preparation of the cAMP standards

cAMP AChE Tracer

Reconstitute the 100 dtn cAMP AChE Tracer (Item No. 481000) with 6 ml EIA Buffer. Store the reconstituted cAMP AChE Tracer at 4°C *(do not freeze!)* and use within one week. A 20% surplus of tracer has been included to account for any incidental losses.

cAMP EIA Antiserum

Reconstitute the 100 dtn cAMP EIA Antiserum (Item No. 481002) with 6 ml EIA Buffer. Store the reconstituted cAMP EIA Antiserum at 4°C. It will be stable for at least four weeks. A 20% surplus of antiserum has been included to account for any incidental losses.

EIA Plate Set Up

The 96-well plate(s) included with this kit is supplied ready to use. It is not necessary to rinse the plate(s) prior to adding the reagents. *NOTE: If you do not need to use all the strips at once, place the unused strips back in the plate packet and store at 4°C. Be sure the packet is sealed with the desiccant inside.*

Each plate or set of strips must contain a minimum of two blanks (Blk), two non-specific binding wells (NSB), two maximum binding wells (B_0), and an eight point standard curve run in duplicate. *NOTE: Each assay must contain this minimum configuration in order to ensure accurate and reproducible results.* Each sample should be assayed at two dilutions and each dilution should be assayed in duplicate. For statistical purposes, we recommend assaying samples in triplicate.

A suggested plate format is shown in Figure 4, below. The user may vary the location and type of wells present as necessary for each particular experiment. The plate format provided below has been designed to allow for easy data analysis using a convenient spreadsheet offered by Cayman (see page 18, for more details). We suggest you record the contents of each well on the template sheet provided (see page 27).

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blk	S1	S1	1	1	1	9	9	9	17	17	17
B	Blk	S2	S2	2	2	2	10	10	10	18	18	18
C	NSB	S3	S3	3	3	3	11	11	11	19	19	19
D	NSB	S4	S4	4	4	4	12	12	12	20	20	20
E	B_0	S5	S5	5	5	5	13	13	13	21	21	21
F	B_0	S6	S6	6	6	6	14	14	14	22	22	22
G	B_0	S7	S7	7	7	7	15	15	15	23	23	23
H	TA	S8	S8	8	8	8	16	16	16	24	24	24

Blk - Blank
TA - Total Activity
NSB - Non-Specific Binding
B_0 - Maximum Binding
S1-S8 - Standards 1-8
1-24 - Samples

Figure 4. Sample plate format

Performing the 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.

Addition of the Reagents

1. **EIA Buffer**

   Add 100 µl EIA Buffer to NSB wells. Add 50 µl EIA Buffer to B_0 wells.

2. **cAMP EIA Standard**

   Add 50 µl from tube #8 to both of the lowest standard wells (S8). Add 50 µl from tube #7 to each of the next two standard wells (S7). Continue with this procedure until all the standards are aliquoted. The same pipette tip should be used to aliquot all the standards. Before pipetting each standard, be sure to equilibrate the pipette tip in that standard.

3. **Samples**

   Transfer 50 µl of the supernatant from each well of the Reverse Transfection plate (prepared under Cell Stimulation Section) to a corresponding well in the Precoated (Mouse Anti-Rabbit IgG) EIA 96-Well Strip Plate.

4. **cAMP AChE Tracer**

   Add 50 µl to each well *except* the TA and the Blk wells.

5. **cAMP EIA Antiserum**

   Add 50 µl to each well *except* the TA, the NSB, and the Blk wells.

Well	EIA Buffer	Standard/ Sample	Tracer	Antiserum
Blk	-	-	-	-
TA	-	-	5 µl (at devl. step)	-
NSB	100 µl	-	50 µl	-
B_0	50 µl	-	50 µl	50 µl
Std/Sample	-	50 µl	50 µl	50 µl

Table 1. Pipetting summary

Incubation of the Plate

Cover each plate with a 96-Well Cover Sheet (Item No. 400012) and incubate 18 hours at 4°C.

Development of the Plate

1. Reconstitute Ellman's Reagent immediately before use (20 ml of reagent is sufficient to develop 100 wells):

   100 dtn vial Ellman's Reagent (Item No. 400050): Reconstitute with 20 ml of UltraPure water.

NOTE: Reconstituted Ellman's Reagent is unstable and should be used the same day it is prepared; protect the Ellman's Reagent from light when not in use. Extra vials of the reagent have been provided should a plate need to be re-developed or multiple assays be run on different days.

