Prostaglandin E_2 EIA Kit - Monoclonal

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CONTENTS OF THE KIT

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

PRECAUTIONS

- Please read these instructions carefully before beginning this assay.
- The reagents in this kit have been tested and formulated to work exclusively with 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.

STORAGE AND STABILITY

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

ADDITIONAL ITEMS REQUIRED

1. A plate reader with a 405-420 nm filter.
2. An adjustable 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.

4. Materials used for sample preparation (see page 6).

ABOUT THIS ASSAY...

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

Our PGE_2 EIA has been validated for use with urine, plasma, and culture media samples. In general, urine and culture media samples can be diluted, if necessary, and added directly to the assay well. Plasma samples should be purified prior to use. Because of the rapid metabolism of PGE_2, the determination of *in vivo* PGE_2 biosynthesis is often best accomplished by the measurement of PGE_2 metabolites. Our Bicyclo PGE_2 assay converts all major PGE_2 metabolites into a single stable derivative which is easily measurable by EIA (see Figure 1).

Proper sample handling and preparation is the most important aspect of this assay. Please read the section of this booklet on sample preparation carefully before beginning.

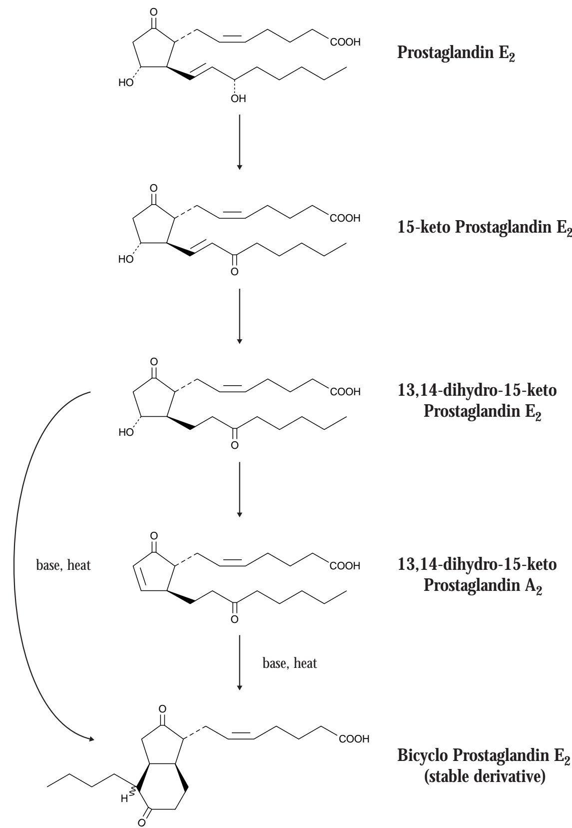


Figure 1. The metabolism of PGE_2

INTRODUCTION TO ACE™ EIAs

Description of the ACE™ Competitive Enzyme Immunoassay[4,5]

This assay is based on the competition between PGE_2 and a PGE_2-acetylcholinesterase 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 a goat anti-mouse polyclonal antibody 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 acetylcholinesterase) is added to the well. The product of this enzymatic reaction has a distinct yellow color and absorbs strongly at 412 nm. The intensity of this color, determined spectrophotometrically, is proportional to the amount of PGE_2 tracer bound to the well, which is inversely proportional to the amount of free PGE_2 present in the well during the incubation; or

$$\textbf{Absorbance} \propto \textbf{[Bound PGE}_2\textbf{ Tracer]} \propto \textbf{1/[PGE}_2\textbf{]}$$

A schematic of this process is shown in Figure 2.

