Prostaglandin E_2 Express EIA Kit - Monoclonal

Catalog No. 500141 (Strip Plate)
Catalog No. 500141.1 (Solid Plate)

ACE™

TABLE OF CONTENTS

Materials Supplied

Number	Item	96 wells Quantity/Size	480 wells Quantity/Size
1	Prostaglandin E_2 Express EIA Monoclonal Antibody	1 vial/100 dtn	1 vial/500 dtn
2	Prostaglandin E_2 Express AChE Tracer	1 vial/100 dtn	1 vial/500 dtn
3	Prostaglandin E_2 Express EIA Standard	1 vial	1 vial
4	EIA Buffer Concentrate (10X)	2 vials/10 ml	4 vials/10 ml
5	Wash Buffer Concentrate (400X)	1 vial/5 ml	1 vial/12.5 ml
5a	Tween 20	1 vial/3 ml	1 vial/3 ml
6	Goat Anti-Mouse IgG Coated Plate	1 plate	5 plates
7	Plate Cover	1 cover	5 covers
8	Ellman's Reagent	3 vials/100 dtn	6 vials/250 dtn
9	EIA Tracer Dye	1 vial	1 vial
10	EIA Antiserum Dye	1 vial	1 vial

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 975-3999. We cannot accept any returns without prior authorization.

WARNING: Not for human or animal disease diagnosis or therapeutic drug use.

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 ACE™ EIA Kits. This kit may not perform as described if any reagent or procedure is replaced or modified.

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

E-Mail: 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

This kit will perform as specified if stored as directed at -20°C and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader capable of measuring absorbance between 405-420 nm.
2. Adjustable pipettes and a repeat pipettor.
3. 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 (Catalog No. 400000).*
4. Materials used for **Collection and Storage** (see page 12).

INTRODUCTION

Biochemistry of Prostaglandin E_2

Prostaglandin E_2 (PGE_2) is a primary product of arachidonic acid metabolism in many cells. Like most eicosanoids, it does not exist preformed in any cellular reservoir. When cells are activated or exogenous free arachidonate is supplied, PGE_2 is synthesized *de novo* and released into the extracellular space. *In vivo*, PGE_2 is rapidly converted to an inactive metabolite (13,14-dihydro-15-keto PGE_2) by the PG 15-dehydrogenase pathway.[1,2] (see Figure 1, page 7) The half-life of PGE_2 in the circulatory system is approximately 30 seconds and normal plasma levels are 3-12 pg/ml.[3]

About This Assay

Cayman's PGE_2 Express EIA has been validated for use with urine, plasma, and culture media samples. In general, urine and culture media samples can be diluted, if necessary, and added directly to the assay well. Plasma samples should be purified prior to use. Because of the rapid metabolism of PGE_2, the determination of *in vivo* PGE_2 biosynthesis is often best accomplished by the measurement of PGE_2 metabolites. Our PGE Metabolite assay (Catalog No. 514531) converts all major PGE_2 metabolites into a single stable derivative which is easily measurable by EIA (see Figure 1, page 7).Proper sample handling and preparation is the most important aspect of this assay. *NOTE: Please read the section of this booklet on sample preparation carefully before beginning.*

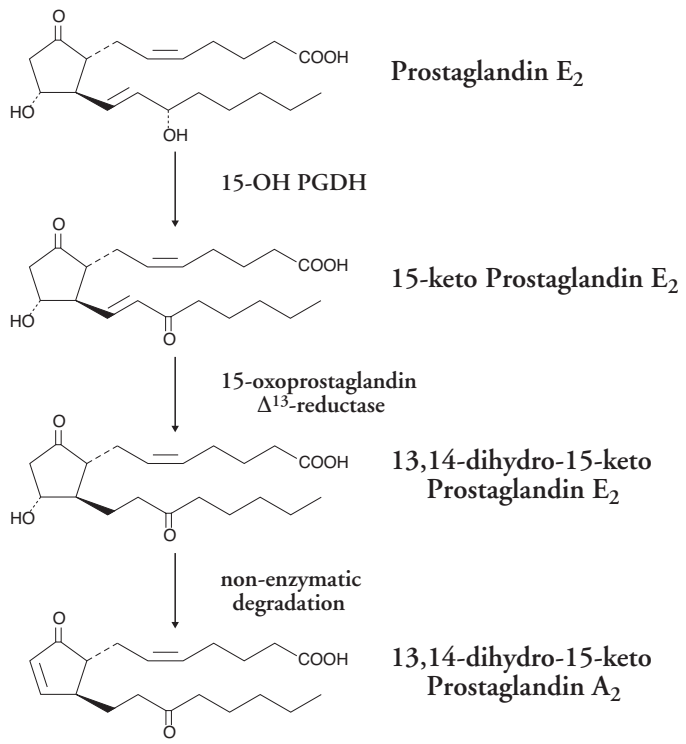


Figure 1. Metabolism of PGE_2

Description of ACE™ Competitive EIAs[4,5]

This assay is based on the competition between PGE_2 and a PGE_2-acetylcholinesterase (AChE) conjugate (PGE_2 tracer) for a limited amount of PGE_2 monoclonal antibody. Because the concentration of the PGE_2 tracer is held constant while the concentration of PGE_2 varies, the amount of PGE_2 tracer that is able to bind to the PGE_2 monoclonal antibody will be inversely proportional to the concentration of PGE_2 in the well. This antibody-PGE_2 complex binds to goat polyclonal anti-mouse 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 PGE_2 tracer bound to the well, which is inversely proportional to the amount of free PGE_2 present in the well during the incubation; or

$$\textbf{Absorbance} \propto [\textbf{Bound } PGE_2 \textbf{ Tracer}] \propto 1/[PGE_2]$$

A schematic of this process is shown in Figure 2, below.