2. Empty the wells and rinse five times with Wash Buffer.
3. Add 200 µl of Ellman's Reagent to each well.
4. Add 5 µl of tracer to the TA wells.
5. Cover the plate with plastic film. Optimum development is obtained by using an orbital shaker equipped with a large, flat cover to allow the plate(s) to develop in the dark. This assay typically develops (*i.e.*, B_0 wells ≥0.3 A.U. (blank subtracted)) in 90-120 minutes.

Reading the Plate

1. Wipe the bottom of the plate with a clean tissue to remove fingerprints, dirt, etc.
2. Remove the plate cover being careful to keep Ellman's Reagent from splashing on the cover. *NOTE: Any loss of Ellman's Reagent will affect the absorbance readings. If Ellman's Reagent is present on the cover, use a pipette to transfer the Ellman's Reagent into the well. If too much Ellman's Reagent has splashed on the cover to easily redistribute back into the wells, wash the plate three times with wash buffer and repeat the development with fresh Ellman's Reagent.*
3. Read the plate at a wavelength between 405 and 420 nm. The absorbance may be checked periodically until the B_0 wells have reached a minimum of 0.3 A.U. (blank subtracted). The plate should be read when the absorbance of the B_0 wells are in the range of 0.3-1.0 A.U. (blank subtracted). If the absorbance of the wells exceeds 1.5, wash the plate, add fresh Ellman's Reagent and let it develop again.

ANALYSIS

Many plate readers come with data reduction software that plot data automatically. Alternatively a spreadsheet program can be used. The data should be plotted as $\%B/B_0$ *versus* log concentration using either a 4-parameter logistic or log-logit curve fit. *NOTE: Cayman has a computer spreadsheet available for data anaylsis. Please contact Technical Service or visit our website (www.caymanchem.com/analysis/eia) to obtain a free copy of this convenient data analysis tool.*

cAMP EIA Calculations

Preparation of the Data

The following procedure is recommended for preparation of the data prior to graphical analysis.

NOTE: If the plate reader has not subtracted the absorbance readings of the blank wells from the absorbance readings of the rest of the plate, be sure to do that now.

1. Average the absorbance readings from the NSB wells.
2. Average the absorbance readings from the B_0 wells.
3. Subtract the NSB average from the B_0 average. This is the corrected B_0 or corrected maximum binding.
4. Calculate the $\%B/B_0$ (% Sample or Standard Bound/Maximum Bound) for the remaining wells. To do this, subtract the average NSB absorbance from the S1 absorbance and divide by the corrected B_0 (from Step 3). Multiply by 100 to obtain $\%B/B_0$. Repeat for S2-S8 and all sample wells.

NOTE: The TA values are not used in the standard curve calculations. Rather, they are used as a diagnostic tool; the corrected B_0 divided by the actual TA (10X measured absorbance) will give the % Bound. This value should closely approximate the % Bound that can be calculated from the ***Sample Data*** *(see page 20). Erratic absorbance values and a low (or no) % Bound could indicate the presence of organic solvents in the buffer or other technical problems (see page 24 for* ***Troubleshooting****).*

Plot the Standard Curve

Plot $\%B/B_0$ for standards S1-S8 *versus* cAMP concentration using linear (y) and log (x) axes and perform a 4-parameter logistic fit.

Alternative Plot - The data can also be lineraized using a logit transformation. The equation for this conversion is shown below. *NOTE: Do not use $\%B/B_0$ in this calculation.*

$$\textbf{logit } (B/B_0) = \ln [B/B_0/(1 - B/B_0)]$$

Plot the data as logit (B/B_0) *versus* log concentrations and perform a linear regression fit.

Determine the Sample Concentration

Calculate the B/B_0 (or $\%B/B_0$) value for each sample. Determine the concentration of each sample using the equation obtained from the standard curve plot. *NOTE: Remember to account for any concentration or dilution of the sample prior to the addition to the well.* Samples with $\%B/B_0$ values greater than 80% or less than 20% should be re-assayed as they generally fall out of the linear range of the standard curve. A 20% or greater disparity between the apparent concentration of two different dilutions of the same sample indicates interference which could be eliminated by purification.

