



## Prostaglandin D<sub>2</sub>-MOX Express ELISA Kit

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Item No. 500151

[www.caymanchem.com](http://www.caymanchem.com)

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## GENERAL INFORMATION

### Materials Supplied

Item Number	Item	96 wells Quantity/Size	480 wells Quantity/Size
400152	Prostaglandin D <sub>2</sub> -MOX Express ELISA Antiserum	1 vial/100 dtn	1 vial/500 dtn
400150	Prostaglandin D <sub>2</sub> -MOX AChE Express Tracer	1 vial/100 dtn	1 vial/500 dtn
412014	Prostaglandin D <sub>2</sub> ELISA Standard (MOX Kit)	1 vial	1 vial
400060	ELISA Buffer Concentrate (10X)	2 vials/10 ml	4 vials/10 ml
400062	Wash Buffer Concentrate (400X)	1 vial/5 ml	1 vial/12.5 ml
400035	Polysorbate 20	1 vial/3 ml	1 vial/3 ml
400004/400006	Mouse Anti-Rabbit IgG Coated Plate	1 plate	5 plates
400012	96-Well Cover Sheet	1 cover	5 covers
400050	Ellman's Reagent	3 vials/100 dtn	6 vials/250 dtn
412015	Prostaglandin D <sub>2</sub> -MOX Control	1 vial	1 vial
400036	Methoxylamine HCl	1 vial/100 dtn	1 vial/500 dtn
400037	Sodium Acetate	1 vial/100 dtn	1 vial/500 dtn
400040	ELISA Tracer Dye	1 vial	1 vial
400042	ELISA 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) 971-3335. We cannot accept any returns without prior authorization.



**WARNING:** THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

## Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

## Precautions

**Please read these instructions carefully before beginning this assay.**

The reagents in this kit have been tested and formulated to work exclusively with Cayman Chemical's AChE ELISA Kits. This kit may not perform as described if any reagent or procedure is replaced or modified.

When compared to quantification by LC/MS or GC/MS, it is not uncommon for immunoassays to report higher analyte concentrations. While LC/MS or GC/MS analyses typically measure only a single compound, antibodies used in immunoassays sometimes recognize not only the target molecule, but also structurally related molecules, including biologically relevant metabolites. In many cases, measurement of both the parent molecule and metabolites is more representative of the overall biological response than is the measurement of a short-lived parent molecule. It is the responsibility of the researcher to understand the limits of both assay systems and to interpret their data accordingly.

## 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

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 repeating pipettor.
3. A source of 'UltraPure' water. Water used to prepare all ELISA 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 ELISA. *NOTE: UltraPure water is available for purchase from Cayman (Item No. 400000).*
4. Materials used for Sample Preparation (see page 14).

## Background

Prostaglandin D<sub>2</sub> (PGD<sub>2</sub>) is biosynthesized in the brain by a soluble, 26 kDa glutathione-independent lipocalin-type PGD<sub>2</sub> synthase.<sup>1</sup> This PGD<sub>2</sub> accumulates in the cerebrospinal fluid (CSF), where it induces physiologic sleep in rats and humans.<sup>2</sup> PGD<sub>2</sub> is also synthesized in mast cells and leukocytes by a cellular, myeloid-type, glutathione-dependent PGD synthase. The PGD<sub>2</sub> which is formed in the intracellular and vascular compartments is rapidly metabolized to 11β-PGF<sub>2α</sub>.<sup>3</sup> Thus, urinary measurements of PGD synthesis are most appropriately focused on the measurement of 11β-PGF<sub>2α</sub> or tetranor-PGDM. Measurement of the parent eicosanoid PGD<sub>2</sub> is appropriate in the supernatants of cell cultures, where PGD<sub>2</sub> levels may reach several ng/ml, and in CSF, where concentrations of several hundred pg/ml have been measured.<sup>4</sup>

All studies of PGD<sub>2</sub> biosynthesis should take into consideration the chemical instability of PGD<sub>2</sub> and its rapid degradation in the presence of serum proteins such as albumin.<sup>5</sup> PGD<sub>2</sub> also readily degrades in both acidic and basic media to give a variety of decomposition products. These include PGJ<sub>2</sub>, Δ<sup>12</sup>-PGJ<sub>2</sub>, and 15-deoxy-Δ<sup>12,14</sup>-PGJ<sub>2</sub> (see Figure 1, on page 8). Similarly, antigenic protein conjugates of PGD<sub>2</sub>, synthesized for the production of antisera, also show considerable amounts of decomposition. Thus, the resulting antibody response is heterogeneous with poor specificity. This makes PGD<sub>2</sub> assay systems based on the parent compound unreliable and difficult to interpret.

