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Prostaglandin E₁ Enzyme Immunoassay Kit

Catalog No. 900-005

96 Well Kit

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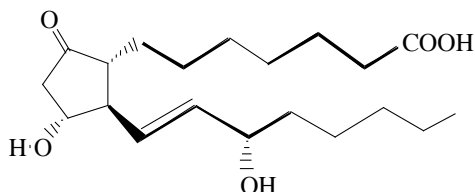
Description

The Assay Designs™ Prostaglandin E₁ enzyme immunoassay (EIA) kit is a competitive immunoassay for the quantitative determination of Prostaglandin E₁ in biological fluids. Please read the complete kit insert before performing this assay. The EIA kit uses a polyclonal antibody to PGE₁ to bind, in a competitive manner, the PGE₁ in the sample or an alkaline phosphatase molecule which has PGE₁ covalently attached to it. After a simultaneous incubation at room temperature the excess reagents are washed away and substrate is added. After a short incubation time the enzyme reaction is stopped and the yellow color generated is read on a microplate reader at 405nm. The intensity of the bound yellow color is inversely proportional to the concentration of PGE₁ in either standards or samples. The measured optical density is used to calculate the concentration of PGE₁. For further explanation of the principles and practice of immunoassays please see the excellent books by Chard¹ or Tijssen².

Introduction

Prostaglandin E₁ (PGE₁) is synthesized from DGLA, dihomo- γ -linolenic acid³. PGE₁ has been shown to have a number of biological actions, including vasodilation⁴, proliferation of vascular smooth muscle cells⁵, platelet aggregation⁶ and has been shown to have insulin-like actions^{7,8}. Its effects are induced by receptor mediated elevation of cAMP⁹. It is the major prostaglandin in semen^{10,11}.

Prostaglandin E₁



Precautions

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

1. Some kit components contain azide, which may react with lead or copper plumbing. When disposing of reagents always flush with large volumes of water to prevent azide build-up.
2. Stop Solution is a solution of trisodium phosphate. This solution is caustic; care should be taken in use.
3. The activity of the alkaline phosphatase conjugate is dependent on the presence of Mg²⁺ and Zn²⁺ ions. The activity of the conjugate is affected by concentrations of chelators (>10 mM) such as EDTA and EGTA.
4. We test this kit's performance with a variety of samples, however it is possible that high levels of interfering substances may cause variation in assay results.
5. The Prostaglandin E₁ Standard provided, Catalog No. 80-0085, is supplied in ethanolic buffer at a pH optimized to maintain PGE₁ integrity. Care should be taken handling this material because of the known and unknown effects of prostaglandins.

Materials Supplied

- 1. Donkey anti-Sheep IgG Microtiter Plate, One Plate of 96 Wells, Catalog No. 80-0045**
A plate using break apart strips coated with donkey antibody specific to sheep IgG.
- 2. PGE₁ EIA Conjugate, 6 mL, Catalog No. 80-0094**
A blue solution of alkaline phosphatase conjugated with PGE₁.
- 3. PGE₁ EIA Antibody, 6 mL, Catalog No. 80-0095**
A yellow solution of a sheep polyclonal antibody to PGE₁.
- 4. Assay Buffer, 30 mL, Catalog No. 80-0010**
Tris buffered saline, containing proteins and sodium azide as preservative.
- 5. Wash Buffer Concentrate, 30 mL, Catalog No. 80-1286**
Tris buffered saline containing detergents.
- 6. Prostaglandin E₁ Standard, 0.5 mL, Catalog No. 80-0085**
A solution of 50,000 pg/mL PGE₁.
- 7. pNpp Substrate, 20 mL, Catalog No. 80-0075**
A solution of p-nitrophenyl phosphate in buffer. Ready to use.
- 8. Stop Solution, 6 mL, Catalog No. 80-0247**
A solution of trisodium phosphate in water. Keep tightly capped. Caution: **Caustic.**
- 9. PGE₁ Assay Layout Sheet, 1 each, Catalog No. 30-0016**
- 10. Plate Sealer, 1 each, Catalog No. 30-0012**

Storage

All components of this kit, **except the PGE₁ Conjugate**, are stable at 4 °C until the kit's expiration date. The PGE₁ Conjugate **must** be stored at -20 °C.