cAMP EIA Performance Characteristics

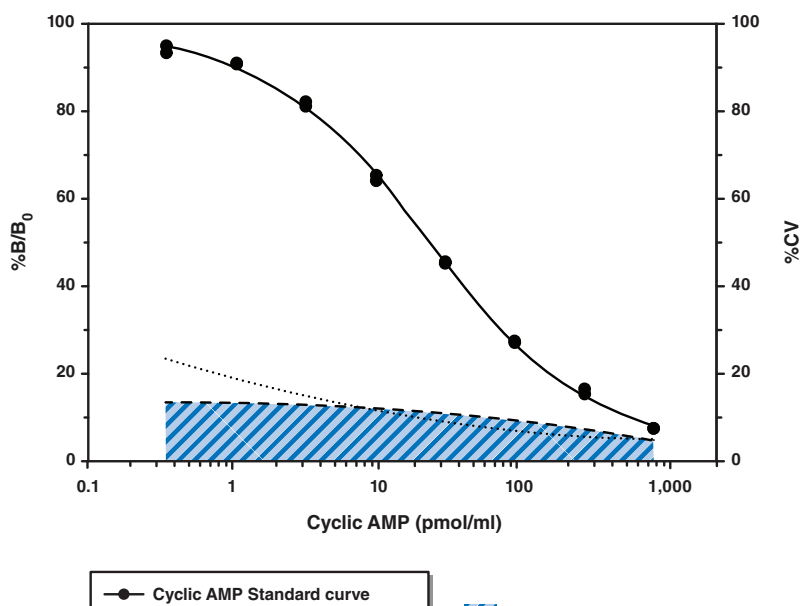
The standard curves presented here are examples of the data typically produced with this kit; however, your results will not be identical to these. You **must** run a new standard curve. Do not use the data below to determine the values of your samples. Your results could differ substantially.

Sample Data

	Raw Data		Average	Corrected
Total Activity	3.312	3.308	3.310	3.312
NSB	-0.003	-0.001	-0.002	
B_0	0.956	0.972		
	0.960	1.011	0.975	0.977

Dose (pmol/ml)	Raw Data		Corrected		%B/B_0	
750	0.053	0.054	0.055	0.056	5.7	5.7
250	0.148	0.135	0.150	0.137	15.4	14.1
83.3	0.256	0.255	0.258	0.257	26.5	26.4
27.8	0.435	0.436	0.437	0.438	44.8	45.0
9.3	0.621	0.632	0.623	0.634	63.9	65.1
3.1	0.792	0.801	0.794	0.803	81.4	82.4
1.0	0.888	0.887	0.890	0.889	91.3	91.2
0.3	0.927	0.913	0.929	0.915	95.3	93.9

Table 2. Typical results for the cAMP EIA



50% B/B_0 - 20.4 pmol/ml
Detection Limit (80% B/B_0) - 3.1 pmol/ml

Figure 5. Typical standard curve for the cAMP EIA

Precision:

The intra- and inter-assay CVs have been determined at multiple points on the standard curve. These data are summarized in the graph on page 21 and in the table below.

Dose (pmol/ml)	%CV* Intra-assay variation	%CV* Inter-assay variation
750	6.7	6.3
250	7.3	5.3
83.3	7.6	8.3
27.8	10.9	5.4
9.3	12.1	16.0
3.1	18.5	15.2
1.0	12.9	23.0
0.3	11.6	20.3

Table 3. Intra- and inter-assay variation of the cAMP assay.
*%CV represents the variation in concentration (not absorbance) as determined using a reference standard curve.

PGE_2 Concentration Response Curve

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. Normalize the results to run from 0% (untreated cells) to 100% (positive control) response. Graph % response *versus* log (drug concentration). In the resulting sigmodial dose-response curve find the best-fit values of the log EC_{50} (the concentration that gives a 50% response; the middle of the curve).