## About This Assay

This PGD<sub>2</sub> Methoxime (MOX) ELISA is based on the conversion of PGD<sub>2</sub> to a stable MOX derivative. Treatment of the sample with MOX hydrochloride (HCl) converts PGD<sub>2</sub> into PGD<sub>2</sub>-MOX, preventing its further chemical degradation. The antiserum used in the assay was developed using conjugates of this derivative and is very specific for PGD<sub>2</sub>-MOX. The assay has been validated against stable isotope dilution GC-mass spectrometry. Measurements using both techniques on identical samples showed a correlation coefficient of 0.97.

Careful sample collection and preparation is critical for the successful measurement of PGD<sub>2</sub>. Samples should be collected into medium containing a cyclooxygenase inhibitor, methoximated, and assayed at once. For additional details on sample handling, refer to the procedure beginning on page 14.

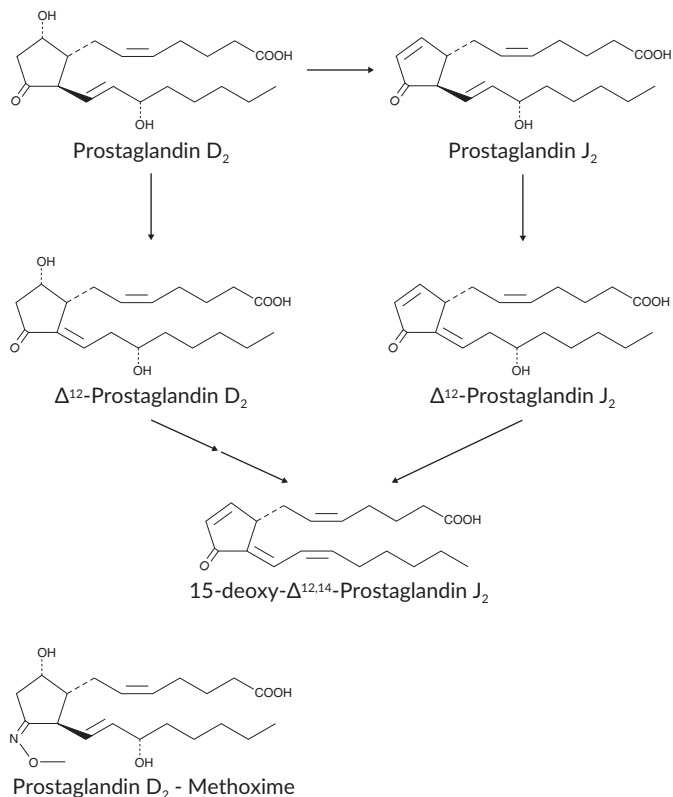


Figure 1. Metabolism of PGD<sub>2</sub>

## Description of AChE Competitive ELISAs<sup>6,7</sup>

This assay is based on the competition between PGD<sub>2</sub>-MOX and a PGD<sub>2</sub>-MOX-acetylcholinesterase (AChE) conjugate (PGD<sub>2</sub>-MOX Tracer) for a limited number of PGD<sub>2</sub>-MOX-specific rabbit antiserum binding sites. Because the concentration of the PGD<sub>2</sub>-MOX Tracer is held constant while the concentration of PGD<sub>2</sub>-MOX varies, the amount of PGD<sub>2</sub>-MOX Tracer that is able to bind to the rabbit antiserum will be inversely proportional to the concentration of PGD<sub>2</sub>-MOX in the well. This rabbit antiserum-PGD<sub>2</sub>-MOX (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 PGD<sub>2</sub>-MOX Tracer bound to the well, which is inversely proportional to the amount of free PGD<sub>2</sub>-MOX present in the well during the incubation; or