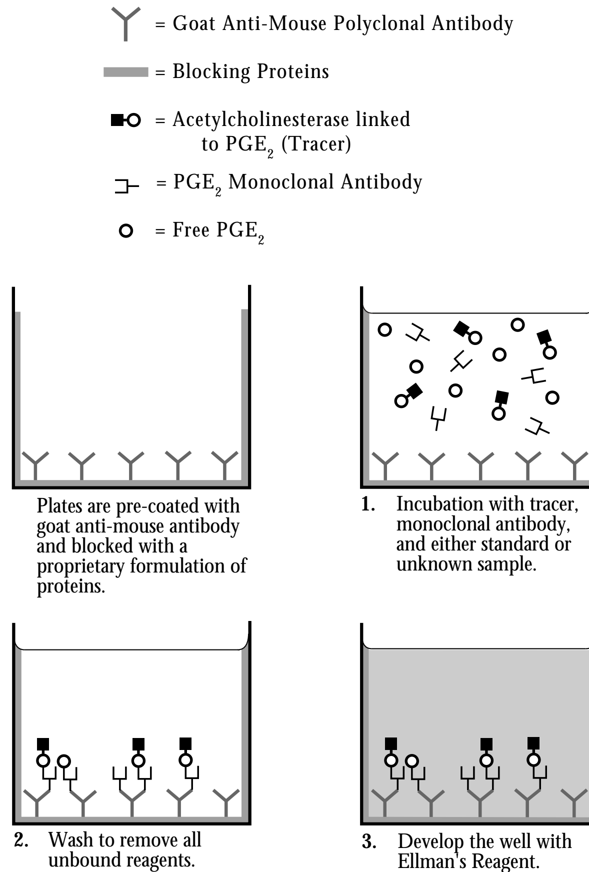


Figure 2. Schematic of the ACE™ EIA

Biochemistry of ACE™ EIAs

The electric organ of the electric eel, *Electrophorus electricus*, contains an avid acetylcholinesterase capable of massive catalytic turnover during the generation of its electrochemical discharges. The electric eel acetylcholinesterase 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 acetylcholinesterase serves as the tracer in ACE™ enzyme immunoassays. Quantification of the tracer is achieved by measuring its acetylcholinesterase activity with Ellman's Reagent. This reagent consists of acetylthiocholine and 5,5'-dithio-*bis*-(2-nitrobenzoic acid). Hydrolysis of acetylthiocholine by acetylcholinesterase 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 (ε = 13,600).

Acetylcholinesterase has several advantages over other enzymes commonly used for enzyme immunoassays. Unlike horseradish peroxidase, acetylcholinesterase does not auto-inactivate during turnover. This property of acetylcholinesterase allows multiple development of the assay. 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 and preservatives.

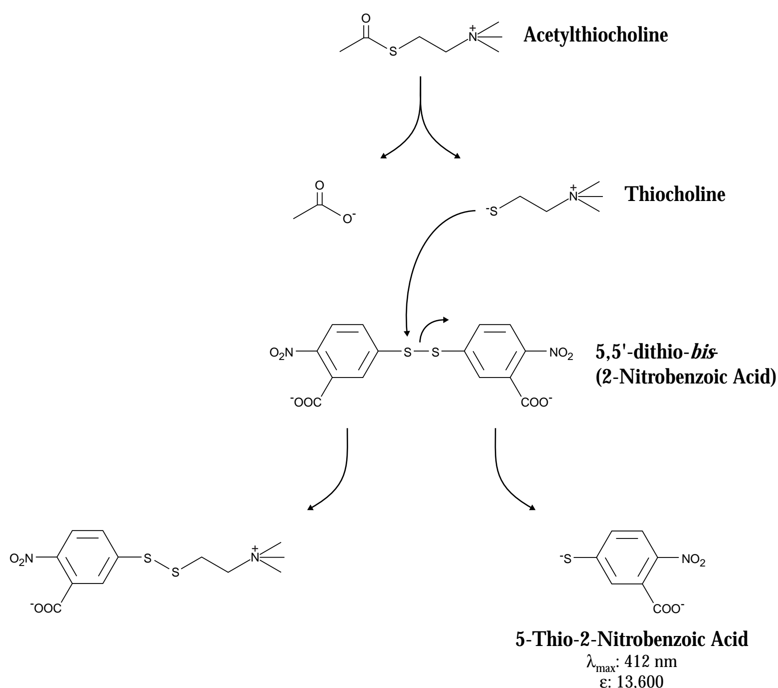


Figure 3. Reaction catalyzed by Acetylcholinesterase

Definition of Key Terms

Blank: background absorbance caused by Ellman's Reagent. Even freshly prepared Ellman's Reagent has some measurable absorbance, approximately 0.1 Absorbance Units (A.U.). The blank absorbance should be subtracted from the absorbance readings of all the other wells.

