

Prostaglandin D₂ EIA Kit

Catalog No. 512021 (Strip Plate)

Catalog No. 10006693 (Solid Plate)



ACE

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GENERAL INFORMATION

Materials Supplied

Kit will arrive packaged as a -80°C kit, for best results, remove components and store as stated below.

Catalog Number	Item	96 wells Quantity/Size	480 wells Quantity/Size	Storage
10006621	Prostaglandin D ₂ Monoclonal Antibody	1 vial/100 dtn	1 vial/500 dtn	-20°C
10006622	Prostaglandin D ₂ AChE Tracer	1 vial/100 dtn	1 vial/500 dtn	-20°C
10006623	Prostaglandin D ₂ EIA Standard	1 vial	1 vial	-80°C
400060	EIA Buffer Concentrate (10X)	2 vials/10 ml	4 vials/10 ml	RT
400062	Wash Buffer Concentrate (400X)	1 vial/5 ml	1 vial/12.5 ml	RT
400035	Tween 20	1 vial/3 ml	1 vial/3 ml	RT
400009	Goat Anti-Mouse IgG Coated Plate	1 plate	5 plates	4°C
400012	96-Well Cover Sheet	1 cover	5 covers	RT
400050	Ellman's Reagent	3 vials/100 dtn	6 vials/250 dtn	-20°C
400040	EIA Tracer Dye	1 vial	1 vial	RT
400042	EIA Antiserum Dye	1 vial	1 vial	RT

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: 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.

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 -80°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 **Sample Preparation** (see page 11).

INTRODUCTION

Background

Prostaglandin D₂ (PGD₂) is biosynthesized in the brain by a soluble, 26 kDa glutathione-independent lipocalin-type PGD₂ synthase.¹ This PGD₂ accumulates in the cerebrospinal fluid (CSF), where it induces physiologic sleep in rats and humans.² PGD₂ is also synthesized in mast cells and leukocytes by a cellular, myeloid-type, glutathione-dependent PGD synthase. This PGD₂ which is formed in the intracellular and vascular compartments is rapidly metabolized to 11β-PGF_{2α}.³ Thus, urinary measurements of PGD₂ synthesis are most appropriately focused on the measurement of 11β-PGF_{2α}.

Measurement of the parent eicosanoid PGD₂ is appropriate in the supernatants of cell cultures, where PGD₂ levels may reach several ng/ml, and in CSF, where concentrations of several hundred pg/ml have been measured.⁴

All studies of PGD₂ biosynthesis should take into consideration the chemical instability of PGD₂ and its rapid degradation in the presence of serum proteins such as albumin. PGD₂ also readily degrades in both acidic and basic media to give a variety of decomposition products including PGJ₂, Δ¹²-PGJ₂, and 15-deoxy-Δ^{12,14}-PGJ₂. For these reasons, antibodies against PGD₂ have generally been prepared against conjugates of a stable methoximine derivative (*i.e.*, PGD₂-MOX). Our PGD₂-MOX and PGD₂-MOX Express EIA Kits (Catalog Nos. 512011 and 500151) are based on this chemistry and require the PGD₂ in all samples to be converted to the stable methoximine derivative prior to performing the assay. These assays are well suited and recommended for the measurement of PGD₂ in complex matrices such as plasma and tissue extracts. The antibody utilized in this PGD₂ EIA was generated in a unique way allowing the direct measure of PGD₂ without prior conversion to the methoximine compound.

About This Assay

Cayman's Prostaglandin D₂ EIA Kit is a competitive assay that can be used for quantification of PGD₂ in cell culture supernatants and enzymatic reactions. The EIA typically displays an IC₅₀ (50% B/B₀) of approximately 850 pg/ml and a detection limit (80% B/B₀) of approximately 200 pg/ml.