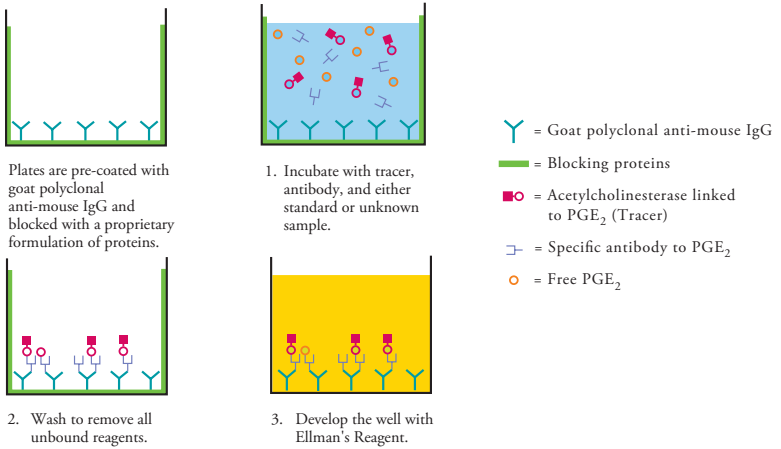


Figure 2. Schematic of the ACE™ EIA

Biochemistry of Acetylcholinesterase

The electric organ of the electric eel, *Electrophorus electricus*, contains an avid acetylcholinesterase (AChE) capable of massive catalytic turnover during the generation of its electrochemical discharges. The electric eel AChE has a clover leaf-shaped tertiary structure consisting of a triad of tetramers attached to a collagen-like structural fibril. This stable enzyme is capable of high turnover (64,000 s^{-1}) for the hydrolysis of acetylthiocholine.

A molecule of the analyte covalently attached to a molecule of AChE serves as the tracer in ACE™ enzyme immunoassays. Quantification of the tracer is achieved by measuring its AChE activity with Ellman's Reagent. This reagent consists of acetylthiocholine and 5,5'-dithio-*bis*-(2-nitrobenzoic acid). Hydrolysis of acetylthiocholine by AChE produces thiocholine (see Figure 3, on page 10). The non-enzymatic reaction of thiocholine with 5,5'-dithio-*bis*-(2-nitrobenzoic acid) produces 5-thio-2-nitrobenzoic acid, which has a strong absorbance at 412 nm (ε = 13,600).

AChE has several advantages over other enzymes commonly used for enzyme immunoassays. Unlike horseradish peroxidase, AChE does not self-inactivate during turnover. This property of AChE also allows redevelopment of the assay if it is accidentally splashed or spilled. In addition, the enzyme is highly stable under the assay conditions, has a wide pH range (pH 5-10), and is not inhibited by common buffer salts or preservatives. Since AChE is stable during the development step, it is unnecessary to use a 'stop' reagent, and the plate may be read whenever it is convenient.

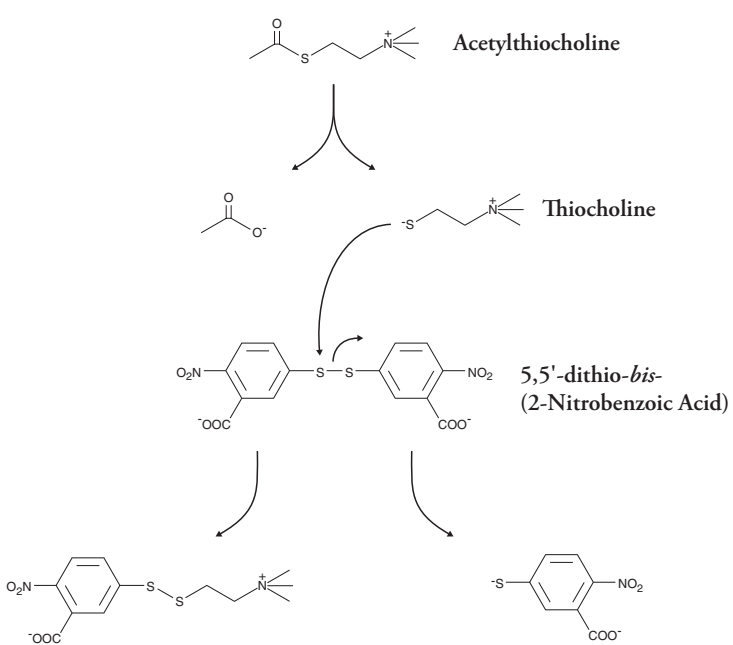


Figure 3. Reaction catalyzed by acetylcholinesterase

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.