Materials Needed but Not Supplied

1. Deionized or distilled water.
2. Precision pipets for volumes between 5 μ L and 1,000 μ L.
3. Repeater pipets for dispensing 50 μ L and 200 μ L.
4. Disposable beakers for diluting buffer concentrates.
5. Graduated cylinders.
6. A microplate shaker.
7. Adsorbent paper for blotting.
8. Microplate reader capable of reading at 405 nm, preferably with correction between 570 and 590 nm.

Sample Handling

The Assay Designs™ PGE₁ EIA Kit is compatible with PGE₁ samples in a wide range of matrices after dilution in Assay Buffer. Please refer to the Sample Recovery recommendations on page 11 for details of suggested dilutions. However, the end user **must verify** that the recommended dilutions are appropriate for their samples. **Samples containing sheep IgG may interfere with the assay.**

Samples in the majority of tissue culture media, including those containing fetal bovine serum, can also be read in the assay, provided the standards have been diluted into the tissue culture media instead of Assay Buffer. There will be a small change in binding associated with running the standards and samples in media. Users should only use standard curves generated in media or buffer to calculate concentrations of PGE₁ in the appropriate matrix. For tissue, urine and plasma samples, prostaglandin synthetase inhibitors, such as, indomethacin or meclofenamic acid at concentrations up to 10 µg/mL should be added to either the tissue homogenate or urine and plasma samples.

Some samples normally have very low levels of PGE₁ present and extraction may be necessary for accurate measurement. A suitable extraction procedure is outlined below:

Materials Needed

1. PGE₁ Standard to allow extraction efficiency to be accurately determined.
2. 2M hydrochloric acid, deionized water, ethanol, hexane and ethyl acetate.
3. 200 mg C₁₈ Reverse Phase Extraction Columns.

Procedure

1. Acidify the plasma, urine or tissue homogenate by addition of 2M HCl to pH of 3.5. Approximately 50 µL of HCl will be needed per mL of plasma. Allow to sit at 4 °C for 15 minutes. Centrifuge samples in a microcentrifuge for 2 minutes to remove any precipitate.
2. Prepare the C₁₈ reverse phase column by washing with 10 mL of ethanol followed by 10 mL of deionized water.
3. Apply the sample under a slight positive pressure to obtain a flow rate of about 0.5 mL minute. Wash the column with 10 mL of water, followed by 10 mL of 15% ethanol, and finally 10 mL hexane. Elute the sample from the column by addition of 10 mL ethyl acetate.
4. If analysis is to be carried out immediately, evaporate samples under a stream of nitrogen. Add at least 250 µL of Assay Buffer to the dried samples. Vortex well then allow to sit for five minutes. Repeat twice more. If analysis is to be delayed, store samples as the eluted ethyl acetate solutions at -80 °C until the immunoassay is to be run. Evaporate the organic solvent under a stream of nitrogen prior to running assay and reconstitute as above

Please refer to references 12-15 for details of extraction protocols.

Procedural Notes

1. Do not mix components from different kit lots or use reagents beyond the kit expiration date.
2. Allow all reagents to warm to room temperature for at least 30 minutes before opening.
3. Standards can be made up in either glass or plastic tubes.
4. Pre-rinse the pipet tip with reagent, use fresh pipet tips for each sample, standard and reagent.
5. Pipet standards and samples to the bottom of the wells.
6. Add the reagents to the side of the well to avoid contamination.
7. This kit uses break-apart microtiter strips, which allow the user to measure as many samples as desired. Unused wells must be kept desiccated at 4 °C in the sealed bag provided. The wells should be used in the frame provided.
8. **Care must be taken to minimize contamination by endogenous alkaline phosphatase.** Contaminating alkaline phosphatase activity, especially in the substrate solution, may lead to high blanks. Care should be taken not to touch pipet tips and other items that are used in the assay with bare hands.
9. **Prior to addition of substrate, ensure that there is no residual wash buffer in the wells. Any remaining wash buffer may cause variation in assay results.**