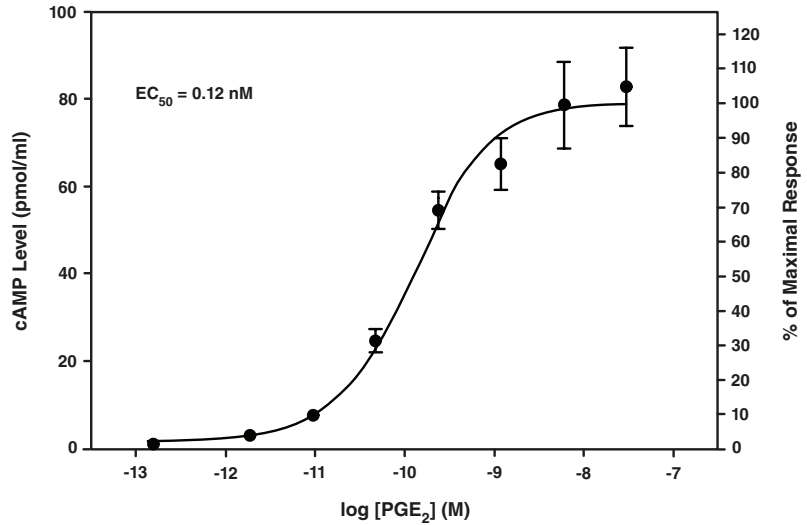


Figure 6. cAMP levels in HEK293T cells transiently-transfected with EP_4 in response to PGE_2 stimulation. HEK293T cells were plated in an EP_4 Reverse Transfection Plate at a density of 8 x 10^4 cells/well and incubated overnight. The next day, cells were treated with different doses of PGE_2. After 30 minutes of stimulation, the cells from each well were processed for cAMP measurement according to the protocol described above. The calculated EC_{50} from the fitted curve is 120 pM.

Troubleshooting

Problem	Possible Causes	Recommended Solutions
No signal	A. Contamination B. Cells lost during medium change	A. Keep cells in sterile environment B. Gently aspirate supernatant; do not disturb cell layer
Erratic response curve of compound treatments	Unequal number of cells in each well	Make sure each well contains the same number of cells
Erratic values; dispersion of duplicates	A. Trace organic contaminants in the water source B. Poor pipetting/technique	Replace activated carbon filter or change source of UltraPure water
High NSB (>0.035)	A. Poor washing B. Exposure of NSB wells to specific antibody	Rewash plate and redevelop
Very low B_0	A. Trace organic contaminants in the water source B. Plate requires additional development time C. Dilution error in preparing reagents	A. Replace activated carbon filter or change source of UltraPure water B. Return plate to shaker and re-read later
Low sensitivity (shift in dose response curve)	Standard is degraded	Replace standard
Only Total Activity (TA) wells develop	Trace organic contaminants in the water source	Replace activated carbon filter or change source of UltraPure water

Additional Reading

Go to **www.caymanchem.com/581001/references** for a list of publications citing the use of Cayman's Cyclic AMP EIA Kit.

References

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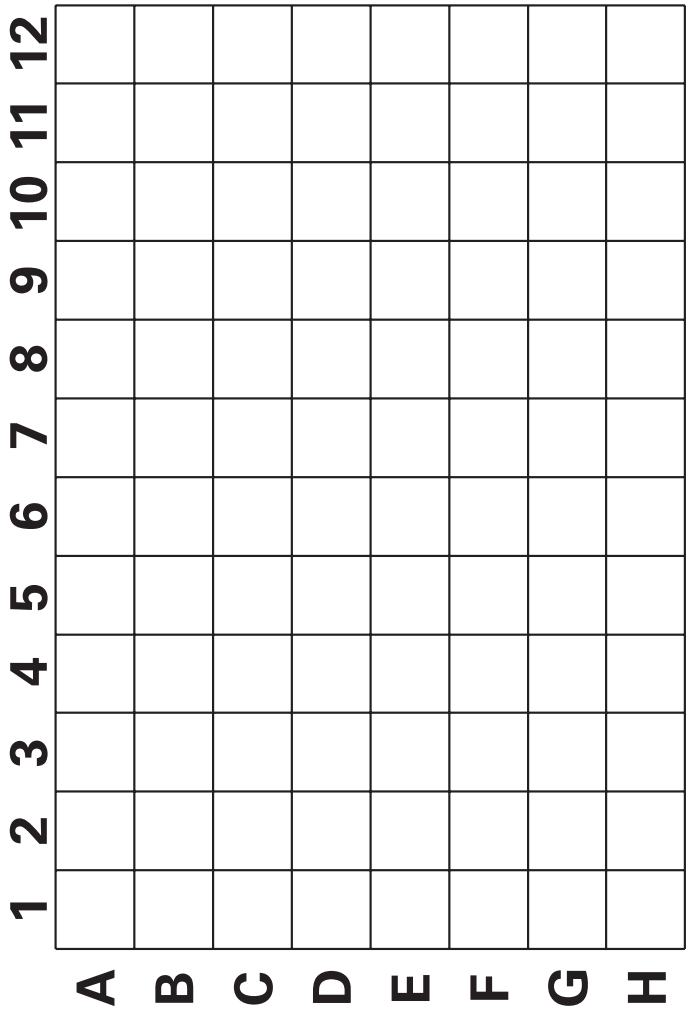

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