11β-Prostaglandin F$_{2\alpha}$ ELISA

Enzyme immunoassay for the quantitative determination of 11β-Prostaglandin F$_{2\alpha}$.

**REF** CM516521

Σ 96/480

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

Distributed by:

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11β-Prostaglandin F$_{2α}$ EIA Kit
Catalog No. 516521 (Strip Plate)
Catalog No. 516521.1 (Solid Plate)
Materials Supplied

<table>
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<th>Catalog Number</th>
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<th>96 wells Quantity/Size</th>
<th>480 wells Quantity/Size</th>
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<td>400062</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 D\(_2\) (PGD\(_2\)) is the major eicosanoid released from stimulated mast cells. PGD\(_2\) is an unstable compound which is rapidly metabolized and eliminated from the circulation. For this reason, quantitation of PGD\(_2\) is an unreliable indicator of its in vivo production. \(\text{PGD}_2\) is the primary plasma metabolite of PGD\(_2\) in vivo, the levels of which can increase from 6 pg/ml in a normal healthy volunteer to 490 ng/ml in patients with systemic mastocytosis. The normal human urinary excretion of \(\text{PGF}_{2\alpha}\) is about 11 ng/mmol creatinine (approximately 400 ng/24 hr.), which is increased nearly three-fold upon allergen-induced bronchoconstriction in asthmatics. Unlike most PG metabolites, \(\text{PGF}_{2\alpha}\) retains potent biological activity. \(\text{PGF}_{2\alpha}\) is equipotent to PGF\(_{2\alpha}\) in inducing human bronchial smooth muscle contractions and inhibition of adipose differentiation. About This Assay

Cayman's \(\text{PGF}_{2\alpha}\) EIA Kit is a competitive assay that provides accurate measurements of \(\text{PGF}_{2\alpha}\) within the range of 4.1-400 pg/ml, typically with a detection limit (80% B/B\(_0\)) of -5-10 pg/ml. Inter- and intra-assay CV's of less than 20% can be achieved at most concentrations of the standard curve. This assay allows sensitive detection of \(\text{PGF}_{2\alpha}\) in urine, the most common sample matrix. Plasma concentrations of \(\text{PGF}_{2\alpha}\) are generally below the detection limit of the assay. For plasma and other complex sample matrices, we recommend purification of the sample prior to analysis. A purification protocol is included in this kit booklet (see page 12). Measurements of \(\text{PGF}_{2\alpha}\) in urine using our EIA gives values in the same range but slightly higher than those obtained by GC-MS. This appears to be due to measurement of \(\text{PGF}_{2\alpha}\) plus the 2,3-dinor metabolite of \(\text{PGF}_{2\alpha}\). Description of ACE\(^\text{TM}\) Competitive EIAs

This assay is based on the competition between \(\text{PGF}_{2\alpha}\) and an \(\text{PGF}_{2\alpha}\)-acetylcholinesterase (AChE) conjugate (\(\text{PGF}_{2\alpha}\) tracer) for a limited number of \(\text{PGF}_{2\alpha}\)-specific rabbit antiserum binding sites. Because the concentration of the \(\text{PGF}_{2\alpha}\) tracer is held constant while the concentration of \(\text{PGF}_{2\alpha}\) varies, the amount of \(\text{PGF}_{2\alpha}\) tracer that is able to bind to the rabbit antiserum will be inversely proportional to the concentration of \(\text{PGF}_{2\alpha}\) in the well. This rabbit antiserum-11\(\beta\)-\(\text{PGF}_{2\alpha}\) (either free or tracer) complex binds to the rabbit IgG mouse monoclonal 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 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 \(\text{PGF}_{2\alpha}\) tracer bound to the well, which is inversely proportional to the amount of free \(\text{PGF}_{2\alpha}\) present in the well during the incubation; or

\[
\text{Absorbance} \propto \frac{\text{Bound } \text{PGF}_{2\alpha} \text{ Tracer}}{1/\text{PGF}_{2\alpha}}
\]

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

Figure 1. Schematic of the ACE\(^\text{TM}\) EIA
Biochemistry of Acetylcholinesterase

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

A molecule of the analyte covalently attached to a molecule of AChE serves as the tracer in ACE™ enzyme immunoassays. Quantification of the tracer is achieved by measuring its AChE activity with Ellman's Reagent. This reagent consists of acetylthiocholine and 5,5'-dithio-bis-(2-nitrobenzoic acid). Hydrolysis of acetylthiocholine by AChE produces thiocholine (see Figure 2, on page 9). 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.

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**Figure 2. Reaction catalyzed by acetylcholinesterase**
Definition of Key Terms

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

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

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

**B₀ (Maximum Binding)**: maximum amount of the tracer that the antiserum 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.

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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).
     