Total Activity: total enzymatic activity of the acetylcholinesterase-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 antiserum 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 antiserum can bind in the absence of free analyte.

%B/B_0 (%Bound/Maximum Bound): ratio of the absorbance of a particular 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.

PRE-ASSAY PREPARATION

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.

Buffer Preparation *(Store all buffers at 4°C)*

1. **EIA Buffer Preparation**

   Dilute the contents of one vial of EIA Buffer Concentrate (vial #4) with 90 ml of UltraPure water. Be certain to rinse the vial to remove any salts that may have precipitated (NOTE: It is normal for the concentrated buffer to contain crystalline salts after thawing. These will completely dissolve upon dilution with water.).

2. **Wash Buffer Preparation**

   Dilute the contents of the vial (5 ml) of Wash Buffer Concentrate (vial #5) to a total volume of 2 liters with UltraPure water and add 1 ml of Tween 20 (vial #5a), or dilute the contents of the vial (12.5 ml) of Wash Buffer Concentrate (vial #5) to a total volume of 5 liters with UltraPure water and add 2.5 ml of Tween 20 (vial #5a). (NOTE: Tween 20 is a viscous liquid and cannot be measured by a pipet. A positive displacement device such as a syringe should be used to deliver small quantities accurately.) A smaller volume 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).

Sample Preparation

This assay has been validated for a wide range of samples including urine (diluted at least 1:2), plasma (diluted at least 1:50), and tissue culture media (see Figure 4). Proper sample storage and preparation are essential for consistent and accurate results. Please read this section thoroughly before beginning the assay.

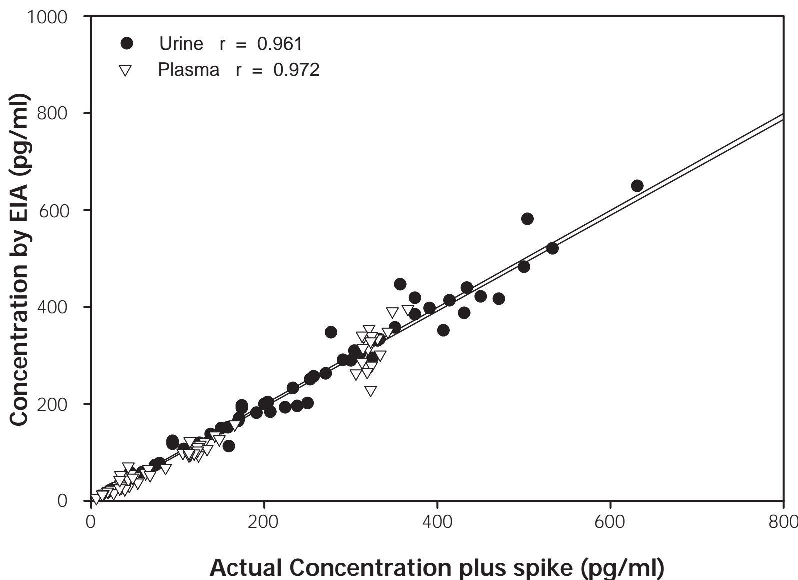


Figure 4. Validation curves for this PGE_2 assay

General Precautions

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

1. Urine Samples

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 Bicyclo PGE_2 assay

2. Plasma Samples

Plasma is a complex matrix that contains many substances that can interfere with this assay. We have found that plasma samples must be diluted at least 1:50 in EIA Buffer before interference is minimized and consistent results are obtained. However, at this dilution the PGE_2 content of normal samples (3-12 pg/ml) will be well below the detection limit of this assay.[3] These samples must be purified prior to assay (see the purification protocol in step 5 below). By subjecting a large volume of sample (5-10 ml) to this procedure, the PGE_2 content can be concentrated into as little as 0.5 ml of EIA Buffer. This will bring the PGE_2 concentration into the readable range of the standard curve.