Description of ACE™ Competitive EIAs^{5,6}

This assay is based on the competition between PGD₂ and a PGD₂-acetylcholinesterase (AChE) conjugate (PGD₂ tracer) for a limited number of PGD₂ monoclonal antibody binding sites. Because the concentration of the PGD₂ tracer is held constant while the concentration of PGD₂ varies, the amount of PGD₂ tracer that is able to bind to the monoclonal antibody will be inversely proportional to the concentration of PGD₂ in the well. This antibody-PGD₂ complex (either free or tracer) binds to the 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 PGD₂ tracer bound to the well, which is inversely proportional to the amount of free PGD₂ present in the well during the incubation; or

$$\text{Absorbance} \propto [\text{Bound PGD}_2 \text{ Tracer}] \propto 1/[\text{PGD}_2]$$

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

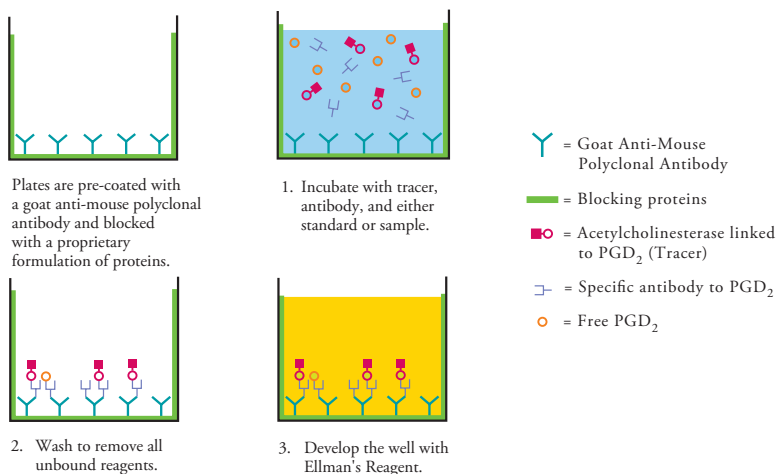


Figure 1. Schematic of the ACE™ EIA

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 2, on page 8). 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 ($\epsilon = 13,600$).

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 re-development 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.

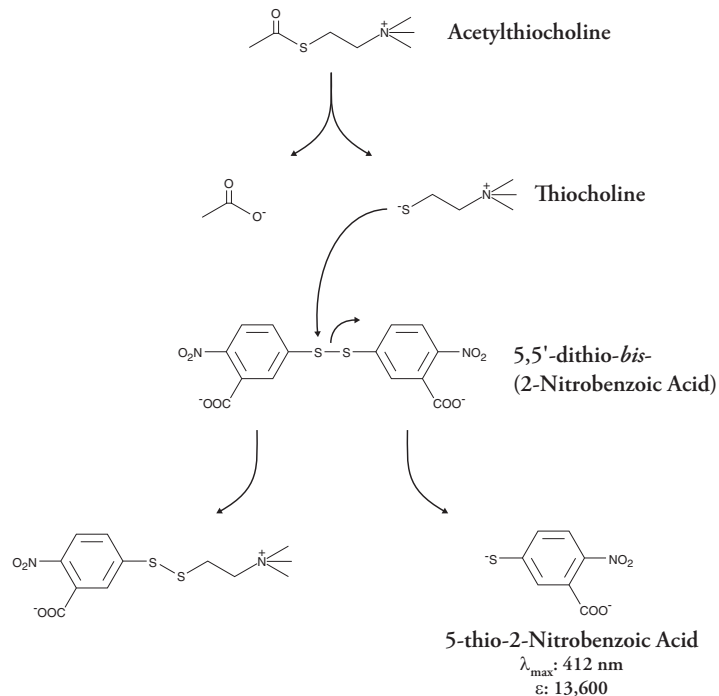


Figure 2. Reaction catalyzed by acetylcholinesterase

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 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) (Catalog 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

5 ml vial Wash Buffer Concentrate (400X) (96-well kit; Catalog No. 400062):
Dilute to a total volume of 2 liters with UltraPure water and add 1 ml of Tween 20 (Catalog No. 400035).

OR

12.5 ml vial Wash Buffer Concentrate (400X) (480-well kit; Catalog No. 400062):
Dilute to a total volume of 5 liters with UltraPure water and add 2.5 ml of Tween 20 (Catalog No. 400035).

