



# Prostaglandin F<sub>2α</sub> Enzyme Immunoassay Kit

Catalog No. 133-17333

96 Well Kit

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FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

## Description

Sapphire's Prostaglandin  $F_{2\alpha}$  kit is a competitive immunoassay for the quantitative determination of prostaglandin  $F_{2\alpha}$  in biological samples. This assay is based on the competition between prostaglandin  $F_{2\alpha}$  and a prostaglandin  $F_{2\alpha}$ -alkaline phosphatase tracer for a limited amount of prostaglandin  $F_{2\alpha}$ -specific antiserum. Because of the competition between prostaglandin  $F_{2\alpha}$  in the sample and prostaglandin  $F_{2\alpha}$  tracer for the prostaglandin  $F_{2\alpha}$  antiserum, the signal obtained with the assay will be inversely proportional to the amount of  $PGF_{2\alpha}$  in each sample. This equilibration is performed in the wells of a 96-well plate pre-coated with mouse monoclonal anti-rabbit IgG, which binds all of the  $PGF_{2\alpha}$  antiserum added to the well. After the equilibration step, the plate is washed, and a solution of *para*-nitrophenyl phosphate (*p*NPP), a substrate for alkaline phosphatase, is added. The product of this enzymatic reaction has a distinct yellow color which absorbs at 412 nm, allowing quantification of prostaglandin  $F_{2\alpha}$  in each sample.

## Introduction

Prostaglandin  $F_{2\alpha}$  ( $PGF_{2\alpha}$ ) is produced from arachidonic acid by the sequential activity of COX-1/COX-2 and prostaglandin F synthase.<sup>1,2</sup> It causes contraction of vascular, bronchial, intestinal, and myometrial smooth muscle, and also exhibits potent luteolytic activity.<sup>3</sup>  $PGF_{2\alpha}$  exerts its activity by binding to a cell-surface G-protein coupled receptor, the FP receptor, with affinity of less than 10 nM.<sup>4</sup> Maximal ovine myometrial contraction can be achieved at 125 nM  $PGF_{2\alpha}$  *in vitro*.<sup>5</sup>  $PGF_{2\alpha}$  has a very short half-life in the general circulation with typical plasma concentrations of <10 pg/ml.<sup>6</sup>  $PGF_{2\alpha}$  is metabolized *via* the 15-hydroxy prostaglandin dehydrogenase pathway to 13,14-dihydro-15-keto  $PGF_{2\alpha}$  *in vivo*.

## Precautions

1. Please read all instructions carefully before beginning this assay.
2. Some reagents contain azide, which may react with lead or copper plumbing. If disposing of reagents in the sanitary sewer, please flush with a large volume of water to prevent azide build-up.
3. This kit is for research use only. It is not intended for human or diagnostic use.

## Materials Supplied

|  |           |           |
|--|-----------|-----------|
| 1. Prostaglandin $F_{2\alpha}$ Antiserum | 1 vial    | 133-73333 |
| 2. Prostaglandin $F_{2\alpha}$ AP Tracer | 1 vial    | 133-73332 |
| 3. Prostaglandin $F_{2\alpha}$ Standard  | 1 vial    | 133-73331 |
| 4. Mouse Anti-rabbit IgG Coated Plate    | 1 plate   | 133-00008 |
| 5. Tris Buffer Concentrate               | 2 vials   | 133-00003 |
| 6. AP Wash Buffer Concentrate            | 1 vial    | 133-00004 |
| 7. DEA Buffer Concentrate                | 1 vial    | 133-00001 |
| 8. <i>p</i> NPP Tablets                  | 5 tablets | 133-00002 |
| 9. Plate Cover                           | 1 cover   | 133-00005 |

## Storage

This kit should be stored at -20°C and used before the expiration date printed on the box.

## Materials Needed but Not Supplied

1. A source of Millipore water
2. Adjustable pipettors
3. Materials used for sample preparation
4. An orbital shaker
5. A plate reader capable of measuring absorbance between 405-420 nm

### **Materials needed for sample purification**

1. Prostaglandin F<sub>2α</sub> standard to use in determination of recovery
2. 1.0 M HCl, methanol, Millipore water, hexane, and ethyl acetate
3. 6 ml SPE C-18 cartridges

### **Purification Procedure**

While the following purification protocol works well for many samples, it may not be sufficient for the purification of prostaglandin F<sub>2α</sub> from all samples.

