



Prostaglandin E₂ Enzyme Immunoassay Kit

Catalog No. 133-16359

96 Well Kit

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Description

Sapphire's Prostaglandin E₂ (PGE₂) kit is a competitive immunoassay for the quantitative determination of PGE₂ in biological samples. This assay is based on the competition between PGE₂ and a PGE₂-alkaline phosphatase tracer for a limited amount of PGE₂-specific monoclonal antibody. Because of the competition between PGE₂ in the sample and PGE₂ tracer for the PGE₂ antibody, the signal obtained with the assay will be inversely proportional to the amount of PGE₂ in each sample. This equilibration is performed in the wells of a 96-well plate pre-coated with goat polyclonal anti-mouse IgG, which binds all of the PGE₂ monoclonal antibody 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 PGE₂ in each sample.

Introduction

PGE₂ is a potent lipid mediator produced by metabolism of arachidonic acid *via* the cyclooxygenase (COX) pathway. Following cellular stimulation, arachidonic acid is hydrolyzed from phospholipids stores by one of a family of phospholipase A₂ enzymes and converted to the unstable endoperoxide PGH₂ by either COX-1 or COX-2.^{1,2} PGH₂ is isomerized to PGE₂ by one of 3 distinct enzymes - microsomal PGES-1 (mPGES-1), mPGES-2, or a cytosolic enzyme, cPGES.³ PGE₂ is active in a number of physiological systems and settings including inflammation, immune regulation, generation of fever, pain perception, protection of the gastric mucosa, fertility and parturition, as well as sodium and water retention.⁴⁻⁹ The effects of PGE₂ are transduced by four subtypes of G protein-coupled receptors designated EP₁, EP₂, EP₃, EP₄, with K_d values for PGE₂ ranging from 1-10 nM.¹⁰ PGE₂ is rapidly metabolized *in vivo* by the prostaglandin 15-dehydrogenase pathway (15-hydroxy PGDH) to the inactive metabolite 13,14-dihydro-15-keto PGE₂.^{11,12} The half-life of PGE₂ in the circulatory system is approximately 30 seconds and normal plasma levels are 3-12 pg/ml.¹³

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 E ₂ Monoclonal Antibody	1 vial	133-63593
2. Prostaglandin E ₂ AP Tracer	1 vial	133-63592
3. Prostaglandin E ₂ Standard	1 vial	133-63591
4. Goat Anti-mouse IgG Coated Plate	1 plate	133-00007
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

Sample Handling

[*Note: Samples should be stored at -80°C if unable to be assayed immediately following collection.*]

Materials needed for sample purification

1. Prostaglandin E₂ 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 PGE₂ 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 PGE₂.
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 PGE₂ 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 E₂ 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 200 µl of the PGE₂ standard into a clean test tube. Dilute with 300 µl Millipore water. The concentration of this standard is 40 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 40 ng/ml standard into tube #1 and vortex to mix. Transfer 500 µl from tube #1 to tube #2. Vortex to mix. Transfer 500 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 E₂ Alkaline Phosphatase Tracer

Reconstitute the Prostaglandin E₂ 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 E₂ Monoclonal Antibody

Reconstitute the Prostaglandin E₂ Monoclonal Antibody 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 μ l Tris Buffer into non-specific binding (NSB) wells, and 100 μ l Tris Buffer into zero standard (B_0) wells. If tissue culture medium was used to dilute the standard curve, substitute 100 μ l of this same medium for the Tris Buffer in the NSB and B_0 wells.

2. Prostaglandin E_2 Standard

Pipet 100 μ l of standards into the appropriate wells.

3. Samples

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

4. Prostaglandin E_2 Alkaline Phosphatase Tracer

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

5. Prostaglandin E_2 Antibody

Pipet 50 μ l of antibody into each well except the blank wells, TA wells and NSB wells.

Well	Tris Buffer	Std/Sample	Tracer	Antibody
Blank				
TA			5 μ l (at development)	
NSB	150 μ l		50 μ l	
B_0	100 μ l		50 μ l	50 μ l
Std/Sample		100 μ l	50 μ l	50 μ l

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 μ l pNPP solution to each well including blank and TA wells. Add 5 μ l 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/B₀ 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 B₀ wells.
- c. Subtract the average NSB from the average B₀. This is the corrected B₀.

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

- d. Calculate the %B/B₀ for each standard and sample. To do this, subtract the average NSB absorbance from the absorbance and divide by the corrected B₀ (from step c). Multiply by 100 to obtain %B/B₀. 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/B₀ for all standards *versus* PGE₂ 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/B₀ in this calculation.]