Plasma samples should be collected in vacutainers containing sodium heparin, EDTA, or sodium citrate. Indomethacin should be added immediately after plasma collection (sufficient to give a 10 µM final concentration).

As with urine, the amount of PGE_2 in normal plasma is very low in comparison with other potentially immunoreactive metabolites.[2] A more accurate index of PGE_2 biosynthesis can be obtained using our Bicyclo PGE_2 assay.

3. Culture Media Samples

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

4. Tissue Samples

A. Add 1 ml homogenization buffer (0.1 M phosphate, pH 7.4, containing 1 mM EDTA and 10 µM indomethacin) to 1 g of tissue.

B. Add 10,000 cpm of tritium-labeled PGE_2 ($[^3H]$-PGE_2). We recommend that a high specific activity tracer be used in order to minimize the amount of radioactive PGE_2 added. The EIA will be able to detect the added PGE_2 and therefore the amount added should be insignificant in comparison to the endogenous analyte, yet should be sufficient for accurate scintillation counting.

C. Homogenize the sample with either a Polytron-type homogenizer or a sonicator.

D. Add an equal volume of ethanol to the sample and vortex. Leave the sample at room temperature for 5 minutes.

E. Remove the precipitate by centrifugation at 1500 x g for 10 minutes. Carefully pour the supernatant into a clean test tube.

F. Dilute the sample with 50 mM acetate buffer or citrate buffer (pH 4) until the ethanol concentration is below 15%. Proceed to step D in the Purification Protocol.

5. **Purification Protocol**

In general, heterogeneous mixtures (e.g., lavage fluids and plasma) must be purified before assay. If the concentration of the analyte is too low to be assayed directly, the best way to concentrate the sample is to purify a larger volume of the sample and reconstitute in a small amount of EIA Buffer. Although this assay has been validated for urine and plasma, sample-specific interferences may lead to erroneous results at times. Hence, it is prudent to purify the sample if consistent results are not obtained.[6] If purification is necessary for your samples, follow the steps listed below. NOTE: An immunoaffinity column may also be used to purify samples. In general, this column is easier to use and offers better recovery than solid phase extraction (SPE).

A. 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 aliquot will be concentrated by a factor of 10, a 10 ml aliquot will be concentrated by a factor of 20, etc.).

B. Add 10,000 cpm of tritium-labeled PGE_2 ([^{3}H]-PGE_2). We recommend that a high specific activity tracer be used in order to minimize the amount of radioactive PGE_2 added. The EIA will be able to detect the added PGE_2 and therefore the amount added should be insignificant in comparison to the endogenous analyte, yet should be sufficient for accurate scintillation counting.

C. Adjust the pH of the sample to ~4.0 using 50 mM acetate buffer or citrate buffer (pH 4). 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, resulting in loss of the sample.

D. Activate a 6 ml SPE cartridge by rinsing with 5 ml methanol and then with 5 ml UltraPure water. Do not allow the SPE cartridge to dry.

E. Pass the sample through the SPE cartridge. Rinse the cartridge with 5 ml UltraPure water (allow the cartridge to become dry after this step) followed by 5 ml of HPLC grade hexane. Discard both washes. Elute the PGE_2 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.

F. Remove 10% of the eluate for scintillation counting.*

G. Evaporate the ethyl acetate to dryness either by vacuum centrifugation or by evaporation under a stream of dry nitrogen. It is imperative that all of the organic solvent be removed as even trace quantities will adversely affect the EIA.