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

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

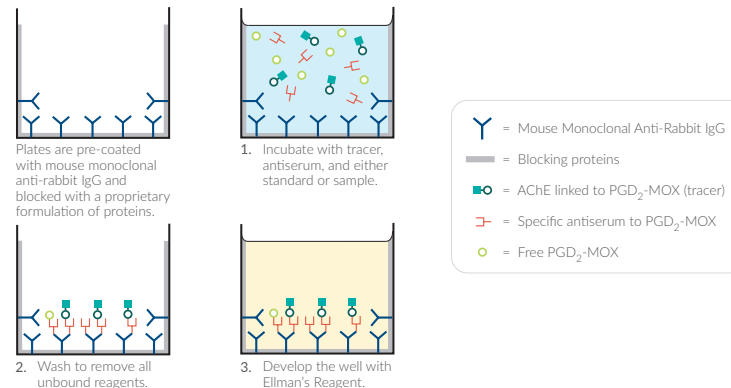


Figure 2. Schematic of the AChE ELISA

## Biochemistry of Acetylcholinesterase

The electric organ of the electric eel, *E. electricus*, contains an avid 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 ( $64,000 \text{ s}^{-1}$ ) for the hydrolysis of acetylthiocholine.

A molecule of the analyte covalently attached to a molecule of AChE serves as the tracer in AChE 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 11). 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 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.

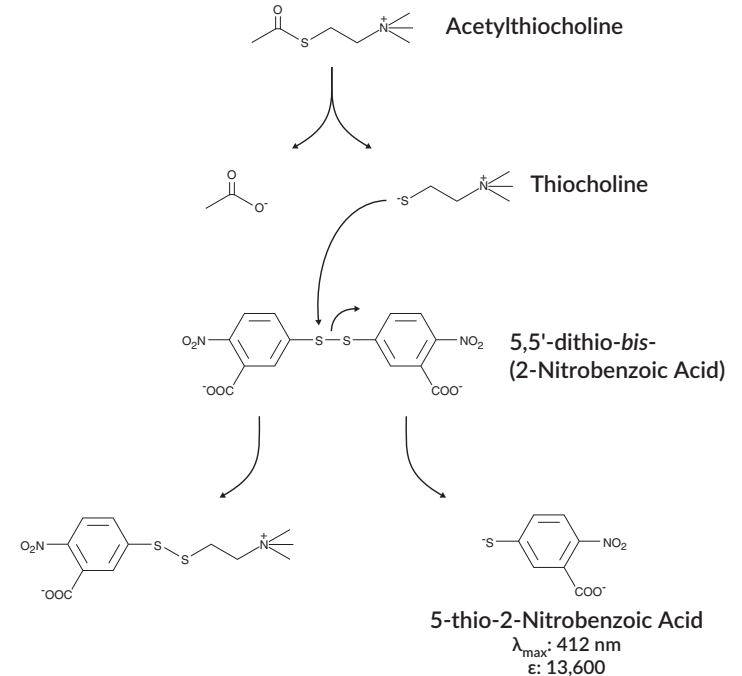


Figure 3. Reaction catalyzed by acetylcholinesterase

## 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, 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<sub>0</sub> (Maximum Binding):** maximum amount of the tracer that the antibody can bind in the absence of free analyte.

**%B/B<sub>0</sub> (%Bound/Maximum Bound):** ratio of the absorbance of a particular sample or standard well to that of the maximum binding (B<sub>0</sub>) well.

**Standard Curve:** a plot of the %B/B<sub>0</sub> 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.

**Cross Reactivity:** numerical representation of the relative reactivity of this assay towards structurally related molecules as compared to the primary analyte of interest. Biomolecules that possess similar epitopes to the analyte can compete with the assay tracer for binding to the primary antibody. Substances that are superior to the analyte in displacing the tracer result in a cross reactivity that is greater than 100%. Substances that are inferior to the primary analyte in displacing the tracer result in a cross reactivity that is less than 100%. Cross reactivity is calculated by comparing the mid-point (50% B/B<sub>0</sub>) value of the tested molecule to the mid-point (50% B/B<sub>0</sub>) value of the primary analyte when each is measured in assay buffer using the following formula:

$$\% \text{ Cross Reactivity} = \left[ \frac{50\% \text{ B/B}_0 \text{ value for the primary analyte}}{50\% \text{ B/B}_0 \text{ value for the potential cross reactant}} \right] \times 100\%$$

## PRE-ASSAY PREPARATION

*NOTE: Water used to prepare all ELISA 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 ELISA. 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. ELISA Buffer Preparation

Dilute the contents of one vial of ELISA 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

