Prostaglandin E$_2$ Metabolite ELISA

Enzyme immunoassay for the quantitative determination of Prostaglandin E Metabolite in urine, whole blood, plasma and tissue culture supernatants.

REF  CM514531

For illustrative purposes only.
To perform the assay the instructions for use provided with the kit have to be used.

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Prostaglandin E Metabolite EIA Kit
Catalog No. 514531 (Strip Plate)
Catalog No. 514531.1 (Solid Plate)
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GENERAL INFORMATION

Materials Supplied

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<tr>
<th>Catalog Number</th>
<th>Item</th>
<th>96 wells Quantity/Size</th>
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<td>Prostaglandin E Metabolite EIA Antiserum</td>
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<td>1 vial/500 dtn</td>
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<td>414530</td>
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<td>Tween 20</td>
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<td>EIA Antiserum Dye</td>
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If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 975-3999. We cannot accept any returns without prior authorization.

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

Please read these instructions carefully before beginning this assay.
The reagents in this kit have been tested and formulated to work exclusively with Cayman Chemical’s ACE™ EIA Kits. This kit may not perform as described if any reagent or procedure is replaced or modified.
For research use only. Not for human or diagnostic use.

If You Have Problems

Technical Service Contact Information
Phone: 888-526-5351 (USA and Canada only) or 734-975-3888
Fax: 734-971-3641
E-Mail: techserv@caymanchem.com
Hours: M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

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

Materials Needed But Not Supplied
1. A plate reader capable of measuring absorbance between 405-420 nm.
2. Adjustable pipettes and a repeat pipettor.
3. A source of ‘UltraPure’ water. Water used to prepare all EIA reagents and buffers must be deionized and free of trace organic contaminants (‘UltraPure’). Use activated carbon filter cartridges or other organic scavengers. Glass distilled water (even if double distilled), HPLC-grade water, and sterile water (for injections) are not adequate for EIA. NOTE: UltraPure water is available for purchase from Cayman (Catalog No. 400000).
4. Materials used for Sample Preparation (see page 12).

INTRODUCTION

Background
Prostaglandin E₂ (PGE₂) is produced by a variety of cell types which, in general, do not contain the enzymes required for metabolism of PGE₂. Thus, cultured endothelial cells or osteoblasts will release PGE₂ into the culture medium where it will accumulate without appreciable metabolism. The direct assay of PGE₂ from the medium is a good way to measure PGE₂ production from these cells.

PGE₂ is rapidly converted in vivo to its 13,14-dihydro-15-keto metabolite (see Figure 1, on page 6), with more than 90% of circulating PGE₂ cleared by a single passage through the lungs. Unfortunately, this metabolite is not chemically stable and undergoes a variable amount of degradation to PGA products. For this reason, blood, urine, or other samples from whole animals or humans often contain very little intact PGE₂, and measurement of the metabolites is necessary to provide a reliable estimate of actual PGE₂ production.

About This Assay
Cayman’s Prostaglandin E Metabolite (PGEM) assay is a competitive assay that converts 13,14-dihydro-15-keto PGA₃ and 13,14-dihydro-15-keto PGE₂ to a single, stable derivative that can be easily quantified. This assay is, therefore, the method of choice if the samples in question have undergone extensive metabolism prior to collection. The EIA typically displays an IC₅₀ (50% B/B₀) value of approximately 10 pg/ml and a detection limit (80% B/B₀) of approximately 2 pg/ml.
**Description of ACE™ Competitive EIAs**

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

\[
A_{\text{absorbance}} \propto \frac{[\text{Bound PGEM Tracer}]}{[\text{PGEM}]} \propto \frac{1}{[\text{PGEM}]}
\]

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

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**Figure 1. Metabolism of PGE2**

**Figure 2. Schematic of the ACE™ EIA**
Biochemistry of Acetylcholinesterase

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

A molecule of the analyte covalently attached to a molecule of AChE serves as the tracer in ACE™ enzyme immunoassays. Quantification of the tracer is achieved by measuring its AChE activity with Ellman’s Reagent. This reagent consists of acetylthiocholine and 5,5’-dithio-bis-(2-nitrobenzoic acid). Hydrolysis of acetylthiocholine by AChE produces thiocholine (see Figure 3). 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.