H. Add 450 µl of EIA Buffer and vortex. Use this for EIA analysis. It is common for an insoluble precipitate to remain after the addition of EIA buffer; this will not affect the assay.

*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.

The sample may be further purified by thin-layer chromatography (TLC) to ensure the accuracy of the results. This procedure may remove other eicosanoid metabolites or structurally unrelated compounds that may exhibit significant crossreactivity or interference with the assay. To purify the sample by TLC, skip step F above, dry the sample (step G), and follow the steps outlined below:

1. Dissolve the sample in a small amount of acetone and spot in the preadsorbent zone of a channeled 20 x 20 cm TLC plate without any fluorescent indicator (e.g., Analtech 31911, Whatman 4865-821). The preadsorbent zone of the plates will concentrate the sample into a thin line at the solvent front so there is no need for special precautions when spotting the sample. Authentic PGE_2 must be spotted on one of the edge lanes of each plate to help locate the appropriate band in your sample. Develop the plate using chloroform/methanol/acetic acid/water (80/18/1/0.8).
2. After the solvent has traveled to the top of the plate, remove the plate from the solvent chamber and allow to dry. The band containing PGE_2 may be detected by masking the sample lanes and spraying the ones containing authentic standard with phosphomolybdic acid (3.5% in methanol) and carefully heating, or by spreading a mixture of iodine and silica gel on the standard lane and removing after 5 minutes (preferred).
3. Carefully scrape the band corresponding to PGE_2 from each lane onto separate pieces of weighing paper and transfer into clean test tubes. Elute the samples by adding 2 ml ethanol, vortexing, and then centrifuging at 1500 x g for 10 minutes. Decant the supernatant into a clean test tube and remove 10% for scintillation counting. Evaporate the remaining solvent using either a vacuum centrifuge or a stream of dry nitrogen.
4. Add 450 µl EIA buffer to the sample tubes and vortex. Use this for EIA analysis. It is normal for an insoluble precipitate to remain after the addition of EIA buffer; this will not affect the assay.

Reconstitution of Reagents

1. Prostaglandin E_2 Standard

Equilibrate a pipet tip in ethanol by repeatedly filling and expelling the tip with ethanol several times. Using the equilibrated pipet tip, transfer 100 µl of the PGE_2 Standard (vial #3) into a clean test tube, then dilute with 900 µl UltraPure water. The concentration of this solution (the bulk standard) will be 10 ng/ml. Store this solution at 4°C; it will be stable for approximately six weeks.

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

To prepare the standard for use in EIA: Obtain 8 clean test tubes and number them #1 through #8. Aliquot 900 µl EIA Buffer to tube #1 and 500 µl EIA Buffer to tubes #2-8. Transfer 100 µl of the bulk standard (10 ng/ml) 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.

2. Prostaglandin E_2 Acetylcholinesterase Tracer

Reconstitute the 100 dtn PGE_2 Tracer (vial #2) with 6 ml EIA Buffer or the 500 dtn PGE_2 Tracer (vial #2) with 30 ml EIA Buffer. Store the reconstituted PGE_2 Tracer at 4°C (do not freeze!) and use within one week. A 20% surplus of PGE_2 Tracer has been included to account for any incidental losses.

3. Prostaglandin E_2 Monoclonal Antibody

Reconstitute the 100 dtn PGE_2 Antibody (vial #1) with 6 ml EIA Buffer or the 500 dtn PGE_2 Antibody (vial #1) with 30 ml EIA Buffer. Store the reconstituted PGE_2 Antibody at 4°C. It will be stable for at least four weeks. A 20% surplus of PGE_2 Antibody has been included to account for any incidental losses.

PERFORMING THE ASSAY

Plate Set Up

Each plate or set of strips must contain a minimum of two blanks (B), two non-specific binding wells (NSB), two maximum binding wells (B_0), and an eight point standard curve run in duplicate. *(NOTE: Each assay must contain this minimum configuration in order to ensure accurate and reproducible results.)* Each sample should be assayed at two dilutions and each dilution should be assayed in duplicate.