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

OR

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

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

*NOTE: Polysorbate 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 a wide range of samples including plasma (diluted at least 1:50) and tissue culture medium. Proper sample storage and preparation are essential for consistent and accurate results. PGD<sub>2</sub> is chemically unstable in biological samples, especially those containing albumin.<sup>5</sup> For this reason, it is necessary to convert PGD<sub>2</sub> to a stable methoxime derivative prior to assay to obtain accurate quantitation of this prostanoid. 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; they will be stable for approximately six months.
- Samples of rabbit origin may contain antibodies which interfere with the assay by binding to the mouse anti-rabbit plate. We recommend that all rabbit samples be purified prior to use in this assay.
- AEBSF (Pefabloc SC®) and PMSF inhibit AChE. Samples containing these protease inhibitors should not be used in this assay.

## 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 35)). Plasma samples and tissue homogenates should be extracted immediately after collection to remove proteins and stabilize the PGD<sub>2</sub>.



## Sample Extraction

### Materials Needed

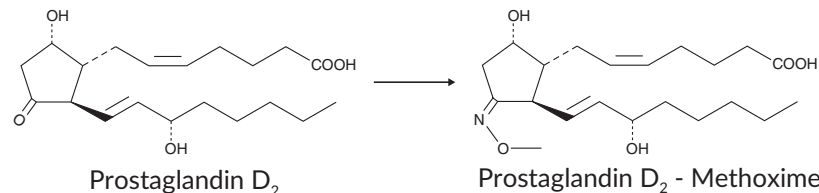
1. Tritium-labeled PGD<sub>2</sub> (optional)
2. Acetone

### Sample Extraction

The following protocol to deproteinate, or to concentrate the samples, is a suggestion only. You may choose a different protocol based on your own requirements, sample type and expertise. If the samples require further purification after methoximation (see suggested SPE (C-18) Purification Protocol following Derivatization of Prostaglandin D<sub>2</sub> to Prostaglandin D<sub>2</sub>-MOX) you may choose to track recovery by spiking samples with tritium-labeled PGD<sub>2</sub> ([<sup>3</sup>H]-PGD<sub>2</sub>) and follow the calculations in the Analysis section on page 31. Otherwise omit step 2 below.

1. Aliquot a known amount of sample in a clean test tube (500 µl is recommended). If your sample needs to be concentrated, a larger volume should be used.
2. Add 10,000 cpm of tritium-labeled PGD<sub>2</sub> ([<sup>3</sup>H]-PGD<sub>2</sub>). Use a high specific activity tracer to minimize the amount of radioactive PGD<sub>2</sub> as the ELISA will be able to detect the added PGD<sub>2</sub>.
3. Dilute the samples 1:1 with cold acetone and incubate on ice for five minutes.
4. Remove any precipitated proteins by centrifugation at 3,000 x g for 10 minutes.
5. Decant the supernatant into a clean test tube and extract the pellet once with 0.5 ml acetone. Repeat Step 4 and combine the supernatant with that from the first extraction. The combined supernatants are relatively stable and may be frozen at -80°C for at least six months.
6. Evaporate the sample to dryness under a stream of nitrogen gas.
7. Resuspend the sample in 100 µl of ELISA Buffer and perform the methoximation according to the following protocol.

## Derivatization of Prostaglandin D<sub>2</sub> to Prostaglandin D<sub>2</sub>-MOX



### 1. Preparation of the Methyl Oximating Reagent (Uses Item Nos. 400036 and 400037)

*NOTE: Methyloximating reagent is not stable. Prepare fresh reagent each time you wish to use it. The instructions below are written for preparation of the entire contents of the vials supplied in the kit. Smaller amounts of methoximating reagent can be prepared by proportionally decreasing the amount of each reagent used.*

**To prepare 100 dtn of methyloximating reagent (sufficient for 1 x 96-well plate):** Prepare 10 ml of a 10:90 solution of ethanol:water. Add a small amount of the ethanol:water solution to the vial labeled 100 dtn (0.1 g) of methoxylamine HCl. Vortex to mix. Transfer to the vial labeled 100 dtn (0.82 g) of sodium acetate. Vortex. Transfer this mixture to a tube capable of holding at least 10 ml. Rinse each of the vials with a portion of the remaining ethanol:water solution and add to the same tube. Transfer the rest of the ethanol:water solution to this same tube. Mix thoroughly before use.

