Prostaglandin E1 ELISA Kit

Catalog Number KA0296

96 assays

Version: 02

Intended for research use only

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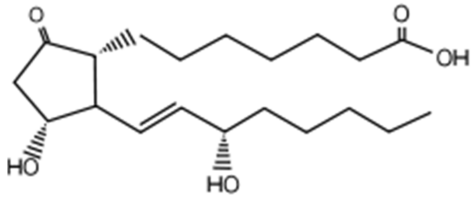
Introduction

Background

Prostaglandin E1 (PGE_1) is synthesized from DGLA, dihomo-γ-linolenic acid[3]. PGE1 has been shown to have a number of biological actions, including vasodilation[4], proliferation of vascular smooth muscle cells[5], platelet aggregation[6] and has been shown to have insulin-like actions[7,8]. Its effects are induced by receptor mediated elevation of cAMP[9]. It is the major prostaglandin in semen[10,11].

Prostaglandin E_1



Principle of the Assay

The 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 Chard[1] or Tijssen[2].

General Information

Materials Supplied

List of component

Component	Description	Amount
Donkey anti-Sheep IgG Microtiter Plate	A plate using break apart strips coated with donkey antibody specific to sheep IgG.	One Plate of 96 Wells
PGE1 EIA Conjugate	A blue solution of alkaline phosphatase conjugated with PGE1	6 mL
PGE1 EIA Antibody	A yellow solution of a sheep polyclonal antibody to PGE1	6 mL
Assay Buffer	Tris buffered saline, containing proteins and sodium azide as preservative.	30 mL
Wash Buffer Concentrate	Tris buffered saline containing detergents.	30 mL
Prostaglandin E1 Standard	A solution of 50,000 pg/mL PGE1	0.5 mL
pNpp Substrate	A solution of p-nitrophenyl phosphate in buffer. Ready to use.	20 mL
Stop Solution	A solution of trisodium phosphate in water. Keep tightly capped. Caution: Caustic.	6 mL
Plate Sealer,	-	1 each

Storage Instruction

All components of this kit, except the PEG1, conjugate, are stable at 4 ℃ until the kit's expiration date. The PEG1, conjugate must be stored at -20 ℃.

Materials Required but Not Supplied

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

Precautions for Use

- Precautions

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

✓ Some kit components contain azide, which may react with lead or copper plumbing. When disposing of reagents always fush with large volumes of water to prevent azide build-up.

✓ Stop Solution is a solution of trisodium phosphate. This solution is caustic; care should be taken in use.

✓ 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.

✓ 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.

✓ The Prostaglandin E1 Standard provided, 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.

- Procedural Notes:

✓ Do not mix components from different kit lots or use reagents beyond the kit expiration date.

✓ Allow all reagents to warm to room temperature for at least 30 minutes before opening.

✓ Standards can be made up in either glass or plastic tubes.

✓ Keep unused plate strips sealed in bag with desiccant.

✓ Pre-rinse the pipet tip with the reagent, use fresh pipet tips for each sample, standard and reagent.

✓ Pipet standards and samples to the bottom of the wells.

✓ Add the reagents to the side of the well to avoid contamination.

✓ 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 ℃ in the sealed bag provided. The wells should be used in the frame provided.

✓ 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.

✓ 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.

Assay Protocol

Reagent Preparation

- 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 #through #6 will be 5,000, 1,250, 313, 78.1, 19.5 and 4.88 pg/mL respectively.
Diluted standards should be used within 60 minutes of preparation.

- 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.

Sample Preparation

The PGE1 samples in a wide range of matrices after dilution in Assay Buffer. Please refer to the Sample Recovery recommendations 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 effciency to be accurately determined.
2. 2M hydrochloric acid, deionized water, ethanol, hexane and ethyl acetate.

3. 200 mg C_{18} 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 ℃ 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 ℃ until the immunoassay is to be run. Evaporate the organic solvent under a stream of nitrogen prior to running assay and reconstitute as above

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 ℃.
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.

Data Analysis

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:

- 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
- Calculate the binding of each pair of standard wells as a percentage of the maximum binding wells (Bo), using the following formula:
  Percent Bound = Net OD/ Net Bo OD x 100
- 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.

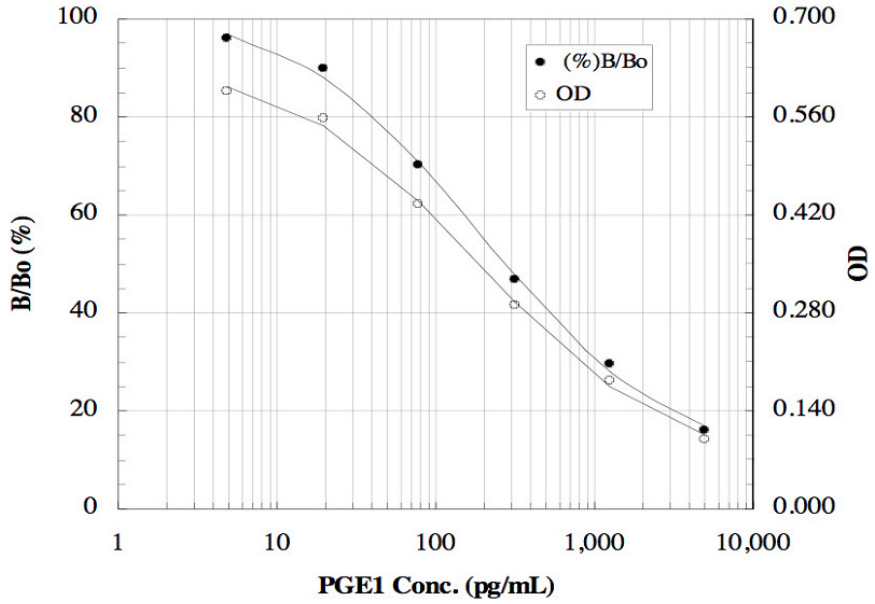
- Typical Results

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

	Mean	Average	Percent	PGE1
Sample	OD (-Blank)	Net OD	Bound	(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
S7	0.379	0.379	60.7%	143
Unknown 1	0.199	0.199	31.9%	894
Unknown 2	(0.072)	0.848	0.00%	0

- 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 ft)		
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 Protocols[16].

- Sensitivity

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	=	0.024/0.021 x 4.88 pg/mL	5.58 pg/mL

- 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.

	PGE1 (pg/mL)	Intra-assay %CV	Inter-assay %CV
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.

Compound	Cross Reactivity
PGE1	100%
PGE2	6.50%
PGE3	2.22%
13,14-dihydro-PGE1*	1.50%
PGE0	1.45%
15-keto-PGE1*	1.15%
13,14-dihydro-15-keto-PGE1*	0.19%
PGF1α	0.14%
PGF2α	0.04%
6-keto-PGF1α	<0.1%
PGA2	<0.1%
PGD2	<0.1%
PGB1	<0.1%
13,14-dihydro-15-keto-PGF2α	<0.1%
6,15-keto-13,14-dihydro-PGF1α	<0.1%
Thromboxane B2	<0.1%
Misoprostol	<0.1%
2-Arachidonoylglycerol	<0.1%
Anandamide	<0.1%

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

- Sample Recoveries

Please refer to 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. 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:

Sample	% Recovery*	Recommended Dilution*
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

Resources

References

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Plate Layout

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