Figure 3. Reaction catalyzed by acetylcholinesterase
Definition of Key Terms

**Blank**: background absorbance caused by Ellman’s Reagent. The blank absorbance should be subtracted from the absorbance readings of all the other wells.

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

**NSB (Non-Specific Binding)**: non-immunological binding of the tracer to the well. Even in the absence of specific antibody a very small amount of tracer still binds to the well; the NSB is a measure of this low binding.

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

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

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

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

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**PRE-ASSAY PREPARATION**

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

**Buffer Preparation**

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

1. **EIA Buffer Preparation**

   Dilute the contents of one vial of EIA Buffer Concentrate (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 (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).

   **12.5 ml vial Wash Buffer (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.
3. **Phosphate Buffer**
Prepare a 1 M Phosphate Buffer solution by dissolving the contents of the 100 dtn vial of Phosphate Buffer (Catalog No. 400032) in 30 ml UltraPure water, or dissolve the contents of one of the 250 dtn vials of Phosphate Buffer (Catalog No. 400032) in 75 ml UltraPure water.

4. **Carbonate Buffer**
Prepare a 1 M Carbonate Buffer solution by dissolving the contents of the 100 dtn vial of Carbonate Buffer (Catalog No. 400027) in 25 ml UltraPure water, or dissolve the contents of the 500 dtn vial of Carbonate Buffer (Catalog No. 400027) in 125 ml UltraPure water.

5. **PGEM Assay Buffer**
Prepare 20 ml of PGEM Assay Buffer by combining 13 ml EIA Buffer, 3 ml Carbonate Buffer, and 4 ml Phosphate Buffer. This quantity of buffer should be more than sufficient to complete one 96-well plate.

**Sample Preparation**
In general, after derivitizing urine and tissue culture supernatant samples may be diluted with PGEM Assay Buffer and added directly to the assay well. Plasma and whole blood, 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 ~2 and 50 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 PGEM concentration, purification is not required. If you do not see good correlation of the different dilutions, purification is advised. The Purification Protocol, on page 13-16, is one such method.

**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.

**Sample Purification**
The method of purification is by solid phase extraction (SPE) as described in the protocol below.

**Determination of Recovery**
Determination of percent recovery is recommended when any sample purification is performed. Detailed on page 15 are two methods that can be employed to monitor recovery. If the *hot spike* method (recommended) is used, 10,000 cpm of tritium-labeled PGE<sub>2</sub> is added directly to the sample and 10% is removed for scintillation counting after purification. If the *cold spike* method is used, the sample must be split prior to purification and an appropriate amount of 13,14-dihydro-15-keto PGE<sub>2</sub> added to one aliquot. The spiked sample is then assayed *via* EIA alongside the unspiked sample. Calculations for each method are found in the Analysis section on page 25.
C-18 SPE Purification Protocol

Materials Needed
1. Tritium-labeled PGE₂ to use as a hot spike or unlabeled 13,14-dihydro-15-keto PGE₂ to use as a cold spike to allow determination of extraction efficiency.
2. 1 M Acetic acid, deionized water, ethanol, methanol, hexane and ethyl acetate
3. 200 mg C-18 solid phase extraction (SPE) columns (non end-capped)

Sample
1. Add ethanol
2. Centrifuge
3. Acidify

SPE (C-18) Cartridge (non end-capped)
1. Wash with H₂O (very polar)
2. Wash with Hexane (very non-polar)
3. Elute with Ethyl acetate/1% Methanol (intermediate polarity)

Hot Spike
1. Aliquot a known amount of each sample into a clean test tube (500 µl is recommended). If your samples need to be concentrated, a larger volume should be used (e.g., a 5 ml sample will be concentrated by a factor of 10, a 10 ml sample will be concentrated by a factor of 20, etc.).
2. Add 10,000 cpm of tritium-labeled PGE₂ ([³H]-PGE₂).