   - OR

   - 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.
Sample Preparation

In general, urine and tissue culture supernatant samples may be diluted with EIA buffer and added directly to the assay well.

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.

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 \textit{ex vivo} formation of eicosanoids, which have the potential to interfere with this assay.

Testing for Interference

Plasma, serum, whole blood, as well as other heterogeneous mixtures such as lavage fluids and aspirates often contain contaminants which can interfere in the assay. The presence of rabbit IgG in the sample will cause interference 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 10 and 350 pg/ml. If the two dilutions of the sample show good correlation (differ by 20% or less) in the final calculated 11β-PGF\textsubscript{20} concentration, purification is not required. If you do not see good correlation of the different dilutions, purification is advised. The Purification Protocol below is one such method.

Determination of Recovery

Determination of percent recovery is recommended when any sample purification is performed. Detailed below is one method that can be employed to monitor the recovery. The sample should be split prior to purification and an appropriate amount of 11β-PGF\textsubscript{20} added to one aliquot. Both samples are then processed and the spiked sample is assayed \textit{via} EIA alongside the unspiked sample. Calculations are found in the \textit{Analysis} section on page 21.

Extraction - Cold Spike Method

1. Aliquot a known amount of each sample into each of two tubes. Label the first tube ‘sample #’ and the second ‘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 11β-PGF\textsubscript{20} to the ‘sample + spike’ tubes. Follow the procedure below for both spiked and unspiked samples.
3. 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. \textit{NOTE: Precipitation of proteins using ethanol is optional and may not be needed if samples are clean enough to flow through the SPE (solid phase extraction) (C-18) cartridge. Body fluids such as plasma and urine can typically be applied directly to the SPE (C-18) cartridge after acidification (step 4). Transfer the supernatant to a clean test tube. Evaporate the ethanol under nitrogen.}
4. Adjust the pH of the sample to ~4.0 using 1.0 M acetate buffer or citrate buffer (pH 4.0). (Standardize the pH adjustment using the sample matrix prior to proceeding with a large number of samples; approximately 1-2 equivalents of buffer are required for most biological samples.) If the samples are cloudy or contain precipitate, either filter or centrifuge to remove the precipitate. Particulate matter in the sample may clog the SPE (C-18) cartridge, resulting in loss of the sample.
5. Activate a 6 ml SPE (C-18) cartridge (Catalog No. 400020) by rinsing with 5 ml methanol and then with 5 ml UltraPure water. Do not allow the SPE (C-18) cartridge to dry.
6. Pass the sample through the SPE (C-18) cartridge. 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.* Rinse the cartridge with 5 ml UltraPure water followed by 5 ml of HPLC grade hexane (allow the cartridge to become dry after this step). Discard both washes. Elute the 11β-PGF$_{2α}$ with 5 ml ethyl acetate containing 1% methanol.

7. Evaporate the ethyl acetate to dryness 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.

8. Add 500 µl of EIA Buffer and vortex. 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 THF at -20°C or -80°C.

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**ASSAY PROTOCOL**

### Preparation of Assay-Specific Reagents

#### 11β-Prostaglandin F$_{2α}$ 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 11β-PGF$_{2α}$ Standard (Catalog No. 416524) 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.

**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 750 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.

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**Figure 3. Preparation of the 11β-PGF$_{2α}$ standards**
11β-Prostaglandin F<sub>2α</sub> AChE Tracer
Reconstitute the 11β-PGF<sub>2α</sub> Tracer as follows:

100 dtn 11β-PGF<sub>2α</sub> AChE Tracer (96-well kit; Catalog No. 416520): Reconstitute with 6 ml EIA Buffer.

OR

500 dtn 11β-PGF<sub>2α</sub> AChE Tracer (480-well kit; Catalog No. 416520): Reconstitute with 30 ml EIA Buffer.

Store the reconstituted 11β-PGF<sub>2α</sub> Tracer at 4°C (do not freeze!) and use within four weeks. A 20% surplus of 11β-PGF<sub>2α</sub> 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).

11β-Prostaglandin F<sub>2α</sub> EIA Antiserum
Reconstitute the 11β-PGF<sub>2α</sub> Antiserum as follows:

100 dtn 11β-PGF<sub>2α</sub> Antiserum (96-well kit; Catalog No. 416522): Reconstitute with 6 ml EIA Buffer.

OR

500 dtn 11β-PGF<sub>2α</sub> Antiserum (480-well kit; Catalog No. 416522): Reconstitute with 30 ml EIA Buffer.

Store the reconstituted 11β-PGF<sub>2α</sub> Antiserum at 4°C. It will be stable for at least four weeks. A 20% surplus of 11β-PGF<sub>2α</sub> 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 (B<sub>0</sub>), and an eight point standard curve run in duplicate. NOTE: Each assay must contain this minimum configuration in order to ensure accurate and reproducible results. Each sample should be assayed at two dilutions and each dilution should be assayed in duplicate. For statistical purposes, we recommend assaying samples in triplicate.