Two suggested plate formats are shown in Figure 5. The user may vary the location and type of wells present as necessary for each particular experiment. We suggest you record the contents of each well on the template sheet provided (see page 15).

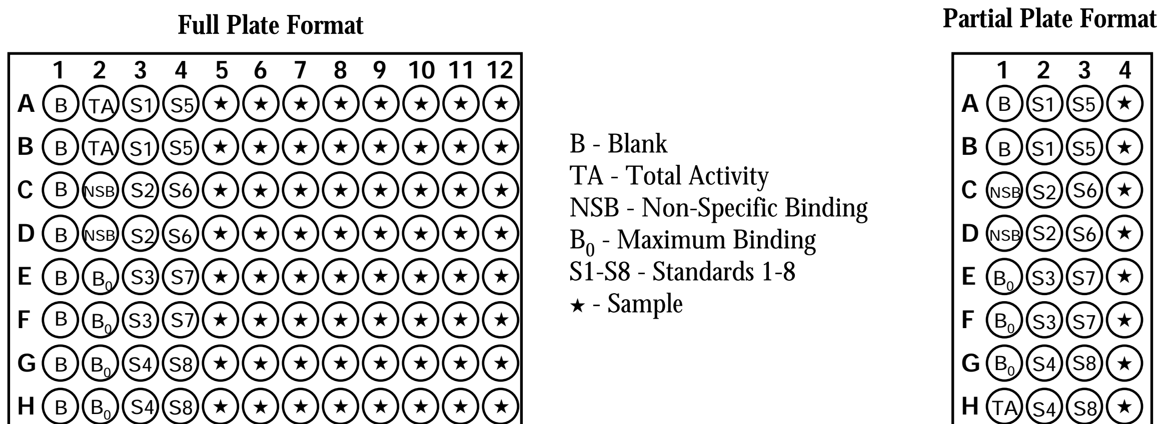


Figure 5. Sample plate formats

Plate Preparation

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.)

Pipet the Reagents

PIPETTING HINTS

- **Use different tips to pipet the buffer, standard, sample, tracer, and antibody.**
- **Before pipetting each reagent, equilibrate the pipet tip in that reagent (i.e., slowly fill the tip and gently expel the contents, repeat several times).**
- **Do not expose the pipet tip to the reagent(s) already in the well.**

1. **EIA Buffer**

   Add 100 µl EIA Buffer to Non-Specific Binding (NSB) wells. Add 50 µl EIA Buffer to Maximum Binding (B_0) wells. If culture medium was used to dilute the standard curve, substitute 50 µl of culture medium for EIA buffer in the NSB and B_0 wells (i.e., add 50 µl culture medium to NSB and B_0 wells and 50 µl EIA Buffer to NSB wells).

2. **Prostaglandin E_2 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 pipet tip should be used to aliquot all the standards. Before pipetting each standard, be sure to equilibrate the pipet 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.

4. **Prostaglandin E_2 Acetylcholinesterase Tracer**

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

5. **Prostaglandin E_2 Monoclonal Antibody**

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

Incubate the Plate

Cover each plate with plastic film (item #7) and incubate for 18 hours at 4°C or room temperature. Incubation at 4°C will significantly increase the sensitivity of the assay.

Develop the Plate

When ready to develop the plate(s), reconstitute one 100 dtn vial of Ellman's Reagent (vial #8) with 20 ml of UltraPure water, or reconstitute one 250 dtn vial of Ellman's Reagent (vial #8) with 50 ml of UltraPure water (20 ml of reagent is sufficient to develop 100 wells). 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.

Empty the wells and rinse five times with Wash Buffer. Add 200 µl of Ellman's Reagent to each well and 5 µl of tracer to the Total Activity wells. 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 equal 0.2 A.U.) in 60-90 minutes.