Cold Spike
1. Aliquot a known amount of each sample into each of two tubes. Label the first tube ‘sample #’ and the second tube ‘sample # + spike’. If your samples need to be concentrated, a larger volume should be used (e.g., a 5 ml sample will be concentrated by a factor of 10, a 10 ml sample will be concentrated by a factor of 20, etc.).
2. Add a cold spike of 13,14-dihydro-15-keto PGE₂ to the ‘sample + spike’ tubes. Follow the procedure below for both spiked and unspiked samples.

Proceed to step 3 below
3. Precipitation of proteins using ethanol is optional and may not be needed if samples are clean enough to flow through the C-18 SPE cartridge. Body fluids such as plasma and urine can typically be applied directly to the C-18 cartridge after the acidification step (step 4) below. To precipitate proteins, add ethanol (approximately four times the sample volume) to each tube. Vortex to mix thoroughly. Incubate samples at 4°C for five minutes, then centrifuge a 3,000 x g for 10 minutes to remove precipitated proteins. Transfer the supernatant to a clean test tube. Evaporate the ethanol under nitrogen.
4. Acidify the sample to ~pH 4 by the addition of 1 M acetic acid. (To avoid having to measure the pH of each individual sample, adjust the pH of an equivalent volume of sample matrix to pH 4.0 using the 1M acetic acid. Add this volume of acetic acid to each sample. NOTE: For samples of different volumes, the amount of acid should be adjusted to maintain this ratio of acid to sample.). If the samples are cloudy or contain precipitate, either filter or centrifuge to remove the precipitate. Particulate matter in the sample may clog the SPE cartridge.
5. Prepare C-18 SPE columns by rinsing with 5 ml methanol followed by 5 ml deionized water. Do not allow the SPE cartridge to dry.

6. Apply the sample to the SPE cartridge and allow the sample to completely enter the packing material.

7. Wash the column with 5 ml deionized water followed by 5 ml HPLC grade hexane (allow the cartridge to become dry after this step). Discard both washes.

8. Elute the PGE$_2$ and PGEM from the column with 5 ml ethyl acetate containing 1% methanol. Higher recovery and better reproducibility may be obtained if the sample is applied and eluted by gravity. The wash steps may be performed under vacuum or pressure.*

9. Evaporate the ethyl acetate to dryness under a stream of nitrogen. It is very important that all of the organic solvent be removed as even small quantities will adversely affect the EIA.

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

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

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

ASSAY PROTOCOL

Derivatization of Standards and Samples to PGEM

Hot Spike (continued)

Derivatization of Standards and Samples to PGEM

Derivatization Hints

- Allow the derivatization to proceed overnight to ensure that all the PGE$_2$ metabolites derivatize completely.
- Derivatize all standards and samples for the same amount of time.

Derivatization of the PGEM EIA 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 PGEM Standard (Catalog No. 414534) into a clean test tube, then dilute with 900 µl UltraPure water. The concentration of this solution (the bulk standard) will be 40 ng/ml.

Aliquot 50 µl of this solution into a clean tube and dilute to a total volume of 1 ml with EIA Buffer (i.e., add 950 µl). Add 300 µl of Carbonate Buffer and incubate at 37°C overnight. Then add 400 µl Phosphate Buffer and 300 µl EIA Buffer. This solution is 1,000 pg/ml.

Derivatization of the Samples

Aliquot 500 µl of each sample into a clean test tube. Add 150 µl of Carbonate Buffer and incubate at 37°C overnight. Then add 200 µl Phosphate Buffer and 150 µl EIA Buffer. The samples are now ready to assay. If you need to dilute your samples after derivatization, be sure to use the PGEM Assay Buffer.
Preparing the Standard Curve

NOTE: Because of the high salt concentration in the 1,000 pg/ml solution, all the points of the standard curve must contain the same salt concentration. Thus, when performing the serial dilution, use the PGEM Assay Buffer.

To prepare the standard for use in EIA: Obtain 8 clean test tubes and number them #1 through #8. Aliquot 950 µl PGEM Assay Buffer to tube #1 and 500 µl PGEM Assay Buffer to tubes #2-8. Transfer 50 µl of the derivatized standard (1,000 pg/ml) to tube #1 and mix thoroughly. Serially dilute the standard by removing 500 µl from tube #1 and placing it 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.

