Prostaglandin E₂ ELISA Kit - Monoclonal

Item No. 514010

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GENERAL INFORMATION

Materials Supplied

<table>
<thead>
<tr>
<th>Item Number</th>
<th>Item</th>
<th>96 wells Quantity/Size</th>
<th>480 wells Quantity/Size</th>
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</thead>
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<td>414013</td>
<td>Prostaglandin E₂ Monoclonal Antibody</td>
<td>1 vial/100 dtn</td>
<td>1 vial/500 dtn</td>
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<tr>
<td>414010</td>
<td>Prostaglandin E₂ AChE Tracer</td>
<td>1 vial/100 dtn</td>
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<td>414014</td>
<td>Prostaglandin E₂ ELISA Standard</td>
<td>1 vial</td>
<td>1 vial</td>
</tr>
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<td>400060</td>
<td>ELISA Buffer Concentrate (10X)</td>
<td>2 vials/10 ml</td>
<td>4 vials/10 ml</td>
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<tr>
<td>400062</td>
<td>Wash Buffer Concentrate (400X)</td>
<td>1 vial/5 ml</td>
<td>1 vial/12.5 ml</td>
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<tr>
<td>400035</td>
<td>Polysorbate 20</td>
<td>1 vial/3 ml</td>
<td>1 vial/3 ml</td>
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<tr>
<td>400008/400009</td>
<td>Goat Anti-Mouse IgG Coated Plate</td>
<td>1 plate</td>
<td>5 plates</td>
</tr>
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<td>400012</td>
<td>96-Well Cover Sheet</td>
<td>1 cover</td>
<td>5 covers</td>
</tr>
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<td>400050</td>
<td>Ellman’s Reagent</td>
<td>3 vials/100 dtn</td>
<td>6 vials/250 dtn</td>
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<td>ELISA Tracer Dye</td>
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<td>ELISA Antiserum Dye</td>
<td>1 vial</td>
<td>1 vial</td>
</tr>
</tbody>
</table>

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 13).
INTRODUCTION

Background

Prostaglandin E\textsubscript{2} (PGE\textsubscript{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\textsubscript{2} is synthesized \textit{de novo} and released into the extracellular space. \textit{In vivo}, PGE\textsubscript{2} is rapidly converted to an inactive metabolite (13,14-dihydro-15-keto PGE\textsubscript{2}) by the PG 15-dehydrogenase pathway.\textsuperscript{1,2} The half-life of PGE\textsubscript{2} in the circulatory system is approximately 30 seconds and normal plasma levels are 3-12 pg/ml.\textsuperscript{3}

Because of the rapid metabolism of PGE\textsubscript{2}, the determination of \textit{in vivo} PGE\textsubscript{2} biosynthesis is often best accomplished by the measurement of PGE\textsubscript{2} metabolites. Our PGE Metabolite Assay Kit (Item No. 514531) converts all major PGE\textsubscript{2} metabolites into a single stable derivative which is easily measurable by ELISA (see Figure 1, on 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.

About This Assay

Cayman’s PGE\textsubscript{2} ELISA Kit - Monoclonal is a competitive assay that can be used for quantification of PGE\textsubscript{2} in urine, plasma, tissue culture supernatants, and other sample matrices. The assay has a range from 7.8-1,000 pg/ml and a sensitivity (80% B/B\textsubscript{0}) of approximately 15 pg/ml.

![Figure 1. Metabolism of PGE\textsubscript{2}](image-url)
INTRODUCTION

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 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 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 (\(\varepsilon = 13,600\)).

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.

**Description of AChE Competitive ELISAs**

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

\[
\text{Absorbance} \propto \frac{\text{Bound PGE}_2 \text{Tracer}}{[\text{PGE}_2]} \propto 1/[\text{PGE}_2]
\]

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

![Figure 2. Schematic of the AChE ELISA](image)

**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 s\(^{-1}\)) for the hydrolysis of acetylthiocholine.

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**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.

**B₀ (Maximum Binding)**: maximum amount of the tracer that the antibody can bind in the absence of free analyte.

**%B/B₀ (%Bound/Maximum Bound)**: ratio of the absorbance of a particular sample or standard well to that of the maximum binding (B₀) well.