Read the Plate

The plate(s) should be read at a single wavelength between 405 and 420 nm. Before reading each plate, wipe the bottom of the plate with a clean tissue to remove finger prints, dirt, etc., as smudges on the bottom of the plate can significantly alter absorbance readings. Be certain that the Ellman's Reagent has not splashed up on the plate cover as any loss of Ellman's Reagent will affect the absorbance readings. If it did, use a pipet to remove the Ellman's Reagent from the cover and place into the well. If too much Ellman's Reagent has splashed on the cover to easily redistribute back into the wells, wash the plate 3 times with Wash Buffer and repeat the development with fresh Ellman's Reagent.

The plate may be checked periodically until the B_0 wells have reached a minimum of 0.2 A.U. The plate should be read when the absorbance of the B_0 wells is in the range of 0.3-0.8 A.U. If the absorbance of the wells exceeds 1.5, wash the plate, add fresh Ellman's Reagent and let it develop again.

CALCULATING THE RESULTS

It is usually more convenient to calculate the assay results by computer; most plate readers come with data reduction software, or a spreadsheet program can be used (4-parameter or log-logit curve fit). If the results need to be calculated manually, the procedure is as follows:

NOTE: If the plate reader has not subtracted the absorbance readings of the blank wells from the absorbance readings of the rest of the plate, be sure to do that now.

1. Average the absorbance readings from the NSB wells.
2. Average the absorbance readings from the B_0 wells.
3. Subtract the NSB average from the B_0 average. This is the corrected B_0 or corrected maximum binding.
4. Calculate the $\%B/B_0$ (% Sample or Standard Bound/Maximum Bound) for the remaining wells. To do this, subtract the average NSB absorbance from the S1 absorbance and divide by the corrected B_0 (from Step 3). Multiply by 100 to obtain $\%B/B_0$ Repeat for S2-S8 and all sample wells.
5. The total activity 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 12). 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 13 for Troubleshooting).

Plotting the Standard Curve

Plot $\%B/B_0$ for standards S1-S8 versus PGE_2 concentration (usually in pg/ml) on semi-log paper.

Determining the Concentration of your Samples

Calculate the $\%B/B_0$ value for each sample. Determine the concentration of each sample by identifying the $\%B/B_0$ on the standard curve and reading the corresponding values on the x-axis. $\%B/B_0$ values greater than 80% and 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.

Calculations

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

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

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

PERFORMANCE CHARACTERISTICS

Precision

The intra- and interassay CV is ≤10%.

Specificity

Compound	Cross Reactivity	Compound	Cross Reactivity
Prostaglandin E_2	100%	Prostaglandin B_1	<0.01%
Prostaglandin E_2 ethanolamide	100%	Prostaglandin B_2	<0.01%
Prostaglandin E_3	43%	Prostaglandin D_2	<0.01%
Prostaglandin E_1	18.7%	15-keto Prostaglandin E_2	<0.01%
6-keto Prostaglandin $F_{1\alpha}$	1%	19(R)-hydroxy Prostaglandin E_2	<0.01%
8-*iso* Prostaglandin $F_{2\alpha}$	0.25%	Prostaglandin $F_{1\alpha}$	<0.01%
Arachidonic acid	<0.01%	Prostaglandin $F_{2\alpha}$	<0.01%
Misoprostol	<0.01%	13,14-dihydro-15-keto Prostaglandin $F_{2\alpha}$	<0.01%
Misoprostol (free acid)	<0.01%	Prostaglandin $F_{3\alpha}$	<0.01%
Prostaglandin A_1	<0.01%	Tetranor PGEM	<0.01%
Prostaglandin A_2	<0.01%	Tetranor PGFM	<0.01%
Prostaglandin A_3	<0.01%	Thromboxane B_2	<0.01%

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. *(NOTE: Raw data is reported in milli-Absorbance Units.)*

Room Temperature Incubation

	Raw Data		Average	Corrected
Total Activity	689	692	690.5	
NSB	3	0	1.5	
B_0	457	456	461	459.5
	468	463		