Preparation of Assay-Specific Reagents

PGEM AChE Tracer

Reconstitute the PGEM Tracer as follows:

100 dnl PGEM AChE Tracer (96-well kit; Catalog No. 414530): Reconstitute with 6 ml EIA Buffer.

OR

500 dnl PGEM AChE Tracer (480-well kit; Catalog No. 414530): Reconstitute with 30 ml EIA Buffer.

Store the reconstituted PGEM Tracer at 4°C (do not freeze!) and use within four weeks. A 20% surplus of PGEM 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).
PGEM EIA Antiserum

Reconstitute the PGEM Antiserum as follows:

100 dtn PGEM Antiserum (96-well kit; Catalog No. 414532): Reconstitute with 6 ml EIA Buffer.

OR

500 dtn PGEM Antiserum (480-well kit; Catalog No. 414532): Reconstitute with 30 ml EIA Buffer.

Store the reconstituted PGEM Antiserum at 4°C. It will be stable for at least four weeks. A 20% surplus of PGEM 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).

Plate Set Up

The 96-well plate(s) included with this kit is supplied ready to use. It is not necessary to rinse the plate(s) prior to adding the reagents. NOTE: If you do not need to use all the strips at once, place the unused strips back in the plate packet and store at 2-4°C. Be sure the packet is sealed with the desiccant inside.

Each plate or set of strips must contain a minimum of two blanks (Blk), two non-specific binding wells (NSB), two maximum binding wells (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 5, below. The user may vary the location and type of wells present as necessary for each particular experiment. The plate format provided below has been designed to allow for easy data analysis using a convenient spreadsheet offered by Cayman (see page 25, for more details). We suggest you record the contents of each well on the template sheet provided (see page 35).

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Blk - Blank
TA - Total Activity
NSB - Non-Specific Binding
B₀ - Maximum Binding
S₁-S₈ - Standards 1-8
1-24 - Samples

Figure 5. Sample plate format
Performing the Assay

**Pipetting Hints**
- Use different tips to pipette the buffer, standard, sample, tracer, and antiserum.
- 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. **PGEM Buffer**
   Add 50 µl EIA Buffer and 50 µl of PGEM Buffer to Non-Specific Binding (NSB) wells. Add 50 µl PGEM Buffer to Maximum Binding ($B_0$) wells.

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

3. **Samples**
   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. **PGEM AChE Tracer**
   Add 50 µl to each well except the Total Activity (TA) and the Blank (Blk) wells.

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

**Incubate the Plate**
Cover each plate with plastic film (Catalog No. 400012) and incubate for 18 hours at room temperature.

**Develop the Plate**

1. Reconstitute Ellman’s Reagent immediately before use (20 ml of reagent is sufficient to develop 100 wells):
   - **100 dtn vial Ellman’s Reagent (96-well kit; 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 be run on different days.

---

<table>
<thead>
<tr>
<th>Well</th>
<th>PGEM Buffer</th>
<th>EIA 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>
<td>-</td>
</tr>
<tr>
<td>TA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5 µl (at devl. step)</td>
<td>-</td>
</tr>
<tr>
<td>NSB</td>
<td>50 µl</td>
<td>50 µl</td>
<td>-</td>
<td>50 µl</td>
<td>-</td>
</tr>
<tr>
<td>$B_0$</td>
<td>50 µl</td>
<td>-</td>
<td>-</td>
<td>50 µl</td>
<td>50 µl</td>
</tr>
<tr>
<td>Std/Sample</td>
<td>-</td>
<td>-</td>
<td>50 µl</td>
<td>50 µl</td>
<td>50 µl</td>
</tr>
</tbody>
</table>

**Table 1. Pipetting Summary**
ASSAY PROTOCOL

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.