**Standard Curve**: a plot of the %B/B₀ 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/B₀) value of the tested molecule to the mid-point (50% B/B₀) value of the primary analyte when each is measured in assay buffer using the following formula:

\[
\% \text{ Cross Reactivity} = \left( \frac{50\% \text{ B}/B_0 \text{ value for the primary analyte}}{50\% \text{ B}/B_0 \text{ value for the potential cross reactant}} \right) \times 100\%
\]
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).
   - **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).

**Sample Preparation**

This assay has been validated for a wide range of samples including urine, plasma, and tissue culture media (see Figure 4, on page 14). 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.
- Samples of mouse and rat origin may contain antibodies which interfere with the assay by binding to the goat anti-mouse plate. We recommend that all mouse and rat samples be purified prior to use in this assay.

**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 PGE Metabolite ELISA Kit (Item 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 PGE₂ in normal plasma is very low in comparison with other potentially immunoreactive metabolites. 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 PGE₂ content can be concentrated into as little as 0.5 ml of ELISA Buffer. This will bring the PGE₂ concentration into the readable range of the standard curve. A more accurate index of PGE₂ biosynthesis in plasma can be obtained using our PGE Metabolite ELISA Kit (Item No. 514531).

**Culture Medium Samples**

Cell culture supernatants may be assayed directly without purification. If the PGE₂ concentration in the medium is high enough to dilute the sample 10-fold with ELISA Buffer, the assay can be performed without any modifications. When assaying less concentrated samples (where samples cannot be diluted with ELISA 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**

Snap-freeze tissues in liquid nitrogen immediately upon collection and then store at -80°C. 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. Proceed to the purification section below. Alternatively, frozen samples can be pulverized in the presence of ethanol for extraction of PGE₂.

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![Figure 4. Validation curves for this PGE₂ assay](image-url)
**Tissue Homogenization using the Precellys 24 Homogenizer**

Snap-freeze tissues in liquid nitrogen immediately upon collection and store at -80°C. Add 1 ml homogenization buffer (0.1 M phosphate buffer, pH 7.4, containing 1 mM EDTA and 10 µM indomethacin) per 100 mg of tissue. Homogenize the sample with the Precellys 24 using the appropriate settings (see Table 1). Spin the tissue homogenates at 8,000 x g for 10 minutes. Collect supernatant and assay as described below. Samples will need to be diluted appropriately for the assay. Tissue samples should be normalized using a protein assay. Cayman’s Protein Determination Kit (Item No. 704002) may be used to normalize protein samples.

**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 10 and 250 pg/ml (i.e., between 20-80% B/B₀). If the two different dilutions of the sample show good correlation (differ by 20% or less) in the final calculated PGE₂ concentration, purification is not required. If you do not see good correlation of the different dilutions, purification is advised.

**SPE (C-18) Purification Protocol**

The following protocol is a suggestion only. You may choose a different protocol based on your own requirements, sample type, and expertise. If desired, recovery may be tracked by spiking samples with tritium-labeled PGE₂ ([^3]H-PGE₂) and follow the spiked-sample recovery calculations in the Analysis section on page 28. Otherwise, omit steps 2 and 11.

**Materials Needed**

1. Tritium-labeled PGE₂ (optional)
2. 1 M acetate buffer, deionized water, ethanol, methanol, and ethyl acetate
3. 500 mg SPE Cartridges (C-18) (Item No. 400020)

**Organ** | **Speed (rpm)** | **Cycle Length (seconds)** | **Beads**  
--- | --- | --- | ---  
Lung | 5,200 | 20 | CK28 Large Ceramic (Item No. 10011151)  
Brain | 5,500 | 15 | CK28 Large Ceramic (Item No. 10011151)  
Liver | 5,200 | 20 | CK14 Small Ceramic (Item No. 10011152)  
Kidney | 5,200 | 20 | CK14 Small Ceramic (Item No. 10011152)  
Heart | 5,200 | 30 | CK14 Small Ceramic (Item No. 10011152)  

**Table 1. Precellys settings**

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**Pre-assy Preparation**

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**Figure 5. Schematic of PGE₂ Purification by SPE (C-18)**
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 PGE₂ ([³H]-PGE₂). Use a high specific activity tracer to minimize the amount of radioactive PGE₂ as the ELISA will be able to detect the added PGE₂.