OR

**To prepare 500 dtn of methyloximating reagent (sufficient for 5 x 96-well plates):** Prepare 50 ml of a 10:90 solution of ethanol:water. Add a small amount of the ethanol:water solution to the vial labeled 500 dtn (0.5 g) of methoxylamine HCl. Vortex to mix. Transfer to the vial labeled 500 dtn (4.1 g) of sodium acetate. Vortex. Transfer this mixture to a tube capable of holding at least 50 ml. Rinse each of the vials with a portion of the remaining ethanol:water solution and add to the same tube. Transfer the rest of the ethanol:water solution to this same tube. Mix thoroughly before use.

## 2. Derivatization of the Prostaglandin D<sub>2</sub> ELISA Standard

Equilibrate a pipette tip in ethanol by repeatedly filling and expelling the tip with ethanol several times. Using the equilibrated pipette tip, transfer 100 µl of the PGD<sub>2</sub> ELISA Standard (Item No. 412014) into a clean test tube, then dilute with 900 µl UltraPure water. Heat a 1:1 solution of the diluted standard and Methyl Oximating Reagent at 60°C for 30 minutes. The concentration of this methoximated solution (the bulk standard) will be 20 ng/ml. The methoximated standard will be used to prepare the standard curve. Store this solution at 4°C; it will be stable for approximately six weeks.

## 3. Derivatization of the Prostaglandin D<sub>2</sub> Samples

Add 100 µl of the reconstituted Methyl Oximating Reagent to 100 µl of sample. Heat this solution at 60°C for 30 minutes. Centrifuge the samples and dilute the supernatant at least 1:5 with ELISA Buffer before addition to the assay well.

## SPE (C-18) Purification Protocol

In general, methoximated tissue culture supernatant samples may be diluted with ELISA Buffer and added directly to the assay well. Plasma and serum, as well as other heterogeneous mixtures such as lavage fluids and aspirates often contain contaminants which can interfere in the assay. It is best to check for interference 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 ~5 and 100 pg/ml (*i.e.*, between 20-80% B/B<sub>0</sub>). If the two different dilutions of the sample show good correlation (differ by 20% or less) in the final calculated PGD<sub>2</sub> concentration, purification is not required. If you do not see good correlation of the different dilutions, purification is advised. The following Purification Protocol, is one such method.

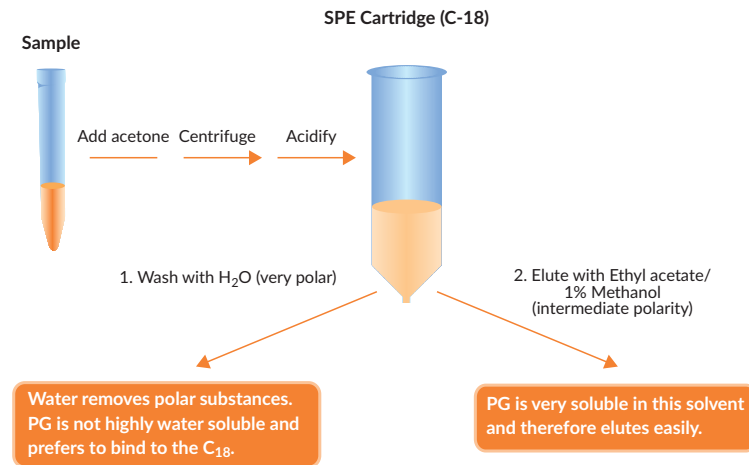


Figure 4. Schematic of PGD<sub>2</sub>-MOX Purification by SPE (C-18)

## Materials Needed

1. SPE Cartridges (C-18) (6 ml) (Item No. 400020)
2. 1 M acetate buffer, pH 4.0, or citrate buffer, methanol, ethyl acetate

## SPE (C-18) Purification Protocol

1. Adjust the pH of the sample to ~4.0 using 1.0 M acetate buffer or citrate buffer (pH 4.0). (Standardize the pH adjustment using the sample matrix prior to proceeding with a large number of samples; approximately 1-2 equivalents of buffer is required for most biological 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 Cartridges (C-18) (6 ml) (Item No. 400020), resulting in loss of the sample.
2. Activate a 6 ml SPE Cartridge (C-18) by rinsing with 5 ml methanol and then with 5 ml UltraPure water. Do not allow the SPE Cartridge (C-18) to dry.
3. Pass the sample through the SPE Cartridge (C-18). Rinse the cartridge with 5 ml UltraPure water. Discard the wash. Elute the PGD<sub>2</sub> 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.\*
4. Evaporate the ethyl acetate to dryness by evaporation under a stream of dry nitrogen gas. It is imperative that all of the organic solvent be removed as even trace quantities will adversely affect the ELISA.
5. Add 500 µl of ELISA Buffer and vortex. Use this for ELISA analysis. If the samples were spiked with tritiated-PGD<sub>2</sub> to track recovery, use 50 µl of this sample for scintillation counting to determine recovery. It is common for an insoluble precipitate to remain after the addition of ELISA Buffer; this will not affect the assay.