A suggested plate format is shown in Figure 4, below. The user may vary the location and type of wells present as necessary for each particular experiment. The plate format provided below has been designed to allow for easy data analysis using a convenient spreadsheet offered by Cayman (see page 21, for more details). We suggest you record the contents of each well on the template sheet provided (see page 31).

![Figure 4. Sample plate format](image-url)
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. **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. **11β-Prostaglandin F$_{2α}$ 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. **11β-Prostaglandin F$_{2α}$ AChE Tracer**  
   Add 50 µl to each well except the Total Activity (TA) and the Blank (Blk) wells.

5. **11β-Prostaglandin F$_{2α}$ 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 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 run on different days.

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<td>-</td>
<td>-</td>
<td>-</td>
</tr>
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<td>TA</td>
<td>-</td>
<td>-</td>
<td>5 µl (at devl. step)</td>
<td>-</td>
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<tr>
<td>NSB</td>
<td>100 µl</td>
<td>-</td>
<td>50 µl</td>
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<td>50 µl</td>
<td>50 µl</td>
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</table>

**Table 1. Pipetting summary**
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., B\text{0} 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 B\text{0} 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 plot data automatically. Alternatively a spreadsheet program can be used. The data should be plotted as %B/B\text{0} 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 B\text{0} wells.
3. Subtract the NSB average from the B\text{0} average. This is the corrected B\text{0} or corrected maximum binding.
4. Calculate the %B/B\text{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\text{0} (from Step 3). Multiply by 100 to obtain %B/B\text{0}. 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 B\text{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 24). 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 28 for Troubleshooting).
Plot the Standard Curve
Plot \( \%B/B_0 \) for standards S1-S8 versus 11\( \beta \)-PGF\(_{2\alpha} \) 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, \( \text{NOTE: Do not use } \%B/B_0 \text{ in this calculation:} \)
\[
\text{logit} \left( \frac{B}{B_0} \right) = \ln \left( \frac{B}{B_0} / (1 - B/B_0) \right)
\]
Plot the data as logit \( (B/B_0) \) 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 plot, \( \text{NOTE: Remember to account for any concentration or dilution of the sample prior to the addition to the well.} \) 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.

Cold Spike Method
The original concentration of the sample and recovery factor can be determined by the following method:
\( V = \text{EIA determined concentration of the unspiked sample (pg/ml)} \)
\( S = \text{concentration of the spike (pg/ml)} \)
\( Y = \text{EIA determined concentration of the spiked sample (pg/ml)} \)

\[
\text{Purification Recovery Factor} = \left[ \frac{Y - V}{S} \right]
\]
\( 11\beta\)-PGF\(_{2\alpha} \) (pg) in purified sample = \left( \frac{V}{\text{Recovery Factor}} \right) \times 0.5 \text{ ml} 
\]
\( 11\beta\)-PGF\(_{2\alpha} \) in original sample (pg/ml) = \[
\frac{11\beta\text{-PGF}_{2\alpha} \text{ (pg) in purified sample}}{\text{Volume of sample used for purification (ml)}}
\]
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>NSB</td>
<td>0.000</td>
<td>0.003</td>
<td>0.002</td>
</tr>
<tr>
<td>B₀</td>
<td>0.780</td>
<td>0.812</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.846</td>
<td>0.872</td>
<td>0.827</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.826</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>0.072</td>
<td>0.065</td>
<td>0.070</td>
</tr>
<tr>
<td>400</td>
<td>0.112</td>
<td>0.102</td>
<td>0.110</td>
</tr>
<tr>
<td>160</td>
<td>0.160</td>
<td>0.163</td>
<td>0.158</td>
</tr>
<tr>
<td>64</td>
<td>0.258</td>
<td>0.252</td>
<td>0.256</td>
</tr>
<tr>
<td>25.6</td>
<td>0.367</td>
<td>0.378</td>
<td>0.365</td>
</tr>
<tr>
<td>10.2</td>
<td>0.531</td>
<td>0.528</td>
<td>0.529</td>
</tr>
<tr>
<td>4.1</td>
<td>0.682</td>
<td>0.674</td>
<td>0.680</td>
</tr>
<tr>
<td>1.6</td>
<td>0.735</td>
<td>0.764</td>
<td>0.733</td>
</tr>
</tbody>
</table>

Table 2. Typical results

50% B/B₀ - 32 pg/ml
Detection Limit (80% B/B₀) - 5.5 pg/ml

Figure 5. Typical standard curve
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 25.