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.

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.

PRE-ASSAY PREPARATION

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 (Catalog No. 400000).

Buffer Preparation

(Store all 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 (vial #4) 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

5 ml vial Wash Buffer (96-well kit vial #5): Dilute to a total volume of 2 liters with UltraPure water and add 1 ml of Tween 20 (vial #5a)

OR

12.5 ml vial Wash Buffer (480-well kit vial #5): Dilute to a total volume of 5 liters with UltraPure water and add 2.5 ml of Tween 20 (vial #5a).

Smaller volumes of Wash Buffer can be prepared by diluting the Wash Buffer Concentrate 1:400 and adding Tween 20 (0.5 ml/liter of Wash Buffer).

NOTE: Tween 20 is a viscous liquid and cannot be measured by a regular pipette. A positive displacement pipette or a syringe should be used to deliver small quantities accurately.

Sample Collection and Storage

This assay has been validated for a wide range of samples including urine (diluted at least 1:2), plasma (diluted at least 1:50), and tissue culture media. Proper sample storage and preparation are essential for consistent and accurate results. Please read this section thoroughly before beginning the assay.

General Precautions

- All samples must be free of organic solvents prior to assay.
- Samples should be assayed immediately after collection; samples that cannot be assayed immediately should be stored at -80°C.

Urine

Since interference in urine is infrequent, dilutions of 1:2 and greater show a direct linear correlation between PGE_2 immunoreactivity and PGE_2 concentration. However, the amount of PGE_2 in normal urine is very low in comparison with other potentially immunoreactive metabolites.[2] A more accurate index of PGE_2 biosynthesis and excretion can be obtained using our Prostaglandin E Metabolite Assay (Catalog No. 514531).

Plasma

Collect blood in vacutainers containing heparin, EDTA, or sodium citrate. Indomethacin should be added immediately after whole blood collection (sufficient to give a 10 µM final concentration). Indomethacin will prevent *ex vivo* formation of eicosanoids, which have the potential to interfere with this assay (although most eicosanoids do not appear to exhibit any cross-reactivity (see page 31)).

The amount of PGE_2 in normal plasma is very low in comparison with other potentially immunoreactive metabolites.[2] In addition, plasma is a complex matrix that contains many substances that can interfere with this assay and, therefore, sample purification is recommended. By purifying a large volume of sample (5-10 ml), the PGE_2 content can be concentrated into as little as 0.5 ml of EIA Buffer. This will bring the PGE_2 concentration into the readable range of the standard curve. A more accurate index of PGE_2 biosynthesis in plasma can be obtained using our Prostaglandin E Metabolite Assay Kit (Catalog No. 514531).

Culture Media Samples

Cell culture supernatants may be assayed directly without purification. If the PGE_2 concentration in the medium is high enough to dilute the sample 10-fold with EIA Buffer, the assay can be performed without any modification. When assaying less concentrated samples (where samples cannot be diluted with EIA Buffer), dilute the standard curve in the same culture medium as that used in the experiment. This will ensure that the matrix for the standards is comparable to the samples. We recommend that a standard curve be run first to ensure that the assay will perform in a particular culture medium.

Tissue Samples

1. Add 5 ml homogenization buffer (0.1 M phosphate, pH 7.4, containing 1 mM EDTA and 10 µM indomethacin) to 1 g of tissue. Homogenize the sample with either a Polytron-type homogenizer or a sonicator.
2. Add a hot or cold spike of PGE_2 as described in the purification protocols below.
3. Add acetone (2-4X the sample volume) to the sample and vortex. Leave the sample at room temperature for 5 minutes.
4. Remove the precipitate by centrifugation at 1,500 x g for 10 minutes. Carefully transfer the supernatant to a clean test tube.
5. Remove the acetone by vacuum centrifugation or using a gentle stream of nitrogen. Proceed to step four of the purification protocol using the hot or cold spike on page 17.

Sample Purification

Samples containing PGE_2 can be purified using two basic methods. The easiest method is affinity purification using Cayman's PGE_2 Affinity Sorbent, Column, or Kit (Catalog Nos. 414020, 414018, and 514018, respectively). Specific literature describing use of these products is available from Cayman Chemical at **www.caymanchem.com.** If you choose to use the PGE_2 Affinity Sorbent or Column, you will need to prepare Eicosanoid Affinity Column Buffer (0.1 M potassium phosphate, pH 7.4, containing 0.5 M sodium chloride, and 0.05% sodium azide) and Eicosanoid Affinity Column Elution Solution (95% EtOH). These solutions are included in the PGE_2 Affinity Purification Kit. The second method for purification is by solid phase extraction (SPE) as described in the protocol below.