Reagent Preparation

1. PGE₁ Standard

Allow the 50,000 pg/mL PGE₁ standard solution to warm to room temperature. Label six 12 x 75 mm glass tubes #1 through #6. Pipet 1mL of standard diluent (Assay Buffer or Tissue Culture Media) into tube #1. Pipet 750 μL of standard diluent into tubes #2 through #6. Remove 100 μL of diluent from tube #1. Add 100 μL of the 50,000 pg/mL standard to tube #1. Vortex thoroughly. Add 250 μL of tube #1 to tube #2 and vortex thoroughly. Add 250 μL of tube #2 to tube #3 and vortex. Continue this for tubes #4 through #6.

The concentration of PGE₁ in tubes #1 through #6 will be 5,000, 1,250, 313, 78.1, 19.5 and 4.88 pg/mL respectively. See PGE₁ Assay Layout Sheet for dilution details.

Diluted standards should be used within 60 minutes of preparation.

2. Wash Buffer

Just before use, prepare the Wash Buffer by diluting 5 mL of the supplied concentrate with 95 mL of deionized water. Discard unused buffer or add up to 0.09% sodium azide (w/v) for storage.

Assay Procedure

Bring all reagents to room temperature for at least 30 minutes prior to opening.

All standards and samples should be run in duplicate.

1. Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells with the desiccant back into the pouch and seal the ziploc. Store unused wells at 4 °C.
2. Pipet 100 μL of standard diluent (Assay Buffer or Tissue Culture Media) into the NSB and the Bo (0 pg/mL Standard) wells.
3. Pipet 100 μL of Standards #1 through #6 into the appropriate wells.
4. Pipet 100 μL of the Samples into the appropriate wells.
5. Pipet 50 μL of Assay Buffer into the NSB wells.
6. Pipet 50 μL of blue Conjugate into each well, except the TA and Blank wells.
7. Pipet 50 μL of yellow Antibody into each well, except the Blank, TA and NSB wells.

NOTE: Every well used should be **Green** in color except the NSB wells which should be **Blue**. The Blank and TA wells are empty at this point and have no color.

8. Incubate the plate at room temperature on a plate shaker for 2 hours at ~500 rpm. The plate may be covered with the plate sealer provided, if so desired.
9. Empty the contents of the wells and wash by adding 400 μL of wash solution to every well. Repeat the wash 2 more times for a total of **3 Washes**.
10. After the final wash, empty or aspirate the wells, and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
11. Add 5 μL of the blue Conjugate to the TA wells.
12. Add 200 μL of the pNpp Substrate solution to every well. Incubate at room temperature for 45 minutes without shaking.
13. Add 50 μL of Stop Solution to every well. This stops the reaction and the plate should be read immediately.
14. Blank the plate reader against the Blank wells, read the optical density at 405 nm, preferably with correction between 570 and 590 nm. If the plate reader is not able to be blanked against the Blank wells, manually subtract the mean optical density of the Blank wells from all readings.

Calculation of Results

Several options are available for the calculation of the concentration of PGE₁ in the samples. We recommend that the data be handled by an immunoassay software package utilizing a 4 parameter logistic curve fitting program. If data reduction software is not readily available, the concentration of PGE₁ can be calculated as follows:

1. Calculate the average net Optical Density (OD) bound for each standard and sample by subtracting the average NSB OD from the average OD bound:
Average Net OD = Average Bound OD - Average NSB OD
2. Calculate the binding of each pair of standard wells as a percentage of the maximum binding wells (Bo), using the following formula:
Percent Bound = $\frac{\text{Net OD}}{\text{Net Bo OD}}$ x 100
3. Using Logit-Log paper plot Percent Bound versus Concentration of PGE₁ for the standards. Approximate a straight line through the points. The concentration of PGE₁ in the unknowns can be determined by interpolation.