Read the Plate

1. Wipe the bottom of the plate with a clean tissue to remove fingerprints, dirt, etc.
2. Remove the plate cover being careful to keep Ellman’s Reagent from splashing on the cover. NOTE: Any loss of Ellman’s Reagent will affect the absorbance readings. If Ellman’s Reagent is present on the cover, use a pipette to transfer the Ellman’s Reagent into the well. If too much Ellman’s Reagent has splashed on the cover to easily redistribute back into the wells, wash the plate three times with Wash Buffer and repeat the development with fresh Ellman’s Reagent.
3. Read the plate at a wavelength between 405 and 420 nm. The absorbance may be checked periodically until the B₀ wells have reached a minimum of 0.3 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 plots data automatically. Alternatively a spreadsheet program can be used. The data should be plotted as %B/B₀ versus log concentration using either a 4-parameter logistic or log-logit curve fit. NOTE: Cayman has a computer spreadsheet available for data analysis. Please contact Technical Service or visit our website (www.caymanchem.com/analysis/eia) 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). Multiply by 100 to obtain %B/B₀. Repeat for S2-S8 and all sample wells.

NOTE: The total activity (TA) values are not used in the standard curve calculations. Rather, they are used as a diagnostic tool; the corrected 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/B_0 for standards S1-S8 versus PGEM concentration using linear (y) and log (x) axis and fit the data to a 4-parameter logistic equation.

Alternative Plot - The data can also be linearized using a logit transformation. The equation for this conversion is as follows, \textit{NOTE: Do not use %B/B_0 in this calculation:}

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

Plot the data as \text{logit } \left( \frac{B}{B_0} \right) \text{ versus log concentrations and perform a linear regression fit.}

Determine the Sample Concentration

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

\textbf{Hot Spike Method}

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

\[
\text{PGEM (pg) in purified sample } = \left[ \frac{\text{Value from EIA (pg/ml)}}{\text{Recovery Factor}} \right] \times 0.5 \text{ ml}
\]

\[
\text{Total PGEM in sample (pg/ml) } = \frac{\text{PGEM (pg) in purified sample}}{\text{Volume of sample used for purification (ml)}}
\]

\textbf{Cold Spike Method}

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

\[ V = \text{EIA determined concentration of the unspiked sample (pg/ml)} \]
\[ S = \text{concentration of the spike (pg/ml)} \]
\[ Y = \text{EIA determined concentration of the spiked sample (pg/ml)} \]

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

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

\[
\text{PGEM in original sample } = \frac{\text{PGEM (pg) in purified sample}}{\text{Volume of sample used for purification (ml)}}
\]
Performance Characteristics

Sample Data

The standard curve presented here is an example of the data typically produced with this kit; however, your results will not be identical to these. You must run a new standard curve - do not use this one to determine the values of your samples. Depending on the development conditions and the purity of the water used, your results could differ substantially from the data presented below.

<table>
<thead>
<tr>
<th>Total Activity</th>
<th>Raw Data</th>
<th>Average</th>
<th>Corrected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.752</td>
<td>1.965</td>
<td>1.859</td>
</tr>
<tr>
<td>NSB</td>
<td>-0.001</td>
<td>-0.003</td>
<td>-0.002</td>
</tr>
<tr>
<td>B₀</td>
<td>0.674</td>
<td>0.726</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.684</td>
<td>0.719</td>
<td>0.701</td>
</tr>
</tbody>
</table>

Table 2. Typical results

<table>
<thead>
<tr>
<th>Dose (pg/ml)</th>
<th>Raw Data</th>
<th>Corrected</th>
<th>%B/B₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>0.138</td>
<td>0.140</td>
<td>0.122</td>
</tr>
<tr>
<td>25</td>
<td>0.230</td>
<td>0.232</td>
<td>0.215</td>
</tr>
<tr>
<td>12.5</td>
<td>0.335</td>
<td>0.337</td>
<td>0.309</td>
</tr>
<tr>
<td>6.25</td>
<td>0.441</td>
<td>0.443</td>
<td>0.433</td>
</tr>
<tr>
<td>3.13</td>
<td>0.518</td>
<td>0.520</td>
<td>0.523</td>
</tr>
<tr>
<td>1.56</td>
<td>0.592</td>
<td>0.594</td>
<td>0.584</td>
</tr>
<tr>
<td>0.78</td>
<td>0.640</td>
<td>0.642</td>
<td>0.620</td>
</tr>
<tr>
<td>0.39</td>
<td>0.649</td>
<td>0.651</td>
<td>0.689</td>
</tr>
</tbody>
</table>

Figure 6. Typical standard curve

50% B/B₀ - 11 pg/ml
Detection Limit (80% B/B₀) - 2 pg/ml
Precision:
The intra- and inter-assay CV’s have been determined at multiple points on the standard curve. These data are summarized in the graph on page 29.