1. Split samples into two equal parts and place each sample into clean test tubes.
2. Spike one set of samples with prostaglandin F<sub>2α</sub>.
3. Perform the following steps on all samples:
  - a. Acidify the sample to pH < 4.0 by the addition of 1.0 M HCl.
  - b. Prepare a C-18 SPE cartridge by conditioning it first with 5 ml of methanol followed by 5 ml Millipore water.
  - c. Apply the sample and allow it to flow through the column.
  - d. Rinse the column with 5 ml Millipore water, followed by 5 ml hexane. Allow the column to become dry following the hexane wash.
  - e. Elute the prostaglandin F<sub>2α</sub> with 5 ml ethyl acetate containing 1% methanol. If unable to run the assay at this time, store the samples in the ethyl acetate/methanol at -80°C.
  - f. Dry the sample under a stream of nitrogen. Reconstitute the sample in Tris Buffer and assay both spiked and unspiked samples in the assay.

### **Reagent Preparation**

All diluted buffers should be stored at 4°C. When stored in this manner, they will be stable for approximately two months.

#### **1. Tris Buffer**

Dilute the contents of one vial of Tris Buffer Concentrate with 90 ml of Millipore water. It is common for the concentrated buffer to contain crystalline salts after thawing. It is important to rinse the vial to obtain any salts that may have precipitated.

#### **2. Wash Buffer**

Dilute the 5 ml vial of Wash Buffer to a final volume of 750 ml with Millipore water.

#### **3. DEA Buffer**

Dilute the 2.5 ml vial of DEA Buffer Concentrate to a final volume of 25 ml with Millipore water.

#### **4. Prostaglandin F<sub>2α</sub> Standard**

*[Note: If assaying culture medium samples that have not been diluted with Tris Buffer, the culture medium rather than Tris Buffer should be used for dilution of the standard curve.]*

Equilibrate a pipet tip in ethanol, by repeatedly filling and expelling. Use the equilibrated pipet tip to transfer 100 µl of the PGF<sub>2α</sub> standard into a clean test tube. Dilute with 900 µl Millipore water. The concentration of this standard is 30 ng/ml. Label eight glass test tubes #1 - #8. Pipet 900 µl of Tris Buffer into tube #1, and 500 µl into tubes #2 - #8. Transfer 100 µl of the 30 ng/ml standard into tube #1 and vortex to mix. Transfer 250 µl from tube #1 to tube #2. Vortex to mix. Transfer 250 µl from tube #2 to tube #3. Vortex to mix. Continue this process for standard tubes #4 - #8. These diluted standards should be used within twenty-four hours.

#### **5. Prostaglandin F<sub>2α</sub> Alkaline Phosphatase Tracer**

Reconstitute the Prostaglandin F<sub>2α</sub> Alkaline Phosphatase Tracer with 6 ml of Tris buffer. Vortex to mix. Store this reconstituted tracer at 4°C and use within four weeks.

#### **6. Prostaglandin F<sub>2α</sub> Antiserum**

Reconstitute the Prostaglandin F<sub>2α</sub> Antiserum with 6 ml of Tris Buffer. Vortex to mix. Store this reconstituted antibody at 4°C and use within four weeks.

## Assay Procedure

[**Note:** All reagents should be allowed to warm to room temperature before use.]

### 1. Tris Buffer

Pipet 150 µl Tris Buffer into non-specific binding (NSB) wells, and 100 µl Tris Buffer into zero standard (B<sub>0</sub>) wells. If tissue culture medium was used to dilute the standard curve, substitute 100 µl of this same medium for the Tris Buffer in the NSB and B<sub>0</sub> wells.

### 2. Prostaglandin F<sub>2α</sub> Standard

Pipet 100 µl of standards into the appropriate wells.

### 3. Samples

Pipet 100 µl of sample into the appropriate wells. Each sample should be assayed in duplicate or triplicate.

### 4. Prostaglandin F<sub>2α</sub> Alkaline Phosphatase Tracer

Pipet 50 µl of tracer into each well except the blank wells and total activity (TA) wells.

### 5. Prostaglandin F<sub>2α</sub> Antiserum

Pipet 50 µl of antiserum into each well except the blank wells, TA wells and NSB wells.

| Well           | Tris Buffer | Std/Sample | Tracer                | Antibody |
|----------------|-------------|------------|-----------------------|----------|
| Blank          |             |            |                       |          |
| TA             |             |            | 5 µl (at development) |          |
| NSB            | 150 µl      |            | 50 µl                 |          |
| B <sub>0</sub> | 100 µl      |            | 50 µl                 | 50 µl    |
| Std/Sample     |             | 100 µl     | 50 µl                 | 50 µl    |

#### Pipetting Summary

### 6. Incubate the plate

Cover each plate with a plate cover and incubate for two hours at room temperature on an orbital shaker.