Testing for Interference

Plasma, serum, as well as other heterogeneous mixtures such as CSF often contain contaminants which can interfere in the assay. It is best to check for interference to evaluate the need for sample purification before embarking on a large number of sample measurements. To test for interference, dilute one or two test samples to obtain at least two different dilutions of each sample between 15 and 250 pg/ml (i.e., between 20-80% B/B_0). If the two different dilutions of the sample show good correlation (differ by 20% or less) in the final calculated PGE_2 concentration, purification is not required. If you do not see good correlation of the different dilutions, purification is advised.

Determination of Recovery

Determination of percent recovery is recommended when any sample purification is performed. Detailed below are two methods that can be employed to monitor the recovery. If the **hot spike method** (recommended) is used, 10,000 cpm of tritium-labeled PGE_2 is added directly to the sample and 10% is removed for scintillation counting after purification. If the **cold spike method** is used, the sample must be split prior to purification and an appropriate amount of PGE_2 added to one aliquot. The spiked sample is then assayed *via* EIA alongside the unspiked sample. Calculations for each method are found in the **Analysis** section on page 25.

SPE (C-18) Purification Protocol

Materials Needed

1. Tritium-labeled PGE_2 to use as a '**hot spike**' or unlabeled PGE_2 Standard to use as a '**cold spike**' to allow determination of extraction efficiency.
2. 2 M hydrochloric acid, UltraPure water, ethanol, methanol, hexane and ethyl acetate
3. 200 mg C-18 solid phase extraction (SPE) columns (non end-capped)

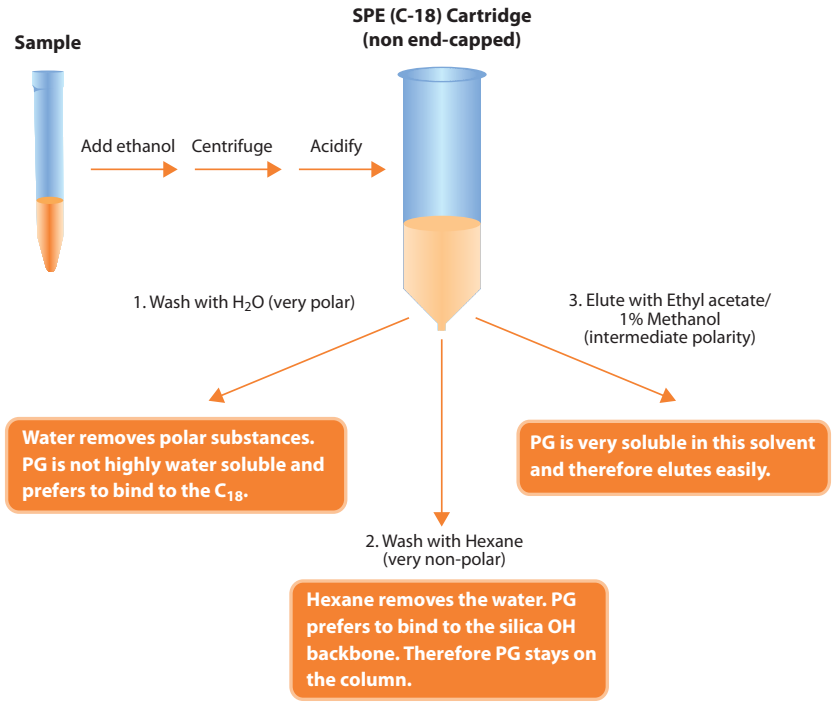


Figure 4. Schematic of PGE_2 Purification by SPE (C-18)

Hot Spike

1. Aliquot a known amount of each sample into a clean test tube (500 µl is recommended). If your samples need to be concentrated, a larger volume should be used (e.g., a 5 ml sample will be concentrated by a factor of 10, a 10 ml sample will be concentrated by a factor of 20, etc.).
2. Add 10,000 cpm of tritium-labeled PGE_2 ($[^3H]$-PGE_2). Use a high specific activity tracer to minimize the amount of radioactive PGE_2 as the EIA will be able to detect the added PGE_2.

Cold Spike

1. Aliquot a known amount of each sample into each of two tubes. Label one 'sample #' and the other 'sample # + spike'. If your samples need to be concentrated, a larger volume should be used (e.g., a 5 ml sample will be concentrated by a factor of 10, a 10 ml sample will be concentrated by a factor of 20, etc.).
2. Add a cold spike of PGE_2 to the 'sample + spike' tubes. Follow the procedure below for both spiked and unspiked samples.

Proceed to step 3 below

3. ***Precipitation of proteins using ethanol is optional and may not be needed if samples are clean enough to flow through the SPE (C-18) cartridge. Body fluids such as plasma and urine can typically be applied directly to the SPE (C-18) cartridge after the acidification step (step 4) below.*** To precipitate proteins, add ethanol (approximately four times the sample volume) to each tube. Vortex to mix thoroughly. Incubate samples at 4°C for five minutes, then centrifuge a 3,000 x g for 10 minutes to remove precipitated proteins. Transfer the supernatant to a clean test tube. Evaporate the ethanol under nitrogen.
4. Acidify the sample to ~pH 3.5 by the addition of 1 M formic acid. (Standardize the pH adjustment using the sample matrix prior to proceeding with a large number of samples). If the samples are cloudy or contain precipitate, either filter or centrifuge to remove the precipitate. Particulate matter in the sample may clog the SPE cartridge.