\*If it is necessary to stop during the purification, samples may be stored in the ethyl acetate/methanol solution at -20°C or -80°C.

## ASSAY PROTOCOL

### Preparation of Assay-Specific Reagents

#### Prostaglandin D<sub>2</sub> ELISA Standard (MOX Kit)

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

To prepare the standard for use in ELISA: Obtain eight clean test tubes and number them #1 through #8. Aliquot 950 µl ELISA Buffer to tube #1 and 500 µl ELISA Buffer to tubes #2-8. Transfer 50 µl of the bulk standard (20 ng/ml), prepared as described in Step 2 on page 18, to tube #1 and mix thoroughly. 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.

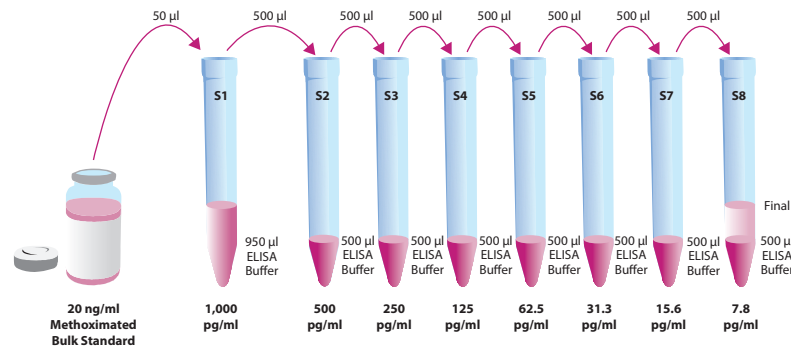


Figure 5. Preparation of the methoximated PGD<sub>2</sub> standards

## Prostaglandin D<sub>2</sub>-MOX AChE Express Tracer

Reconstitute the PGD<sub>2</sub>-MOX AChE Express Tracer as follows:

**100 dtn PGD<sub>2</sub>-MOX AChE Express Tracer (96-well kit; Item No. 400150):**  
Reconstitute with 6 ml ELISA Buffer.

**OR**

**500 dtn PGD<sub>2</sub>-MOX AChE Express Tracer (480-well kit; Item No. 400150):**  
Reconstitute with 30 ml ELISA Buffer.

Store the reconstituted PGD<sub>2</sub>-MOX AChE Express 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).

## Prostaglandin D<sub>2</sub>-MOX Express ELISA Antiserum

Reconstitute the PGD<sub>2</sub>-MOX Express ELISA Antiserum as follows:

**100 dtn PGD<sub>2</sub>-MOX Express ELISA Antiserum (96-well kit; Item No. 400152):** Reconstitute with 6 ml ELISA Buffer.

**OR**

**500 dtn PGD<sub>2</sub>-MOX Express ELISA Antiserum (480-well kit; Item No. 400152):** Reconstitute with 30 ml ELISA Buffer.

Store the reconstituted PGD<sub>2</sub>-MOX Express ELISA 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.

### 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).

## Prostaglandin D<sub>2</sub>-MOX Control

This control is supplied in methoximated form. Do not perform the methoximation procedure. Use of this control is optional and it is provided as a tool to monitor the degree of methoximation of the ELISA Standard and samples (usually 60-70%). To use this control for ELISA, equilibrate a pipette tip in ethanol by repeatedly filling and expelling the tip with ethanol several times. Using the equilibrated pipette tip, transfer 100 µl of the Prostaglandin D<sub>2</sub>-MOX Control (Item No. 412015) to a clean test tube and dilute with 900 µl UltraPure water. The concentration of this solution is 5 ng/ml. Dilute this solution 1:25 (200 pg/ml) and 1:100 (50 pg/ml) in ELISA Buffer and use in the assay.