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 Preparation

This assay has been validated for use with tissue culture media and purified enzyme preparations. Proper sample storage and preparation are essential for consistent and accurate results.

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.
- Samples of mouse origin may contain antibodies which interfere with the assay by binding to the goat anti-mouse plate. We recommend that all mouse samples be purified prior to use in this assay.

Enzyme Reactions

Reactions using purified enzyme can be diluted directly into EIA Buffer and used in the assay without further purification. Suggestions for performing the PGD₂ synthase reactions may be obtained from our technical service staff by phone (888-526-5351, 734-975-3888), fax (734-971-3641), or E-Mail (techserv@caymanchem.com).

Tissue Culture Samples

Tissue culture samples should be assayed immediately after collection without additional sample treatment. Samples that cannot be assayed immediately should be stored at -80°C until ready to assay. *NOTE: Medium containing FBS are likely to demonstrate elevated PGD₂ immunoreactivity. It is best to test the PGD₂ immunoreactivity of your medium before embarking on a large number of sample measurements. To test for immunoreactivity, dilute the sample matrix blank at several different dilutions, and assay these dilutions using PGD₂ diluted in EIA Buffer for your standard curve. The dilution of medium that gives a value between 20-80% B/B₀ is the appropriate starting dilution for assaying your samples.* We recommend that a standard curve be run first in the experiment matrix to ensure compatibility with the assay.

Preparation of Assay-Specific Reagents

Prostaglandin D₂ EIA Standard

Transfer 100 µl of the PGD₂ EIA Standard (Catalog No. 10006623) into a clean test tube and dilute with 900 µl of UltraPure water. The concentration of this solution (the bulk standard) will be 100 ng/ml. This standard should not be stored for more than 24 hours.

NOTE: If assaying culture media 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 eight clean test tubes and number them #1 through #8. Aliquot 900 µl EIA Buffer to tube #1 and 500 µl EIA Buffer to tubes #2-8. Transfer 100 µl of the bulk standard (100 ng/ml) to tube #1 and mix thoroughly. The concentration of this standard, the first point on the standard curve, will be 10 ng/ml (10,000 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.

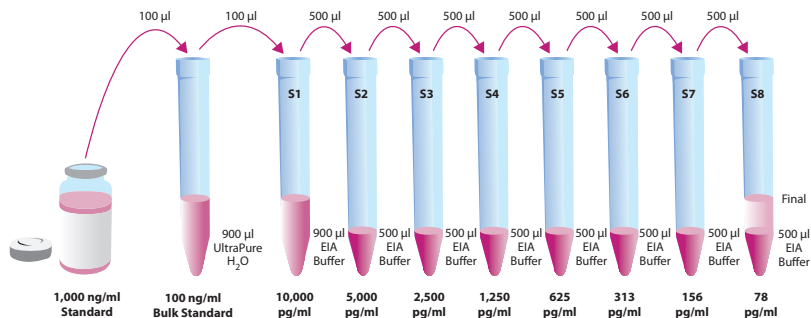


Figure 3. Preparation of the PGD₂ standards

Prostaglandin D₂ AChE Tracer

Reconstitute the PGD₂ AChE Tracer as follows:

100 dtn PGD₂ AChE Tracer (96-well kit; Catalog No. 10006622): Reconstitute with 6 ml EIA Buffer.

OR

500 dtn PGD₂ AChE Tracer (480-well kit; Catalog No. 10006622): Reconstitute with 30 ml EIA Buffer.

Store the reconstituted PGD₂ AChE Tracer at 4°C (*do not freeze!*) and use within four weeks. A 20% surplus of 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). *NOTE: Do not store tracer with dye for more than 24 hours.*

Prostaglandin D₂ Monoclonal Antibody

Reconstitute the PGD₂ Monoclonal Antibody as follows:

100 dtn PGD₂ Monoclonal Antibody (96-well kit; Catalog No. 10006621): Reconstitute with 6 ml EIA Buffer.

OR

500 dtn PGD₂ Monoclonal Antibody (480-well kit; Catalog No. 10006621): Reconstitute with 30 ml EIA Buffer.