5. Prepare SPE (C-18) columns by rinsing with 5 ml methanol followed by 5 ml deionized water. Do not allow the SPE cartridge to dry.
6. Apply the sample to the SPE cartridge and allow the sample to completely enter the packing material.
7. Wash the column with 5 ml deionized water followed by 5 ml HPLC grade hexane (allow the cartridge to become dry after this step). Discard both washes.
8. Elute the PGE_2 from the column with 5 ml ethyl acetate containing 1% methanol. Higher recovery and better reproducibility may be obtained if the sample is applied and eluted by gravity. The wash steps may be performed under vacuum or pressure.
9. Evaporate the ethyl acetate to dryness under a stream of nitrogen. It is very important that all of the organic solvent be removed as even small quantities will adversely affect the EIA.
10. To resuspend the sample, add 500 µl EIA Buffer. Vortex. It is common for insoluble precipitate to remain in the sample after addition of EIA Buffer; this will not affect the assay. This sample is now ready for use in the EIA.

Hot Spike (continued)

11. Use 50 µl of the resuspended sample for scintillation counting.

ASSAY PROTOCOL

Preparation of Assay Specific Reagents

PGE_2 Express EIA Standard

Reconstitute the contents of the PGE_2 Express EIA Standard with 1.0 ml of EIA Buffer. The concentration of this solution (the bulk standard) will be 10 ng/ml. Stored at 4°C; this standard will be stable for up to four weeks.

NOTE: If assaying culture medium samples that have not been diluted with EIA Buffer, culture medium should be used in place of EIA Buffer for dilution of the standard curve.

To prepare the standard for use in EIA: Obtain 8 clean test tubes and number them #1 through #8. Aliquot 800 µl EIA Buffer to tube #1 and 500 µl EIA Buffer to tubes #2-8. Transfer 200 µl of the bulk standard (10 ng/ml) to tube #1 and mix thoroughly. The concentration of this standard, the first point on the standard curve, will be 2 ng/ml (2,000 pg/ml). Serially dilute the standard by removing 500 µl from tube #1 and placing in tube #2; mix thoroughly. Next, remove 500 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.

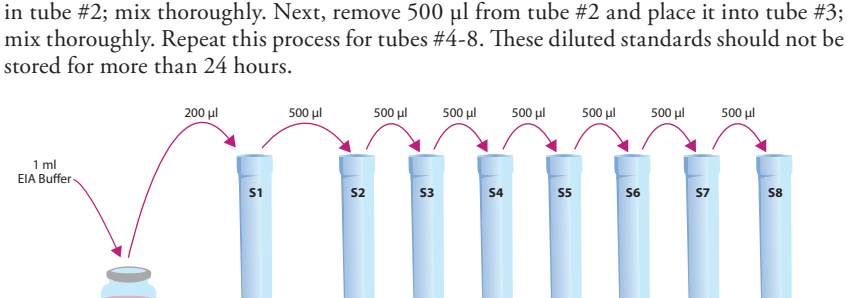


Figure 5. Preparation of the PGE_2 standards

Prostaglandin E_2 Express AChE Tracer

Reconstitute the PGE_2 Tracer as follows:

100 dtn PGE_2 AChE Tracer (96-well kit vial #2): Reconstitute with 6 ml EIA Buffer.

OR

500 dtn PGE_2 AChE Tracer (480-well kit vial #2): Reconstitute with 30 ml EIA Buffer.

Store the reconstituted PGE_2 Tracer at 4°C *(do not freeze!)* and use within four weeks. A 20% surplus of PGE_2 Tracer has been included to account for any incidental losses.

Tracer Dye Instructions (optional)

This dye may be added to the tracer, if desired, to aid in visualization of tracer-containing wells. Add the dye to the reconstituted tracer at a final dilution of 1:100 (add 60 µl of dye to 6 ml tracer or add 300 µl of dye to 30 ml of tracer).

Prostaglandin E_2 Express Monoclonal Antibody

Reconstitute the PGE_2 Monoclonal Antibody as follows:

100 dtn PGE_2 Antibody (96-well kit vial #1): Reconstitute with 6 ml EIA Buffer.

OR

500 dtn PGE_2 Antibody (480-well kit vial #1): Reconstitute with 30 ml EIA Buffer.

Store the reconstituted PGE_2 Antibody at 4°C. It will be stable for at least four weeks. A 20% surplus of PGE_2 Antibody has been included to account for any incidental losses.

Antiserum Dye Instructions (optional)

This dye may be added to the antiserum, if desired, to aid in visualization of antiserum-containing wells. Add the dye to the reconstituted antiserum at a final dilution of 1:100 (add 60 µl of dye to 6 ml antiserum or add 300 µl of dye to 30 ml of antiserum).

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 2-4°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 6, 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 25, for more details). We suggest you record the contents of each well on the template sheet provided (see page 35).