Standard Curve

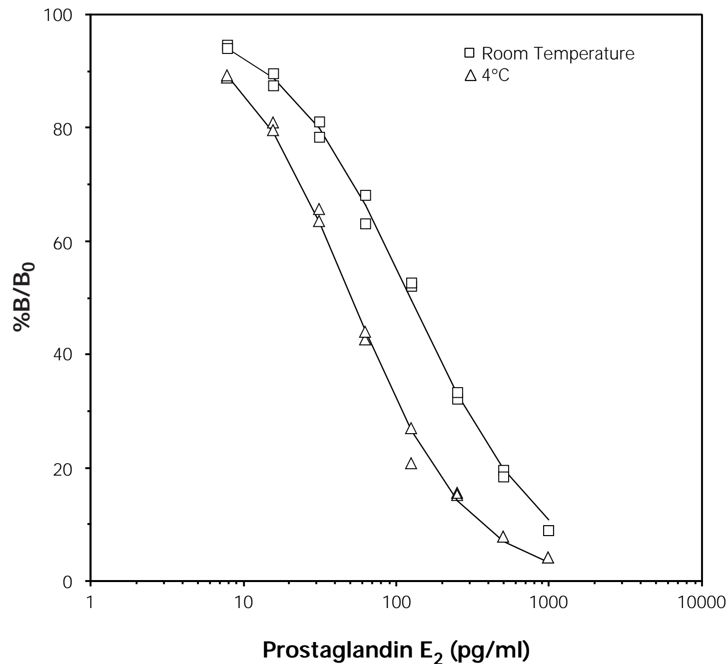
Dose (pg/ml)	Raw Data		Corrected		%B/B_0	
1000	42	42	40.5	40.5	8.8	8.8
500	91	86	89.5	84.5	19.5	18.4
250	149	154	147.5	152.5	32.1	33.2
125	241	243	239.5	241.5	52.1	52.6
62.5	291	314	289.5	312.5	63	68
31.3	374	361	372.5	359.5	81.1	78.2
15.6	403	413	401.5	411.5	87.4	89.6
7.8	437	430	435.5	428.5	94.8	93.3

4°C Incubation

	Raw Data		Average	Corrected
Total Activity	689	692	690.5	
NSB	8	2	5	
B_0	319	327	324.8	319.8
	328	325		

Standard Curve

Dose (pg/ml)	Raw Data		Corrected		%B/B_0	
1000	18	34	13	29	4.1	9.1
500	40	40	35	35	10.9	10.9
250	64	61	59	56	18.5	17.5
125	100	84	95	79	29.7	24.7
62.5	139	147	134	142	41.9	44.4
31.3	230	239	225	234	70.4	73.2
15.6	282	234	277	229	86.6	71.6
7.8	287	260	282	255	88.2	79.7



Room Temperature Incubation

50% B/B_0 - 123 pg/ml
Detection Limit (80% B/B_0) - 31 pg/ml

4°C Incubation

50% B/B_0 - 51 pg/ml
Detection Limit (80% B/B_0) - 15 pg/ml

TROUBLESHOOTING

Problem: Erratic values; dispersion of duplicates.
Causes: Trace organic contaminants in the water source; replace activated carbon filter or change source of UltraPure water. *-or-* Poor pipetting/technique.

Problem: High NSB.
Causes: Poor washing. *-or-* Exposure of NSB wells to specific antibody.

Problem: Very Low B_0.
Causes: Contamination of water with organic solvents. *-or-* Plate requires additional development time. *-or-* Dilution error in preparing reagents.

Problem: Low Sensitivity (shift in dose response curve).
Cause: Standard is degraded.

Problem: Analyses of two dilutions of a biological sample do not agree (i.e. more than 20% difference).
Cause: Interfering substances are present. Sample must be purified prior to analysis by EIA.[6]

Problem: Only Total Activity (TA) wells develop.
Cause: Trace organic contaminants in the water source; replace activated carbon filter or change source of UltraPure water.

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PLATE TEMPLATE

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

NOTES