### 7. Prepare the pNPP Solution

Dissolve 5 pNPP tablets in 25 ml DEA Buffer (25 ml is sufficient to develop 100 wells). [**Note:** Reconstituted pNPP is not stable, so we recommend that you make only the amount that you need at any one time.]

### 8. Wash the plate

Empty the wells and rinse five times with Wash Buffer. After the final wash, firmly tap the inverted plate on a paper towel to remove any recalcitrant drops of buffer.

### 9. Develop the plate

Add 200 µl pNPP solution to each well including blank and TA wells. Add 5 µl of tracer to the TA wells. Cover the plate and allow to develop in the dark on an orbital shaker. This assay typically develops in approximately 60-90 minutes.

### 10. Read the plate

Wipe the bottom of the plate with a paper towel to remove any finger prints, smudges or dirt which may interfere with obtaining an accurate reading of absorbance. Remove the plate cover, and read the plate at a wavelength between 405 and 420 nm.

## Data Analysis

Most 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/B<sub>0</sub> versus log concentration of standard using either a 4-parameter logistic or a log-logit curve fit.

### 1. Prepare the Data

The following procedure is recommended to prepare the data prior to graphing (subtract the absorbance of the blank wells from all wells on the plate, if not already done).

- a. Average the absorbance readings from the NSB wells.
- b. Average the absorbance readings from the B<sub>0</sub> wells.
- c. Subtract the average NSB from the average B<sub>0</sub>. This is the corrected B<sub>0</sub>.

$$\text{Corrected } B_0 = \text{Average } B_0 - \text{Average NSB}$$

- d. Calculate the %B/B<sub>0</sub> for each standard and sample. To do this, subtract the average NSB absorbance from the absorbance and divide by the corrected B<sub>0</sub> (from step c). Multiply by 100 to obtain %B/B<sub>0</sub>. Repeat for all wells.

$$\%B/B_0 = \left[ \frac{\text{Absorbance} - \text{Average NSB}}{\text{Corrected } B_0} \right] \times 100$$

### 2. Plot the Standard Curve

Plot %B/B<sub>0</sub> for all standards *versus* PGF<sub>2α</sub> concentration using log (x) and linear (y) axes, and fit the data to a four parameter logistic equation. Alternatively, the data can be linearized using a logit transformation. [**Note:** Do not use %B/B<sub>0</sub> in this calculation.]

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

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

### 3. Determine the Concentration of your Samples

Calculate the %B/B<sub>0</sub> for each sample. Determine the concentration of each sample using the equations obtained from the analysis of the standard curve. Remember to account for any dilutions made to the sample prior to addition to the well. %B/B<sub>0</sub> values of greater than 80% or less than 20% should be re-assayed as they generally fall outside of the linear range of the standard curve.

### 4. Correct for Recovery (if purification was performed)

Divide the concentration determined in step #3 by the recovery factor. Correct for any volume changes of the sample which may have occurred during purification.

Recovery Factor =

$$\frac{\text{EIA value of spiked sample (pg/ml)} - \text{EIA value of unspiked sample (pg/ml)}}{\text{Concentration of spike (pg/ml)}}$$

## Assay Performance Characteristics

### 1. Precision

Intra-assay precision was determined by measuring samples containing low, medium, and high concentrations of prostaglandin F<sub>2α</sub> multiple times in the same assay (eight samples per plate on a total of five plates). Inter-assay precision was determined by measuring low, medium, and high concentrations of the samples eight times using different reagents.

|        | PGF <sub>2α</sub> (pg/ml) | Intra-assay %CV | Inter-assay %CV |
|--------|---------------------------|-----------------|-----------------|
| Low    | 37.0                      | 14.5            | 9.5             |
| Medium | 111.1                     | 9.0             | 4.4             |
| High   | 333.3                     | 6.2             | 3.0             |