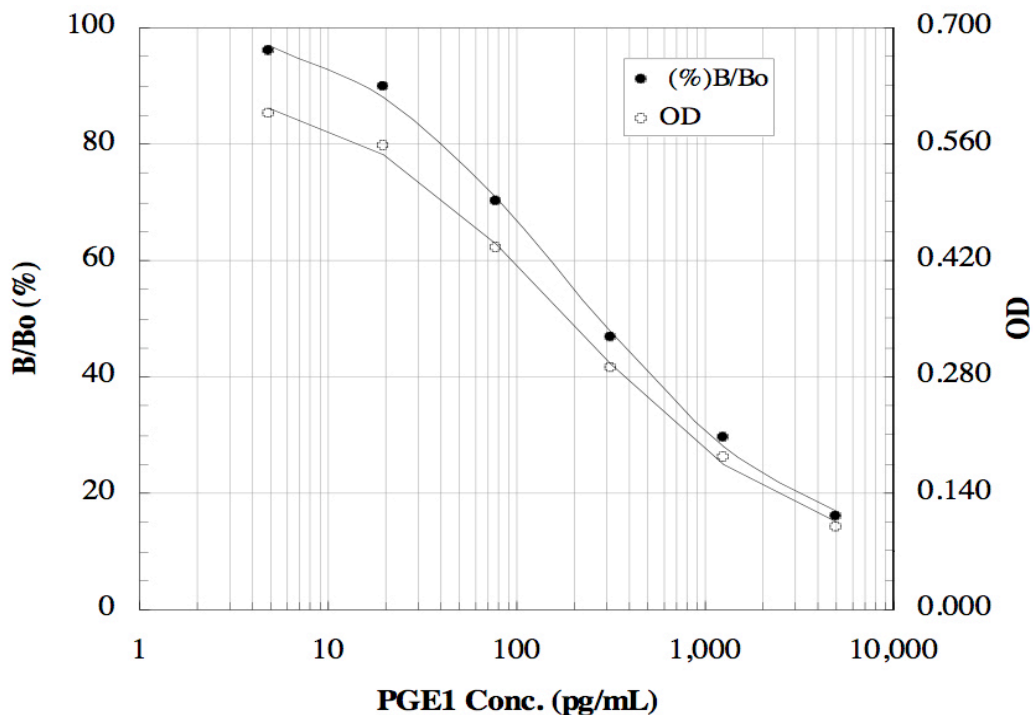
Typical Results

The results shown below are for illustration only and **should not** be used to calculate results from another assay.

Sample	Mean OD (-Blank)	Average Net OD	Percent Bound	PGE₁ (pg/mL)
Blank OD	(0.072)			
TA	0.848	0.848		
NSB	0.000	0.000	0.00%	
Bo	0.621	0.621	100%	0
S1	0.100	0.100	16.0%	5,000
S2	0.184	0.184	29.5%	1,250
S3	0.291	0.291	46.6%	312.5
S4	0.436	0.436	69.9%	78.1
S5	0.557	0.557	89.3%	19.5
S6	0.597	0.597	95.7%	4.88
Unknown 1	0.379	0.379	60.7%	143
Unknown 2	0.199	0.199	31.9%	894

Typical Standard Curve

A typical standard curve is shown below. This curve **must not** be used to calculate PGE₁ concentrations; each user must run a standard curve for each assay.



Typical Quality Control Parameters

Total Activity Added	=	0.848 x 10 = 8.48
%NSB	=	0.0%
%Bo/TA	=	7.4%
Quality of Fit	=	0.999 (Calculated from 4 parameter logistic curve fit)
20% Intercept	=	2,988 pg/mL
50% Intercept	=	268 pg/mL
80% Intercept	=	39 pg/mL

Performance Characteristics

The following parameters for this kit were determined using the guidelines listed in the National Committee for Clinical Laboratory Standards (NCCLS) Evaluation Protocols¹⁶.