<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>50</td>
<td>8.1</td>
<td>18.2</td>
</tr>
<tr>
<td>25</td>
<td>5.4</td>
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<tr>
<td>12.5</td>
<td>5.9</td>
<td>8.3</td>
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<tr>
<td>6.25</td>
<td>5.5</td>
<td>11.2</td>
</tr>
<tr>
<td>3.13</td>
<td>12.8</td>
<td>8.0</td>
</tr>
<tr>
<td>1.56</td>
<td>25.1</td>
<td>13.4</td>
</tr>
<tr>
<td>0.78</td>
<td>23.7</td>
<td>39.3</td>
</tr>
<tr>
<td>0.39</td>
<td>N.D.</td>
<td>123</td>
</tr>
</tbody>
</table>

Table 3. Intra- and inter-assay Variation
*%CV represents the variation in concentration (not absorbance) of 40 repetitions of each point on the standard curve as determined using a reference standard curve.

Specificity:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cross-reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>13,14-dihydro-15-keto PGE₁ (derivatized)</td>
<td>100%</td>
</tr>
<tr>
<td>13,14-dihydro-15-keto PGE₂ (derivatized)</td>
<td>100%</td>
</tr>
<tr>
<td>Bicyclo Prostaglandin E₁</td>
<td>38%</td>
</tr>
<tr>
<td>Leukotriene B₄</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>tetranor-PGEM</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>tetranor-PGFM</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>Prostaglandin D₂</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>Prostaglandin E₁</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>6-keto Prostaglandin E₁</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>Prostaglandin E₂</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>Prostaglandin F₁α</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>6-keto Prostaglandin F₁α</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>Prostaglandin F₂α</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>Thromboxane B₂</td>
<td>&lt;0.01%</td>
</tr>
</tbody>
</table>

Table 4. Specificity of the PGEM Antiserum
## 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 (>0.035) | A. Poor washing  
B. Exposure of NSB wells to specific antibody | A. Rewash plate and redevelop |
| Very low B⁰ | A. Contamination of water with organic solvents  
B. Plate requires additional development time  
C. Dilution error in preparing reagents | A. Replace activated carbon filter or change source of UltraPure water  
B. Return plate to shaker and reread later |
| Low sensitivity (shift in dose response curve) | Standard is degraded | Replace standard |
| Analyses of two dilutions of a biological sample do not agree (i.e., more than 20% difference) | Interfering substances are present | Purify sample prior to analysis by EIA¹ |
| 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/514531/references](http://www.caymanchem.com/514531/references) for a list of publications citing the use of Cayman's PGEM Metabolite EIA Kit.

### References


### Related Products

- Prostaglandin E₂, EIA Kit - Monoclonal - Cat. No. 514010
- Prostaglandin E₂ EIA Kit - Monoclonal (Solid Plate) - Cat. No. 514010.1
- Prostaglandin Screening EIA Kit - Cat. No. 514012
- Prostaglandin Screening EIA Kit (Solid Plate) - Cat. No. 514012.1
- SPE Cartridges (C-18) - Cat. No. 400020
- STAT-Prostaglandin E₂, EIA Kit - Cat. No. 514131
- STAT-Prostaglandin E₂, EIA Kit (Solid Plate) - Cat. No. 514131.1
- UltraPure Water - Cat. No. 400000
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Cayman Chemical Company makes no warranty or guarantee of any kind, whether written or oral, expressed or implied, including without limitation, any warranty of fitness for a particular purpose, suitability and merchantability, which extends beyond the description of the chemicals hereof. Cayman warrants only to the original customer that the material will meet our specifications at the time of delivery. Cayman will carry out its delivery obligations with due care and skill. Thus, in no event will Cayman have any obligation or liability, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if Cayman is informed about their possible existence. This limitation of liability does not apply in the case of intentional acts or negligence of Cayman, its directors or its employees.