## 2. Specificity

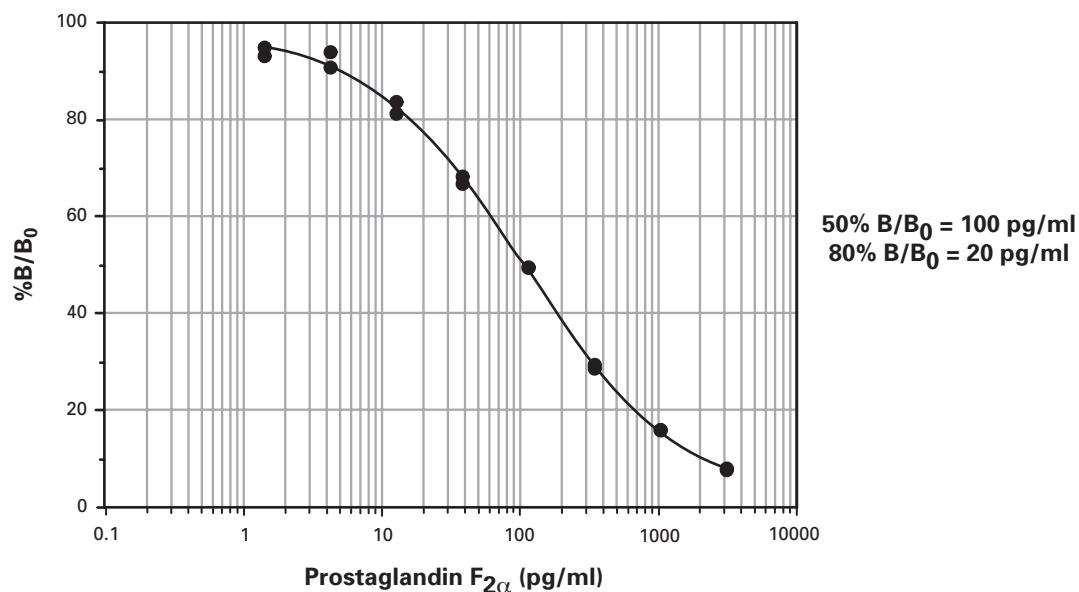
| Analyte                                    | Cross-Reactivity | Analyte   | Cross-Reactivity |
|--|------------------|---|------------------|
| Prostaglandin F <sub>2α</sub> Ethanolamide | 244%             | Prostaglandin D <sub>2</sub>                            | 1.2%             |
| Prostaglandin F <sub>2α</sub>              | 100%             | 19(R)-hydroxy Prostaglandin F <sub>2α</sub>             | 0.69%            |
| Prostaglandin F <sub>1α</sub>              | 61%              | 11β-Prostaglandin F <sub>2α</sub>                       | 0.03%            |
| Prostaglandin F <sub>3α</sub>              | 21%              | 6,15-diketo-13,14-dihydro Prostaglandin F <sub>2α</sub> | 0.02%            |
| 20-hydroxy Prostaglandin F <sub>2α</sub>   | 5.3%             | 11-dehydro Thromboxane B <sub>2</sub>                   | <0.01%           |
| Prostaglandin E <sub>2</sub>               | 2.6%             |   |                  |

## 3. Typical Results

The standard curve shown here is an example of data typically produced by this kit. Your results will vary from these, and it is therefore important that you run a standard curve each time you use the kit.

|                | Raw Data |       | Average | Corrected |
|----------------|----------|-------|---------|-----------|
| Total Activity | 3.153    | 3.238 | 3.196   |           |
| NSB            | 0        | 0     | 0       |           |
| B <sub>0</sub> | 0.901    | 0.940 | 0.949   | 0.949     |
|                | 0.972    | 0.982 |         |           |

| Concentration Std (pg/ml) | Raw Data |       | Corrected |       | %B/B <sub>0</sub> |      |
|---------------------------|----------|-------|-----------|-------|-------------------|------|
| 3,000                     | 0.073    | 0.076 | 0.073     | 0.076 | 8.0               | 7.9  |
| 1,000                     | 0.152    | 0.151 | 0.152     | 0.151 | 15.9              | 16.0 |
| 333.3                     | 0.272    | 0.279 | 0.272     | 0.279 | 29.4              | 29.0 |
| 111.1                     | 0.469    | 0.470 | 0.469     | 0.470 | 49.5              | 49.5 |
| 37.0                      | 0.634    | 0.648 | 0.634     | 0.648 | 68.3              | 67.6 |
| 12.3                      | 0.794    | 0.771 | 0.794     | 0.771 | 81.3              | 82.5 |
| 4.1                       | 0.862    | 0.892 | 0.862     | 0.892 | 94.0              | 92.4 |
| 1.4                       | 0.884    | 0.901 | 0.884     | 0.901 | 95.0              | 94.1 |



## **References**

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## **Warranty and Limitation of Remedy**

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