	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 6. Sample plate format

Performing the Assay

Pipetting Hints

- Use different tips to pipette the buffer, standard, sample, tracer, and antibody.
- 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 Non-Specific Binding (NSB) wells. Add 50 µl EIA Buffer to Maximum Binding (B_0) wells. If culture medium was used to dilute the standard curve, substitute 50 µl of culture medium for EIA Buffer in the NSB and B_0 wells (i.e., add 50 µl culture medium to NSB and B_0 wells and 50 µl EIA Buffer to NSB wells).

2. Prostaglandin E_2 Express 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

Add 50 µl of sample per well. Each sample should be assayed at a minimum of two dilutions. Each dilution should be assayed in duplicate (triplicate recommended).

4. Prostaglandin E_2 Express AChE Tracer

Add 50 µl to each well *except* the Total Activity (TA) and the Blank (Blk) wells.

5. Prostaglandin E_2 Express Monoclonal Antibody

Add 50 µl to each well *except* the Total Activity (TA), the Non-Specific Binding (NSB), and the Blank (Blk) wells.

Well	EIA Buffer	Standard/ Sample	Tracer	Antibody
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

Incubate the Plate

Cover each plate with plastic film (item #7) and incubate 60 minutes at room temperature on an orbital shaker.

Develop 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 (96-well kit vial #8): Reconstitute with 20 ml of UltraPure water.

   OR

   250 dtn vial Ellman's Reagent (480-well kit vial #8): Reconstitute with 50 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 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 Total Activity 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 60-90 minutes.

Read 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/eiatools/promo/kit) to obtain a free copy of this convenient data analysis tool.*

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 total activity (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 28). 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 32 for* ***Troubleshooting****).*

Plot the Standard Curve

Plot %B/B_0 for standards S1-S8 *versus* PGE_2 concentration using linear (y) and log (x) axis and fit the data to a 4-parameter logistic equation.

Alternative Plot - The data can also be lineraized using a logit transformation. The equation for this conversion is as follows, *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 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 would indicate interference which could be eliminated by purification.

Hot Spike Method

$$\text{Recovery Factor} = \frac{10 \times \text{cpm of sample}}{[^3\text{H}]\text{-PGE}_2 \text{ added to sample (cpm)}}$$

$$\text{PGE}_2 \text{ (pg) in purified sample} = \left[\frac{\text{Value from EIA (pg/ml)}}{\text{Recovery Factor}}\right] \times 0.5 \text{ ml} - \text{added } [^3\text{H}]\text{-PGE}_2 \text{ (pg)}$$

$$\text{Total PGE}_2 \text{ in sample (pg/ml)} = \frac{\text{PGE}_2 \text{ (pg) in purified sample}}{\text{Volume of sample used for purification (ml)}}$$

Cold Spike Method

The original concentration of the sample and recovery factor can be determined by the following method:

V = EIA determined concentration of the unspiked sample (pg/ml)

S = concentration of the spike (pg/ml)

Y = EIA determined concentration of the spiked sample (pg/ml)

$$\text{Purification Recovery Factor} = \left[\frac{Y - V}{S}\right]$$

$$\text{PGE}_2 \text{ (pg) in purified sample} = \left[\frac{V}{\text{Recovery Factor}}\right] \times 0.5 \text{ ml}$$

$$\text{PGE}_2 \text{ in original sample (pg/ml)} = \frac{\text{PGE}_2 \text{ (pg) in purified sample}}{\text{Volume of sample used for purification (ml)}}$$

Performance Characteristics

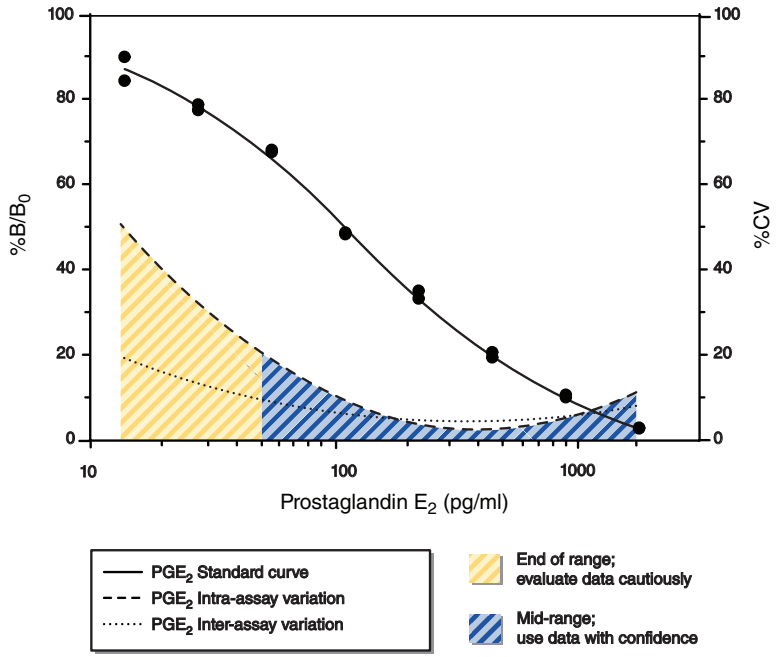
Sample Data

The standard curve presented here is an example 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 this one to determine the values of your samples. Depending on the development conditions and the purity of the water used, your results could differ substantially from the data presented below.