3. Precipitation of proteins using ethanol is optional and may not be needed if samples are clean enough to flow through the SPE Cartridge (C-18). Body fluids such as plasma and urine can typically be applied directly to the SPE Cartridge (C-18) 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 4.0 by the addition of 1 M acetate buffer (or citrate buffer). (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 (C-18).

5. Prepare SPE (C-18) columns by rinsing with 5 ml methanol followed by 5 ml deionized water. Do not allow the SPE Cartridge (C-18) to dry.

6. Apply the sample to the SPE Cartridge (C-18) and allow the sample to completely enter the packing material.

7. Wash the column with 5 ml deionized water. Discard the wash.

8. Elute the PGE₂ 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 ELISA.

10. To resuspend the sample, add 500 µl ELISA Buffer. Vortex. It is common for insoluble precipitate to remain in the sample after addition of ELISA Buffer; this will not affect the assay. This sample is now ready for use in the ELISA.

11. Use 50 µl of the resuspended sample for scintillation counting.
Preparation of Assay-Specific Reagents

Prostaglandin E₂ ELISA Standard

Reconstitute the contents of the PGE₂ ELISA Standard (Item No. 414014) with 1.0 ml of ELISA 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 4 weeks.

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 900 µl ELISA Buffer to tube #1 and 500 µl ELISA Buffer to tubes #2-8. Transfer 100 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 1 ng/ml (1,000 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.

Prostaglandin E₂ AChE Tracer

Reconstitute the PGE₂ AChE Tracer as follows:

100 dtn PGE₂ AChE Tracer (96-well kit; Item No. 414010): Reconstitute with 6 ml ELISA Buffer.

OR

500 dtn PGE₂ AChE Tracer (480-well kit; Item No. 414010): Reconstitute with 30 ml ELISA Buffer.

Store the reconstituted PGE₂ 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 accidental 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 E₂ Monoclonal Antibody

Reconstitute the PGE₂ Monoclonal Antibody as follows:

100 dtn PGE₂ Monoclonal Antibody (96-well kit; Item No. 414013): Reconstitute with 6 ml ELISA Buffer.

OR

500 dtn PGE₂ Monoclonal Antibody (480-well kit; Item No. 414013): Reconstitute with 30 ml ELISA Buffer.

Store the reconstituted PGE₂ 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).
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 (B₀), 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 7, 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 26, for more details). We suggest you record the contents of each well on the template sheet provided (see page 35).

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<td>S15</td>
<td>S16</td>
</tr>
<tr>
<td>G</td>
<td>TA</td>
<td>S7</td>
<td>S8</td>
<td>S9</td>
<td>S10</td>
<td>S11</td>
<td>S12</td>
<td>S13</td>
<td>S14</td>
<td>S15</td>
<td>S16</td>
<td>S17</td>
</tr>
<tr>
<td>H</td>
<td>Blk</td>
<td>S8</td>
<td>S9</td>
<td>S10</td>
<td>S11</td>
<td>S12</td>
<td>S13</td>
<td>S14</td>
<td>S15</td>
<td>S16</td>
<td>S17</td>
<td>S18</td>
</tr>
</tbody>
</table>

Figure 7. 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 µl ELISA Buffer to NSB wells. Add 50 µl ELISA Buffer to B₀ wells. If culture medium was used to dilute the standard curve, substitute 50 µl of culture medium for ELISA Buffer in the NSB and B₀ wells (i.e., add 50 µl culture medium to NSB and B₀ wells and 50 µl ELISA Buffer to NSB wells).

2. **Prostaglandin E₂ ELISA 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 E₂ AChE Tracer**
   - Add 50 µl to each well except the TA and the Blk wells.

5. **Prostaglandin E₂ Monoclonal Antibody**
   - Add 50 µl to each well except the TA, the NSB, and the Blk wells.
Table 2. Pipetting summary

Incubation of the Plate
Cover each plate with plastic film (Item No. 400012) and incubate 18 hours at 4°C.