<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>42.5</td>
<td>14.8</td>
</tr>
<tr>
<td>400</td>
<td>17.8</td>
<td>7.5</td>
</tr>
<tr>
<td>160</td>
<td>9.4</td>
<td>7.7</td>
</tr>
<tr>
<td>64</td>
<td>8.4</td>
<td>8.9</td>
</tr>
<tr>
<td>25.6</td>
<td>8.2</td>
<td>11.9</td>
</tr>
<tr>
<td>10.2</td>
<td>10.3</td>
<td>20.6</td>
</tr>
<tr>
<td>4.1</td>
<td>21.7</td>
<td>†</td>
</tr>
<tr>
<td>1.6</td>
<td>†</td>
<td>†</td>
</tr>
</tbody>
</table>

Table 3. Intra- and inter-assay variation
*%CV represents the variation in concentration (not absorbance) as determined using a reference standard curve.
†Outside of the recommended usable range of the assay.

Specificity:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cross-reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>11β-Prostaglandin F2α</td>
<td>100%</td>
</tr>
<tr>
<td>2,3-dinor-11β-Prostaglandin F2α</td>
<td>10%</td>
</tr>
<tr>
<td>11β-13,14-dihydro-15-keto Prostaglandin F2α</td>
<td>0.5%</td>
</tr>
<tr>
<td>Leukotriene B4</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>Misoprostol</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>tetrano-PGEM</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>tetrano-PGF</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>Prostaglandin D2</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>13,14-dihydro-15-keto Prostaglandin D2</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>Prostaglandin E2</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>Prostaglandin F2β</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>Prostaglandin J2</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 11β-PGF2α 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                        | A. Rewash plate and redevelop                               |
|                                                   | B. Exposure of NSB wells to specific Antiserum |                                                            |
| 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 | Interfering substances are present (i.e., more than 20% difference) | Purify sample prior to analysis by EIA²                        |
| Only Total Activity (TA) wells develop            | Trace organic contaminants in the water source | Replace activated carbon filter or change source of UltraPure water |

References


### Related Products

- Prostaglandin D<sub>2</sub> EIA Kit - Cat. No. 512021
- Prostaglandin D<sub>2</sub>-MOX EIA Kit - Cat. No. 512011
- 11β-Prostaglandin F<sub>2α</sub> - Cat. No. 16520
- SPE Cartridges (C-18) - Cat. No. 400020
- UltraPure Water - Cat. No. 400000

### Warranty and Limitation of Remedy

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.
### Symbols

**REF**  Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.–Cat.: / Αριθμός-Κατ.

**LOT** Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός -Παραγωγή:

**Use by:** / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:

**No. of Tests:** / Kitgröße: / Nb. de Tests: / No. de Determ.: / N.º de Testes: / Quantità dei tests: / Αριθμός εξετάσεων:

**CONC** Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα

**LYO** Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλιασμένο

**IVD** In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Equipo Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση.

**Evaluation kit.** / Nur für Leistungsbewertungszwecke. / Kit pour évaluation. / Juego de Reactivos para Evaluació. / Kit de avaliação. / Kit di evaluazione. / Κιτ Αξιολόγησης.

**Read instructions before use.** / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Leggere le istruzioni prima dell’uso. / Διαβάστε τις οδηγίες πριν την χρήση.

**Keep away from heat or direct sun light.** / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l’abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Non esporre ai raggi solari. / Φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου.

**Store at:** / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Αποθήκευση στους:

**Manufacturer:** / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabricrante: / Παραγωγός:

**Caution!** / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!

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 συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

---

**IBL AFFILIATES WORLDWIDE**

<table>
<thead>
<tr>
<th>IBL International GmbH</th>
<th>IBL International B.V.</th>
<th>IBL International Corp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flughafenstr. 52A, 22335 Hamburg, Germany</td>
<td>Zutphenseweg 55, 7418 AH Deventer, The Netherlands</td>
<td>194 Wildcat Road, Toronto, Ontario M3J 2N5, Canada</td>
</tr>
<tr>
<td>Tel.: +49 (0) 40 532891 -0</td>
<td>Tel.: +49 (0) 40 532891 -0</td>
<td>Tel.: +1 (416) 645 -1703</td>
</tr>
<tr>
<td>E-MAIL: <a href="mailto:IBL@IBL-International.com">IBL@IBL-International.com</a></td>
<td>E-MAIL: <a href="mailto:IBL@IBL-International.com">IBL@IBL-International.com</a></td>
<td>E-MAIL: <a href="mailto:Sales@IBL-International.com">Sales@IBL-International.com</a></td>
</tr>
</tbody>
</table>

**LIABILITY:** Complaints will be accepted in each mode –written or vocal. Preferred is that the complaint is accompanied with the test performance and results. 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 / 2011-07-01