## 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<sub>0</sub>), 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 29, for more details). We suggest you record the contents of each well on the template sheet provided (see page 38).

	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 <sub>0</sub>	S5	S5	5	5	5	13	13	13	21	21	21
F	B <sub>0</sub>	S6	S6	6	6	6	14	14	14	22	22	22
G	B <sub>0</sub>	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<sub>0</sub> - 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 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. ELISA Buffer

Add 100  $\mu$ l ELISA Buffer to NSB wells. Add 50  $\mu$ l ELISA Buffer to B<sub>0</sub> wells. If culture medium was used to dilute the standard curve, substitute 50  $\mu$ l of culture medium for ELISA Buffer in the NSB and B<sub>0</sub> wells (*i.e.*, add 50  $\mu$ l culture medium to NSB and B<sub>0</sub> wells and 50  $\mu$ l ELISA Buffer to NSB wells).

#### 2. Prostaglandin D<sub>2</sub> ELISA Standard (MOX Kit)

Add 50  $\mu$ l from tube #8 to both of the lowest standard wells (S8). Add 50  $\mu$ 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 and Prostaglandin D<sub>2</sub>-MOX Control

Add 50  $\mu$ 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<sub>2</sub>-MOX AChE Express Tracer

Add 50  $\mu$ l to each well *except* the TA and the Blk wells.

#### 5. Prostaglandin D<sub>2</sub> Express ELISA Antiserum

Add 50  $\mu$ l to each well *except* the TA, the NSB, and the Blk wells.

Well	ELISA Buffer	Standard/Control/ Sample	Tracer	Antiserum
Blk	-	-	-	-
TA	-	-	5 $\mu$ l (at devel. step)	-
NSB	100 $\mu$ l	-	50 $\mu$ l	-
B <sub>0</sub>	50 $\mu$ l	-	50 $\mu$ l	50 $\mu$ l
Std/Sample/Control	-	50 $\mu$ l	50 $\mu$ l	50 $\mu$ l

Table 1. Pipetting summary

### Incubation of the Plate

Cover each plate with plastic film (Item No. 400012) and incubate for one hour 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; Item No. 400050):** Reconstitute with 20 ml of UltraPure water.

OR

**250 dtn vial Ellman's Reagent (480-well kit; Item 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  $\mu\text{l}$  of Ellman's Reagent to each well.
4. Add 5  $\mu\text{l}$  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  $\geq 0.3$  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  $B_0$  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 0.3-1.5 A.U. (blank subtracted). If the absorbance of the wells exceeds 2.0, 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  $\%B/B_0$  versus log concentration using a four-parameter logistic fit or as logit  $B/B_0$  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/elisa](http://www.caymanchem.com/analysis/elisa)) 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). Repeat for S2-S8 and all sample wells. (To obtain  $\%B/B_0$  for a logistic four-parameter fit, multiply these values by 100.)

*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 32). 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 36 for Troubleshooting).*

## Plot the Standard Curve

Plot %B/B<sub>0</sub> for standards S1-S8 versus PGD<sub>2</sub>-MOX 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/B<sub>0</sub> in this calculation.*

$$\text{logit (B/B}_0) = \ln [B/B_0/(1 - B/B_0)]$$

Plot the data as logit (B/B<sub>0</sub>) versus log concentrations and perform a linear regression fit.

## Determine the Sample Concentration

Calculate the B/B<sub>0</sub> (or %B/B<sub>0</sub>) 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 of the sample prior to the addition to the well. Samples with %B/B<sub>0</sub> 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.*

## Spiked-Sample Recovery Calculation

Purification Recovery Factor =

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

PGD<sub>2</sub> (pg) in purified sample =

$$\left[ \frac{\text{Value from ELISA (pg/ml)}}{\text{Recovery Factor}} \right] \times 0.5 \text{ ml} - \text{added } [^3\text{H}]\text{-PGD}_2 \text{ (pg)}$$

Total PGD<sub>2</sub> in methoximated sample (pg/ml) =

$$\frac{\text{PGD}_2 \text{ (pg) in purified sample}}{\text{Volume of sample used for purification (ml)}}$$

PGD<sub>2</sub> in Sample (pg/ml) =

$$\text{PGD}_2 \text{ Value from ELISA (pg/ml)}^\dagger \times 2 \times \left[ \frac{0.1 \text{ ml}}{\text{Volume of sample extracted (ml)}} \right]^\ddagger$$

†Use value corrected for recovery if purification was performed. Also account for dilution used after methoximation.