Development of the Plate
1. Reconstitute Ellman’s Reagent immediately before use (20 ml of reagent is sufficient to develop 100 wells):
   **100 dtw vial Ellman’s Reagent (96-well kit; Item No. 400050):** Reconstitute with 20 ml of UltraPure water.

   OR

   **250 dtw 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 µl of Ellman’s Reagent to each well.
4. Add 5 µl of tracer to the TA wells.
5. Cover the plate with plastic film. Optimum development is obtained by using an orbital shaker equipped with a large, flat cover to allow the plate(s) to develop in the dark. This assay typically develops (i.e., 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.

<table>
<thead>
<tr>
<th>Well</th>
<th>ELISA Buffer</th>
<th>Standard/Sample</th>
<th>Tracer</th>
<th>Antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blk</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TA</td>
<td>-</td>
<td>-</td>
<td>5 µl (at devl. step)</td>
<td>-</td>
</tr>
<tr>
<td>NSB</td>
<td>100 µl</td>
<td>-</td>
<td>50 µl</td>
<td>-</td>
</tr>
<tr>
<td>B₀</td>
<td>50 µl</td>
<td>-</td>
<td>50 µl</td>
<td>50 µl</td>
</tr>
<tr>
<td>Std/Sample</td>
<td>-</td>
<td>50 µl</td>
<td>50 µl</td>
<td>50 µl</td>
</tr>
</tbody>
</table>
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/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 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 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 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/B₀ for standards S1-S8 versus PGE₂ 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.

\[
\text{logit} \left( \frac{B}{B_0} \right) = \ln \left[ \frac{B}{B_0} \bigg/ \left(1 - \frac{B}{B_0}\right) \right]
\]

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.

Spiked-Sample Recovery Calculation

10 x cpm of sample [³H]-PGE₂ added to sample (cpm)

\[
\text{Recovery Factor} = \frac{\text{10 x cpm of sample}}{\text{[³H]-PGE₂ added to sample (cpm)}}
\]

\[
\text{PGE₂ (pg) in purified sample} = \left[ \frac{\text{Value from ELISA (pg/ml)}}{\text{Recovery Factor}} \right] \times 0.5\text{ ml} - \text{added [³H]-PGE₂ (pg)}
\]

\[
\text{Total PGE₂ in sample (pg/ml)} = \frac{\text{PGE₂ (pg) in purified sample}}{\text{Volume of sample used for purification (ml)}}
\]
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 the data below to determine the values of your samples. Your results could differ substantially.

<table>
<thead>
<tr>
<th>Raw Data</th>
<th>Average</th>
<th>Corrected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Activity</td>
<td>0.689</td>
<td>0.692</td>
</tr>
<tr>
<td>NSB</td>
<td>0.004</td>
<td>0.002</td>
</tr>
<tr>
<td>B₀</td>
<td>0.871</td>
<td>0.830</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dose (pg/ml)</th>
<th>Raw Data</th>
<th>Corrected</th>
<th>%B/B₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,000</td>
<td>0.021</td>
<td>0.017</td>
<td>0.018</td>
</tr>
<tr>
<td>500</td>
<td>0.058</td>
<td>0.060</td>
<td>0.055</td>
</tr>
<tr>
<td>250</td>
<td>0.106</td>
<td>0.107</td>
<td>0.103</td>
</tr>
<tr>
<td>125</td>
<td>0.207</td>
<td>0.210</td>
<td>0.204</td>
</tr>
<tr>
<td>62.5</td>
<td>0.358</td>
<td>0.342</td>
<td>0.355</td>
</tr>
<tr>
<td>31.3</td>
<td>0.541</td>
<td>0.521</td>
<td>0.538</td>
</tr>
<tr>
<td>15.6</td>
<td>0.691</td>
<td>0.656</td>
<td>0.688</td>
</tr>
<tr>
<td>7.8</td>
<td>0.793</td>
<td>0.767</td>
<td>0.790</td>
</tr>
</tbody>
</table>

Table 3. Typical results

Figure 8. Typical standard curve

Assay Range = 7.8-1,000 pg/ml
Sensitivity (defined as 80% B/B₀) = 15 pg/ml
Mid-point (defined as 50% B/B₀) = 30-70 pg/ml