Sensitivity

Sensitivity was calculated by determining the average optical density bound for twenty (20) wells run as Bo, and comparing to the average optical density for twenty (20) wells run with Standard #6. The detection limit was determined as the concentration of PGE₁ measured at two (2) standard deviations from the zero along the standard curve.

Average Optical Density for the Bo	=	0.579 ± 0.012 (2.07%)
Average Optical Density for Standard #6	=	0.558 ± 0.009 (1.61%)

Delta Optical Density (0-4.88 pg/mL)	=	0.021
2 SD's of the Zero Standard = 2 x 0.012	=	0.024

Sensitivity = $\frac{0.024}{0.021} \times 4.88$ pg/mL	=	5.58 pg/mL
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Linearity

A sample containing 50,000 pg/mL PGE₁ was diluted 7 times 1:2 in the kit Assay Buffer and measured in the assay. The data was plotted graphically as actual PGE₁ concentration versus measured PGE₁ concentration.

The line obtained had a slope of 1.0681 and a correlation coefficient of 1.000.

Precision

Intra-assay precision was determined by taking samples containing low, medium and high concentrations of PGE₁ and running these samples multiple times (n=24) in the same assay. Inter-assay precision was determined by measuring three samples with low, medium and high concentrations of PGE₁ in multiple assays (n=8).

The precision numbers listed below represent the percent coefficient of variation for the concentrations of PGE₁ determined in these assays as calculated by a 4 parameter logistic curve fitting program.

	<u>PGE₁-</u> (pg/mL)	<u>Intra-assay</u> <u>%CV</u>	<u>Inter -assay</u> <u>%CV</u>
Low	53	4.6	
Medium	246	9.5	
High	1,103	13.7	
Low	49		9.3
Medium	214		11.0
High	737		6.2

Cross Reactivities

The cross reactivities for a number of related eicosanoid compounds was determined by dissolving the cross reactant (purity checked by N.M.R. and other analytical methods) in Assay Buffer at concentrations from 500,000 to 4.8 pg/mL. These samples were then measured in the PGE₁ assay, and the measured PGE₁ concentration at 50% B/Bo calculated. The % cross reactivity was calculated by comparison with the actual concentration of cross reactant in the sample and expressed as a percentage.

<u>Compound</u>	<u>Cross Reactivity</u>
PGE ₁	100%
PGE ₂	6.50%
PGE ₃	2.22%
13,14-dihydro-PGE ₁ *	1.50%
PGE ₀	1.45%
15-keto-PGE ₁ *	1.15%
13,14-dihydro-15-keto-PGE ₁ *	0.19%
PGF _{1α}	0.14%
PGF _{2α}	0.04%
6-keto-PGF _{1α}	<0.1%
PGA ₂	<0.1%
PGD ₂	<0.1%
PGB ₁	<0.1%
13,14-dihydro-15-keto-PGF _{2α}	<0.1%
6,15-keto-13,14-dihydro-PGF _{1α}	<0.1%
Thromboxane B ₂	<0.1%
Misoprostol	<0.1%
2-Arachidonoylglycerol	<0.1%
Anandamide	<0.1%

* Data from Covance Laboratories, Inc., Vienna, Virginia.

Sample Recoveries

Please refer to pages 4 and 5 for Sample handling recommendations and Standard preparation.

PGE₁ concentrations were measured in a variety of different samples including tissue culture media, human saliva, serum, plasma, and urine. For samples in tissue culture media, ensure that the standards have been diluted into the same media (refer to page 4). PGE₁ was spiked into the undiluted samples of these media which were then diluted with the kit Assay Buffer and then assayed in the kit. The following results were obtained:

<u>Sample</u>	<u>% Recovery</u>	<u>Recommended Dilution*</u>
Tissue Culture Media	90-110	None
Human Saliva	107.2	1:10
Human Urine	109.9	1:50
Human Serum	87.0	1:20
Human Plasma	107.7	1:20

* See Sample Handling instructions on page 4 for details.

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LIMITED WARRANTY

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Material Safety Data Sheet (MSDS) available on our website or by fax.

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