‡Use only if extraction was done.



## Performance Characteristics

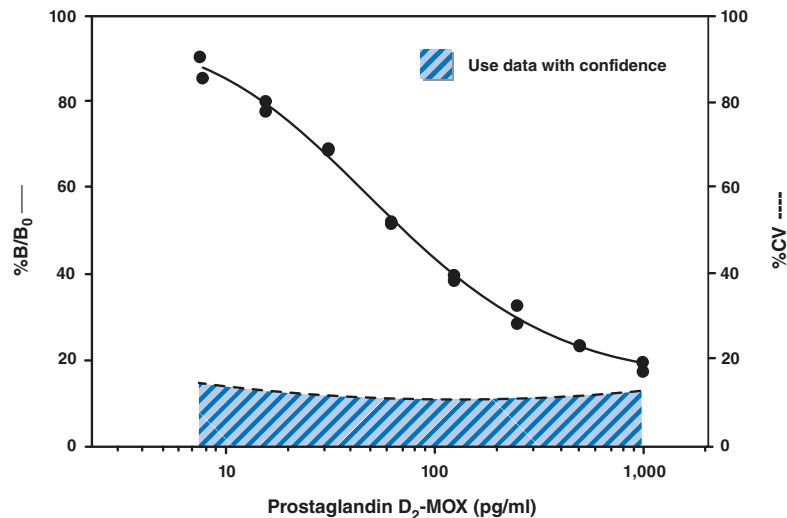
### Sample Data

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	0.414	0.425	0.419	
NSB	0.003	0.003	0.003	
$B_0$	0.789	0.744		
	0.799	0.797	0.779	0.775

Dose (pg/ml)	Raw Data		Corrected		%B/ $B_0$	
1,000	0.144	0.161	0.141	0.158	18.0	20.2
500	0.190	0.190	0.187	0.187	23.9	23.9
250	0.231	0.263	0.228	0.260	29.2	33.3
125	0.318	0.308	0.315	0.305	40.4	39.1
62.5	0.415	0.411	0.412	0.408	52.8	52.3
31.3	0.547	0.543	0.544	0.540	69.8	69.3
15.6	0.614	0.632	0.611	0.629	78.4	80.7
7.8	0.674	0.712	0.671	0.709	86.1	91.0

Table 2. Typical results



**Assay Range** = 7.8-1,000 pg/ml  
**Sensitivity** (defined as 80% B/ $B_0$ ) = 16 pg/ml  
**Mid-point** (defined as 50% B/ $B_0$ ) = 60-100 pg/ml

The sensitivity and mid-point were derived from the standard curve shown above. The standard was diluted with ELISA Buffer.

Figure 7. 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 33 and in the table below.

Dose (pg/ml)	%CV* Intra-assay variation	%CV* Inter-assay variation
1,000	15.0	11.7
500	10.2	6.1
250	11.5	5.8
125	13.8	9.1
62.5	10.3	10.7
31.3	12.5	6.7
15.6	14.5	10.4
7.8	14.6	16.4

**Table 3. Intra- and inter-assay variation**

\*%CV represents the variation in concentration (not absorbance) as determined using a reference standard curve.

### Cross Reactivity:

Compound	Cross Reactivity
Prostaglandin D <sub>2</sub> -MOX	100%
Prostaglandin D <sub>2</sub>	0.3%
Prostaglandin F <sub>2α</sub>	0.02%
tetranor-PGEM	<0.01%
tetranor-PGFM	<0.01%
Prostaglandin E <sub>2</sub> -MOX	<0.01%
6-keto Prostaglandin F <sub>1α</sub> -MOX	<0.01%
Thromboxane B <sub>2</sub> -MOX	<0.01%

**Table 4. Cross Reactivity of the PGD<sub>2</sub>-MOX ELISA**

## 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 (>10% of B <sub>0</sub> )	A. Poor washing B. Exposure of NSB wells to specific antibody	A. Rewash plate and redevelop
Very low B <sub>0</sub>	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 or low efficiency of methoximating agent	
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 ELISA <sup>9</sup>
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/512011/references](http://www.caymanchem.com/512011/references) for a list of publications citing the use of Cayman's PGD<sub>2</sub>-MOX ELISA Kit.

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NOTES

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