Store the reconstituted PGD₂ Monoclonal Antibody at 4°C. It will be stable for at least four weeks. A 20% surplus of antibody has been included to account for any incidental losses.

Antiserum Dye Instructions (optional)

This dye may be added to the antibody, if desired, to aid in visualization of antibody-containing wells. Add the dye to the reconstituted antibody at a final dilution of 1:100 (add 60 µl of dye to 6 ml antibody or add 300 µl of dye to 30 ml of antibody). *NOTE: Do not store antibody with dye for more than 24 hours.*

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 μ l EIA Buffer to Non-Specific Binding (NSB) wells. Add 50 μ l EIA Buffer to Maximum Binding (B_0) wells. If culture medium was used to dilute the standard curve, substitute 50 μ l of culture medium for EIA Buffer in the NSB and B_0 wells (*i.e.*, add 50 μ l culture medium to NSB and B_0 wells and 50 μ l EIA Buffer to NSB wells).

2. Prostaglandin D_2 EIA Standard

Add 50 μ l from tube #8 to both of the lowest standard wells (S8). Add 50 μ l 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 μ l 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 D_2 AChE Tracer

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

5. Prostaglandin D_2 Monoclonal Antibody

Add 50 μ l 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 devel. 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 plastic film (Catalog No. 400012) and incubate two hours at room temperature on an orbital shaker.

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 (96-well kit; Catalog No. 400050): Reconstitute with 20 ml of UltraPure water.

OR

250 dtn vial Ellman's Reagent (480-well kit; Catalog No. 400050): 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.*, B₀ wells ≥0.3 A.U. (blank subtracted)) in 60-90 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 B₀ wells have reached a minimum of 0.3 A.U. (blank subtracted). The plate should be read when the absorbance of the B₀ 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 either %B/B₀ versus log concentration using a four-parameter logistic fit or as logit B/B₀ versus log concentration using a linear fit. *NOTE: Cayman has a computer spreadsheet available for data analysis. Please contact Technical Service or visit our website (www.caymanchem.com/analysis/ia) 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 B₀ wells.
3. Subtract the NSB average from the B₀ average. This is the corrected B₀ or corrected maximum binding.
4. Calculate the B/B₀ (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 B₀ (from Step 3). Repeat for S2-S8 and all sample wells. (To obtain %B/B₀ for a logistic four-parameter fit, multiply these values by 100.)

*NOTE: The total activity (TA) values are not used in the standard curve calculations. Rather, they are used as a diagnostic tool; the corrected B₀ 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/B₀ for standards S1-S8 versus PGD₂ concentration using linear (y) and log (x) axes and perform a 4-parameter logistic fit.

Alternative Plot - The data can also be linearized using a logit transformation. The equation for this conversion is shown below. *NOTE: Do not use %B/B₀ in this calculation.*

$$\text{logit (B/B}_0\text{)} = \ln [\text{B/B}_0\text{}/(1 - \text{B/B}_0\text{)}]$$

Plot the data as logit (B/B₀) versus log concentrations and perform a linear regression fit.

Determine the Sample Concentration

Calculate the B/B₀ (or %B/B₀) 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/B₀ 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.