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

<table>
<thead>
<tr>
<th>Dose (pg/ml)</th>
<th>%CV* Intra-assay variation</th>
<th>%CV* Inter-assay variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,000</td>
<td>4.2</td>
<td>12.4</td>
</tr>
<tr>
<td>500</td>
<td>3.9</td>
<td>6.4</td>
</tr>
<tr>
<td>250</td>
<td>3.8</td>
<td>7.8</td>
</tr>
<tr>
<td>125</td>
<td>3.7</td>
<td>11.6</td>
</tr>
<tr>
<td>62.5</td>
<td>6.6</td>
<td>15.5</td>
</tr>
<tr>
<td>31.3</td>
<td>7.8</td>
<td>15.0</td>
</tr>
<tr>
<td>15.6</td>
<td>10.1</td>
<td>20.9</td>
</tr>
<tr>
<td>7.8</td>
<td>30.4</td>
<td>35.0</td>
</tr>
</tbody>
</table>

Table 4. Intra- and inter-assay variation
*%CV represents the variation in concentration (not absorbance) as determined using a reference standard curve.

Cross Reactivity:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cross Reactivity</th>
<th>Compound</th>
<th>Cross Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostaglandin E₂</td>
<td>100%</td>
<td>tetranor-PGEM</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>Prostaglandin E₂ Ethanolamide</td>
<td>100%</td>
<td>tetranor-PGF</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>Prostaglandin E₂-1-glyceryl ester</td>
<td>100%</td>
<td>Prostaglandin A₁</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>Prostaglandin E₃</td>
<td>43%</td>
<td>8-iso Prostaglandin A₂</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>Prostaglandin E₄</td>
<td>18.7%</td>
<td>Prostaglandin A₃</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>8-iso Prostaglandin E₃</td>
<td>2.5%</td>
<td>Prostaglandin B₁</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>Prostaglandin A₂</td>
<td>1.25%</td>
<td>Prostaglandin B₂</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>6-keto Prostaglandin F₁ₐ</td>
<td>1%</td>
<td>Prostaglandin D₂</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>8-iso Prostaglandin F₂ₐ</td>
<td>0.25%</td>
<td>11-deoxy Prostaglandin E₂</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>Prostaglandin A₃</td>
<td>0.04%</td>
<td>16,16-dimethyl Prostaglandin E₂</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>13,14-dihydro-15-keto Prostaglandin E₂</td>
<td>0.02%</td>
<td>19(R)-hydroxy Prostaglandin E₂</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>Arachidonic Acid</td>
<td>&lt;0.01%</td>
<td>20-hydroxy Prostaglandin E₂</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>Arachidonoyl Ethanolamide</td>
<td>&lt;0.01%</td>
<td>15-keto Prostaglandin E₂</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>O-Arachidonoyl Ethanolamide</td>
<td>&lt;0.01%</td>
<td>Prostaglandin F₁ₐ</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>Butaprost</td>
<td>&lt;0.01%</td>
<td>Prostaglandin F₂ₐ</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>Conjugated Linoleic Acid (10E,12Z)</td>
<td>&lt;0.01%</td>
<td>13,14-dihydro-15-keto Prostaglandin F₂ₐ</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>8(S),15(S)-DIHETE</td>
<td>&lt;0.01%</td>
<td>Prostaglandin F₃ₐ</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>Leukotriene B₄</td>
<td>&lt;0.01%</td>
<td>Prostaglandin J₂</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>Misoprostol</td>
<td>&lt;0.01%</td>
<td>15-deoxy-Δ12,14-Prostaglandin J₂</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>Misoprostol (free acid)</td>
<td>&lt;0.01%</td>
<td>Thromboxane B₂</td>
<td>&lt;0.01%</td>
</tr>
</tbody>
</table>

Table 5. Cross Reactivity of the PGE₂ ELISA
# Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Causes</th>
<th>Recommended Solutions</th>
</tr>
</thead>
</table>
| 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 B₀)         | A. Poor washing  
B. Exposure of NSB wells to specific antibody | A. Rewash plate and redevelop |
| Very low B₀                   | 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 ELISA<sup>5</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/514010/references](http://www.caymanchem.com/514010/references) for a list of publications citing the use of Cayman’s PGE<sub>2</sub> ELISA Kit.

## References

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