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

Plot the data as logit (B/B₀) *versus* log concentration of standard and perform a linear regression fit.

3. Determine the Concentration of your Samples

Calculate the %B/B₀ 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/B₀ 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 PGE₂ 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.

	PGE ₂ (pg/ml)	Intra-assay %CV	Inter-assay %CV
Low	125	9.9	15.9
Medium	500	4.5	9.8
High	1000	3.5	9.3

2. Specificity

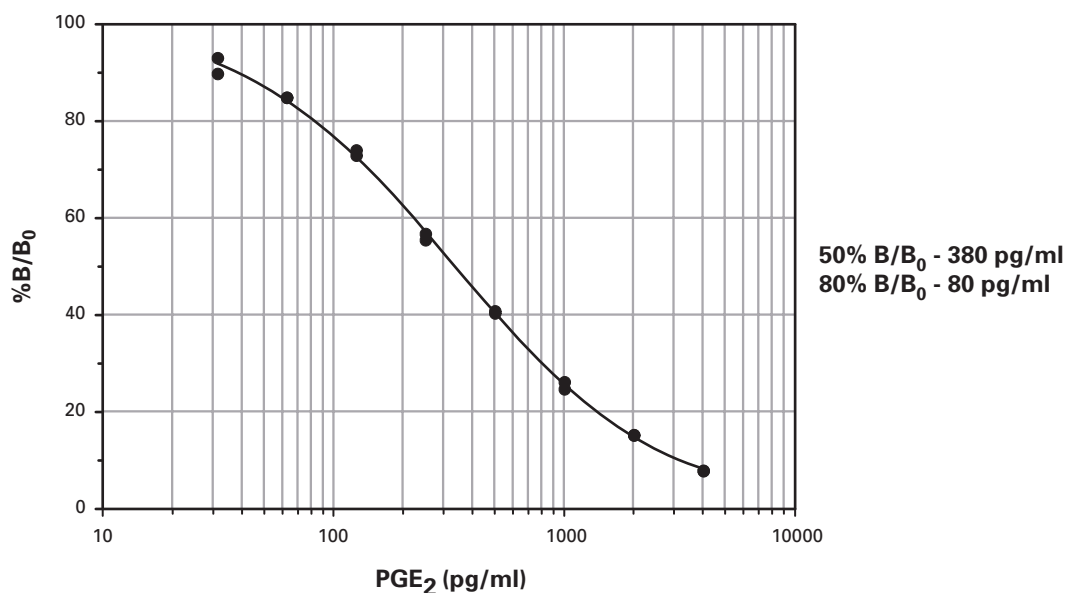
Analyte	Cross-Reactivity	Analyte	Cross-Reactivity
Prostaglandin E ₂	100%	Prostaglandin B ₂	<0.01%
Prostaglandin E ₂ Ethanolamide	100%	Prostaglandin D ₂	<0.01%
Prostaglandin E ₃	43.0%	15-keto Prostaglandin E ₂	<0.01%
8-iso Prostaglandin E ₂	37.4%	Prostaglandin F _{1α}	<0.01%
Prostaglandin E ₁	18.7%	Prostaglandin F _{2α}	<0.01%
6-keto Prostaglandin F _{1α}	1.0%	13,14-dihydro-15-keto Prostaglandin F _{2α}	<0.01%
8-iso Prostaglandin F _{2α}	0.25%	Prostaglandin F _{3α}	<0.01%
Prostaglandin A ₁	<0.01%	Thromboxane B ₂	<0.01%
Prostaglandin A ₂	<0.01%	Tetranor PGEM	<0.01%
Prostaglandin A ₃	<0.01%	Tetranor PGFM	<0.01%
Prostaglandin B ₁	<0.01%		

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 (AU)		Average	Corrected
Total Activity	3.697	3.615	3.615	
NSB	0	0	0	
B ₀	1.126	1.185	1.173	1.173
	1.196	1.185		

Concentration Std (pg/ml)	Raw Data (AU)		Corrected		%B/B ₀	
4,000	0.092	0.093	0.092	0.093	7.8	7.9
2,000	0.179	0.178	0.179	0.178	15.3	15.2
1,000	0.290	0.307	0.290	0.307	24.7	26.2
500	0.474	0.479	0.474	0.479	40.4	40.8
250	0.666	0.651	0.666	0.651	56.8	55.5
125	0.869	0.856	0.869	0.856	74.1	73.0
62.5	0.996	0.996	0.996	0.996	84.9	84.9
31.3	1.092	1.054	1.092	1.054	93.1	89.9



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