Performance Characteristics

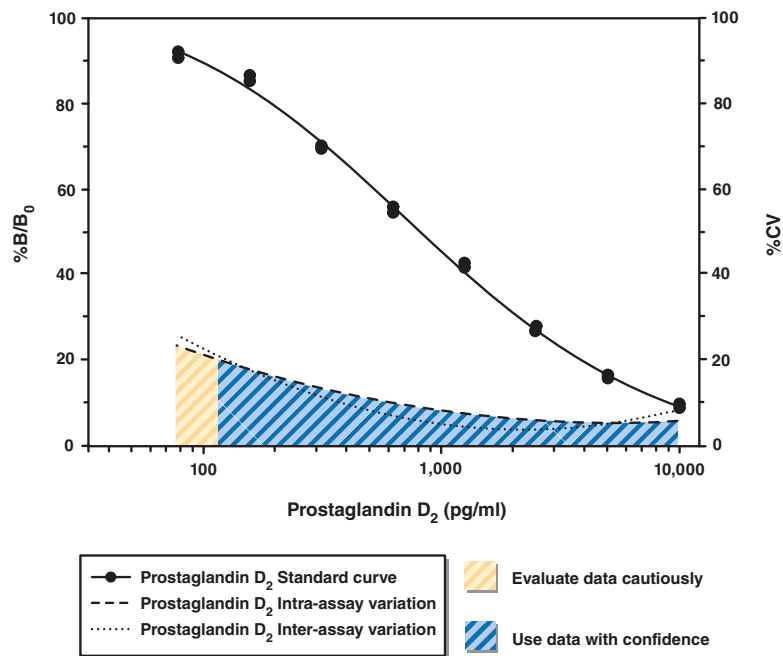
Sample Data

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 the data below to determine the values of your samples. Your results could differ substantially.

	Raw Data		Average	Corrected
Total Activity	0.711	0.740	0.726	
NSB	0.002	0.000	0.001	
B₀	0.582	0.601		
	0.592	0.633	0.602	0.601

Dose (pg/ml)	Raw Data		Corrected		%B/B ₀	
10,000	0.057	0.061	0.056	0.060	9.3	10.0
5,000	0.098	0.102	0.097	0.101	16.1	16.8
2,500	0.164	0.171	0.163	0.170	27.1	28.2
1,250	0.253	0.260	0.252	0.259	42.0	43.0
625	0.331	0.339	0.330	0.338	54.8	56.2
313	0.425	0.421	0.424	0.420	70.4	69.8
156	0.524	0.516	0.523	0.515	86.9	85.6
78	0.549	0.557	0.548	0.556	91.0	92.4

Table 2. Typical results



50% B/B₀ - 840 pg/ml

Detection Limit (80% B/B₀) - 200 pg/ml

Figure 5. Typical standard curve

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.

Dose (pg/ml)	%CV* Intra-assay variation	%CV* Inter-assay variation
10,000	4.5	8.0
5,000	9.9	5.8
2,500	5.6	6.2
1,250	6.8	3.6
625	9.7	6.2
313	13.2	16.6
156	20.2	11.3
78	23.1	28.4

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
Prostaglandin D ₂ Ethanolamide	3,000%	6-keto Prostaglandin F _{1α}	0.6%
Prostaglandin F _{2α}	300%	2,3-dinor-11β-Prostaglandin F _{2α}	0.2%
Prostaglandin D ₂	100%	12(S)-HHTrE	0.14%
Prostaglandin J ₂	17%	Δ ¹² -Prostaglandin J ₂	0.07%
11β-Prostaglandin F _{2α}	10.7%	Arachidonic Acid	<0.01%
Thromboxane B ₂	10%	tetranor-PGEM	<0.01%
Prostaglandin D ₁	1%	tetranor-PGFM	<0.01%
Prostaglandin D ₃	1%	13,14-dihydro-15-keto Prostaglandin D ₂	<0.01%
Prostaglandin E ₂	1%	15-deoxy-Δ ^{12,14} -Prostaglandin J ₂	<0.01%
8-iso Prostaglandin F _{2α}	1%		

Table 4. Specificity of the PGD₂ Monoclonal Antibody

RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic 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 UltraPure 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. 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
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 ⁷
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/512021/references for a list of publications citing the use of Cayman's PGD₂ EIA Kit.

References

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Related Products

Prostaglandin D Synthase

(hematopoietic-type; human recombinant) - Cat. No. 10006593

Prostaglandin D Synthase Inhibitor Screening Assay Kit - Cat. No. 10006595

Prostaglandin D Synthase (lipocalin-type; rat recombinant) - Cat. No. 10010548

Prostaglandin D₂ - Cat. No. 12010

Prostaglandin D₂ Assay Service - Cat. No. 10007574

Prostaglandin D₂ FPLA Kit - Red - Cat. No. 10007835

Prostaglandin D₂-MOX EIA Kit - Cat. No. 512011

Prostaglandin D₂-MOX Express EIA Kit - Cat. No. 500151

UltraPure Water - Cat. No. 400000

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

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