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

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

For further details, please refer to our Warranty and Limitation of Remedy located on our website and in our catalog.
<table>
<thead>
<tr>
<th>REF</th>
<th>Cat.-No.: / Kat.-Nr.: / No.-Cat.: / Cat.-No.: / N.º Cat.: / N.-Cat.:</th>
<th>Αριθµός-Κατ.:</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOT</td>
<td>Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.:</td>
<td>Αριθµός-Παραγωγή:</td>
</tr>
<tr>
<td></td>
<td>Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até:</td>
<td>Da utilizarrre entro: / Χρησιµοποιείται από:</td>
</tr>
<tr>
<td>CONC</td>
<td>Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συµπύκνωµα</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizzato / Λυοφιλιασµένο</td>
<td></td>
</tr>
<tr>
<td>LYO</td>
<td>In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Equipamiento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro.</td>
<td>Ιατρική συσκευή για In-Vitro Διάγνωση.</td>
</tr>
<tr>
<td></td>
<td>Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Leggere le istruzioni prima dell’uso.</td>
<td>Διαβάστε τις οδηγίες πριν την χρήση.</td>
</tr>
<tr>
<td></td>
<td>Keep away from heat or direct sunlight. / Vor Hitze und direkter Sonneneinstrahlung schützen.</td>
<td>Το κοιτάζεται από θερµότητα και άµεση επαφή µε το φως του ηλίου.</td>
</tr>
<tr>
<td></td>
<td>Store at: / Lagern bei: / Stocker à: / Almacenar a: / Armazenar a: / Conservare a:</td>
<td>Αποθήκευση στους:</td>
</tr>
<tr>
<td></td>
<td>Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante:</td>
<td>Παραγωγός:</td>
</tr>
<tr>
<td></td>
<td>Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione!</td>
<td>Προσοχή!</td>
</tr>
</tbody>
</table>

Symbols of the kit components see MATERIALS SUPPLIED.

Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben.

Voir MATERIEL FOURNI pour les symbôles des composants du kit.

Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS.

Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS.

Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT.

Για τα σύµβολα των συστατικών του κιτ συµβουλεύετε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

---

**LIB AFFILIATES WORLDWIDE**

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<thead>
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</tr>
</thead>
<tbody>
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</tr>
<tr>
<td>WEB: <a href="http://www.IBL-International.com">http://www.IBL-International.com</a></td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>IBL Deventer B.V.</th>
<th>Tel.: + 31 570-66 15 15 Fax: -60 73 86</th>
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<tbody>
<tr>
<td>Zutphenseweg 55, NL-7418 AH Deventer, The Netherlands</td>
<td>E-MAIL: <a href="mailto:IBL@IBL-International.com">IBL@IBL-International.com</a></td>
</tr>
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<table>
<thead>
<tr>
<th>IBL - Transatlantic Corp.</th>
<th>Toll free: +1 (866) 645 -6755</th>
</tr>
</thead>
<tbody>
<tr>
<td>288 Wildcat Road, Toronto, Ontario M3J 2N5</td>
<td>Tel.: +1 (416) 645 -1703 Fax: -1704</td>
</tr>
<tr>
<td>E-MAIL: <a href="mailto:IBL@IBL-Transatlantic.com">IBL@IBL-Transatlantic.com</a></td>
<td>WEB: <a href="http://www.IBL-Transatlantic.com">http://www.IBL-Transatlantic.com</a></td>
</tr>
</tbody>
</table>

---

**LIABILITY**: Complaints will only be accepted in written and if all details of the test performance and results are included (complaint form available from IBL or supplier). Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement. Regardless, in the event of any claim, the manufacturer’s liability is not to exceed the value of the test kit. Any damage caused to the kit during transportation is not subject to the liability of the manufacturer.

Symbols Version 3.5 / 2008-10-01