	Raw Data		Average	Corrected
Total Activity	1.100	1.200	1.150	
NSB	0.004	0.003	0.004	
B_0	0.670	0.694		
	0.652	0.708	0.681	0.677

Dose (pg/ml)	Raw Data		Corrected		%B/B_0	
2,000	0.028	0.027	0.024	0.023	3.5	3.4
1,000	0.076	0.080	0.072	0.076	10.6	11.2
500	0.139	0.148	0.135	0.144	19.9	21.3
250	0.233	0.245	0.229	0.241	33.8	35.6
125	0.337	0.335	0.333	0.331	49.2	48.9
62.5	0.465	0.468	0.461	0.464	68.1	68.5
31.3	0.532	0.540	0.528	0.536	78.0	79.2
15.6	0.578	0.616	0.574	0.612	84.8	90.4

Table 2. Typical results



50% B/B_0 - 125 pg/ml
Detection Limit (80% B/B_0) - 36 pg/ml

Figure 7. Typical standard curve

Precision:

The intra- and interassay CV's have been determined at multiple points on the standard curve. These data are summarized in the graph on below.

Dose (pg/ml)	%CV* Intra-assay variation	%CV* Inter-assay variation
2,000	10.6	9.2
1,000	6.1	6.5
500	4.6	4.7
250	7.1	4.0
125	9.4	7.5
62.5	19.5	14.7
31.3	27.1	8.9
15.6	55.3	21.2

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

Specificity:

Compound	Cross-reactivity	Compound	Cross-reactivity
PGE_2	100%	PGA_2	<0.01%
PGE_2 Ethanolamide	100%	PGA_3	<0.01%
PGE_3	43%	PGB_1	<0.01%
8-*iso* PGE_2	37.4%	PGB_2	<0.01%
PGE_1	18.7%	PGD_2	<0.01%
Sulprostone	1.25%	11-deoxy PGE_2	<0.01%
6-keto $PGF_{1\alpha}$	1%	16,16-dimethyl PGE_2	<0.01%
8-*iso* $PGF_{2\alpha}$	0.25%	20-hydroxy PGE_2	<0.01%
13,14-dihydro-15-keto PGE_2	0.02%	15-keto PGE_2	<0.01%
Arachidonic Acid	<0.01%	19(R)-hydroxy PGE_2	<0.01%
Arachidonoyl Ethanolamide	<0.01%	tetranor-PGEM	<0.01%
O-Arachidonoyl Ethanolamide	<0.01%	$PGF_{1\alpha}$	<0.01%
Butaprost	<0.01%	$PGF_{2\alpha}$	<0.01%
8(S),15(S)-DiHETE	<0.01%	13,14-dihydro-15-keto $PGF_{2\alpha}$	<0.01%
Leukotriene B_4	<0.01%	$PGF_{3\alpha}$	<0.01%
Conjugated Linoleic Acid (10E,12Z)	<0.01%	tetranor-PGFM	<0.01%
Misoprostol	<0.01%	PGJ_2	<0.01%
Misoprostol (free acid)	<0.01%	15-deoxy-$\Delta^{12,14}$-PGJ_2	<0.01%
PGA_1	<0.01%	Thromboxane B_2	<0.01%

Table 4. Specificity of the PGE_2 Monoclonal Antibody

RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Eratic values; dispersion of duplicates	A. Trace organic contaminants in the water source B. Poor pipetting/technique	A. Replace activated carbon filter or change source of deionized water
High NSB (>0.035)	A. Poor washing B. Exposure of NSB wells to specific antibody	A. Rewash plate and redevelop
Very low B_0	A. Contamination of water with organic solvents B. Plate requires additional development time C. Dilution error in preparing reagents	A. Replace activated carbon filter or change source of deionized water B. Return plate to shaker and reread later
Low sensitivity (shift in dose response curve)	Standard is degraded	Replace standard
Analyses of two dilutions of a biological sample do not agree (i.e., more than 20% difference)	Interfering substances are present	Purify sample prior to analysis by EIA[6]
Only Total Activity (TA) wells develop	Trace organic contaminants in the water source	Replace activated carbon filter or change source of deionized water

Additional Reading

For a list of publications citing the use of Cayman Chemical's PGE_2 EIA Kit go to **www.caymanchem.com/500141/references**.

References

1. Granström, E., Hamberg, M., Hansson, G., *et al.* Chemical instability of 15-keto-13,14-dihydro-PGE_2: The reason for low assay reliability. *Prostaglandins* **19**, 933-945 (1980).
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3. Fitzpatrick, F.A., Aguirre, R., Pike J.E., *et al.* The stability of 13,14-dihydro-15-keto-PGE_2. *Prostaglandins* **19**, 917-931 (1980).
4. Pradelles, P., Grassi, J., and Maclouf, J.A. Enzyme immunoassays of eicosanoids using acetylcholinesterase as label: An alternative to radioimmunoassay. *Anal. Chem.* **57**, 1170-1173 (1985).
5. Maclouf, J., Grassi, J., and Pradelles, P. Development of enzyme-immunoassay techniques for the measurement of eicosanoids, Chapter 5, in *Prostaglandin and Lipid Metabolism in Radiation Injury.* Walden, T.L., Jr. and Hughes, H.N., editors, Plenum Press, Rockville, 355-364 (1987).
6. Maxey, K.M., Maddipati, K.R. and Birkmeier, J. Interference in enzyme immunoassays. *J. Clin. Immunoassay* **15**, 116-120 (1992).

Related Products

Prostaglandin E Metabolite EIA Kit - Cat. No. 5145311
Prostaglandin E Metabolite EIA Kit (Solid Plate) - Cat. No. 514531.1
Prostaglandin E_2 - Cat. No. 14010
Prostaglandin E_2 Affinity Column - Cat. No. 414018
Prostaglandin E_2 Affinity Purification Kit - Cat. No. 514018
Prostaglandin E_2 Affinity Sorbent - Cat. No. 414020
Prostaglandin E_2 EIA Kit - Monoclonal (Solid Plate) - Cat. No. 514010.1
Prostaglandin E_2 Express EIA Kit - Cat. No. 500141
Prostaglandin E_2 Express EIA Kit (Solid Plate) - Cat. No. 500141.1
Prostaglandin E_2 FPIA Kit - Green - Cat. No. 500501
Prostaglandin E_2 FPIA Kit - Red - Cat. No. 10004517
Prostaglandin Screening EIA Kit - Cat. No. 514012
Prostaglandin Screening EIA Kit (Solid Plate) - Cat. No. 514012.1
SPE Cartridges (C-18) - Cat. No. 400020
UltraPure Water - Cat. No. 400000

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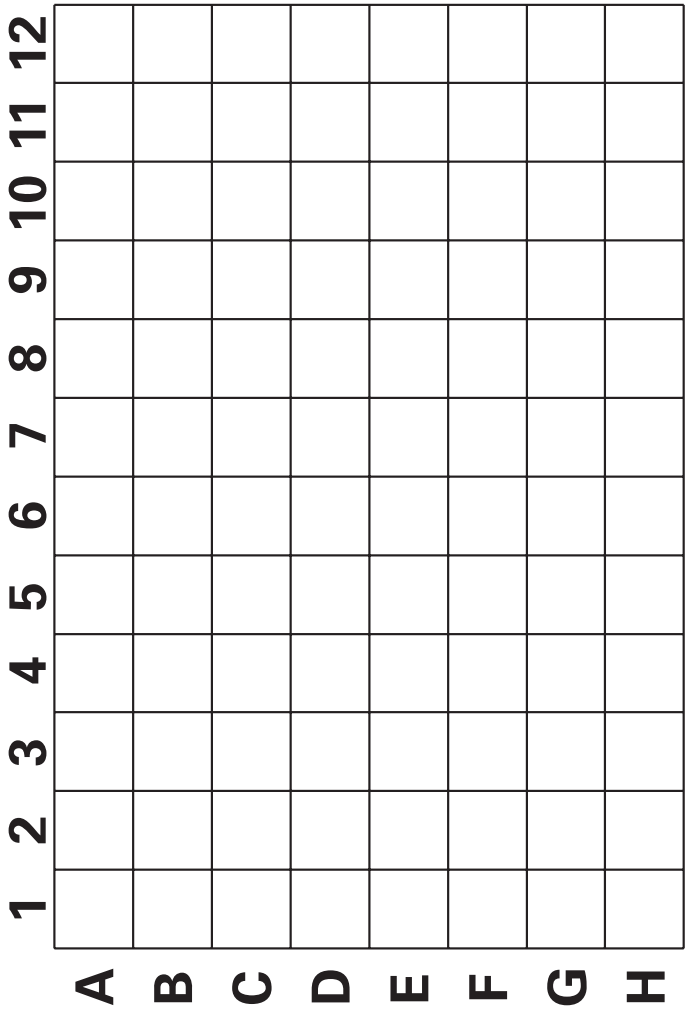
Cayman Chemical Company makes **no warranty or guarantee** of any kind, whether written or oral, expressed or implied, including without limitation, any warranty of fitness for a particular purpose, suitability and merchantability, which extends beyond the description of the chemicals hereof. Cayman **warrants only** to the original customer that the material will meet our specifications at the time of delivery. Cayman will carry out its delivery obligations with due care and skill. Thus, in no event will Cayman have **any obligation or liability**, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if Cayman is informed about their possible existence. This limitation of liability does not apply in the case of intentional acts or negligence of Cayman, its directors or its employees.

Buyer's **exclusive remedy** and Cayman's sole liability hereunder shall be limited to a refund of the purchase price, or at Cayman's option, the replacement, at no cost to Buyer, of all material that does not meet our specifications.

Said refund or replacement is conditioned on Buyer giving written notice to Cayman within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

For further details, please refer to our Warranty and Limitation of Remedy located on our website and in our catalog.



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