PGE2 ELISA

For the quantitative determination of Prostaglandin E2 in Cell Culture, Saliva, Serum, Urine and Whole Blood.

For Research Use Only. Not For Use In Diagnostic Procedures**.**

Catalog Number: 74-PG2HU-E01
Size: 96 wells
Version: 112320 - ALPCO 1.1

Intended Use

The PGE_2 ELISA kit is a competitive immunoassay for the quantitative determination of Prostaglandin E_2 in biological fluids. For Research Use Only. Not for use in diagnostic procedures.

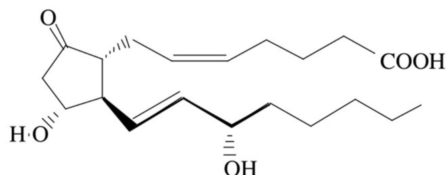
Description

The PGE_2 ELISA kit is a competitive immunoassay for the quantitative determination of Prostaglandin E_2 in biological fluids. Please read the complete kit insert before performing this assay. The kit uses a monoclonal antibody to PGE_2 to bind, in a competitive manner, the PGE_2 in the sample, standard, or an alkaline phosphatase molecule which has PGE_2 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 405 nm. The intensity of the yellow color is inversely proportional to the concentration of PGE_2 in either standards or samples. The measured optical density is used to calculate the concentration of PGE_2. For further explanation of the principles and practice of immunoassays please see the excellent books by Chard[1] or Tijssen[2].

Introduction

Prostaglandin E_2 is formed in a variety of cells from PGH_2, which itself is synthesized from arachidonic acid by the enzyme prostaglandin synthetase[3-]6. PGE_2 has been shown to have several biological actions, including vasodilation[7], both anti- and proinflammatory action[8,9], modulation of sleep/wake cycles[10], and facilitation of the replication of human immunodeficiency virus[11]. It elevates cAMP levels[12,] stimulates bone resorption[13], and has thermoregulatory effects. It has been shown to be a regulator of sodium excretion and renal hemodynamics[14].

Prostaglandin E_2



Precautions

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 Mg^{2+} and Zn^{2+} ions. The activity of the conjugate is affected by concentrations of chelators (>10 mM) such as EDTA and EGTA.
4. The kit's performance was tested with a variety of samples; however it is possible that high levels of interfering substances may cause variation in assay results.
5. The Prostaglandin E_2 Standard provided is supplied in ethanolic buffer at a pH optimized to maintain PGE_2 integrity. Care should be taken handling this material because of the known and unknown effects of prostaglandins.

Materials Supplied

1. **Goat anti-Mouse IgG Microtiter Plate, One Plate 96 Wells**
   A plate using break-apart strips coated with goat antibody specific to mouse IgG.
2. **PGE_2 ELISA Conjugate, 5 mL**
   A blue solution of alkaline phosphatase conjugated with PGE_2.
3. **PGE_2 ELISA Antibody, 5 mL**
   A yellow solution of a monoclonal antibody to PGE_2.

4. **Assay Buffer, 27 mL**
   Tris buffered saline containing proteins and sodium azide as preservative.
5. **Wash Buffer Concentrate, 27 mL**
   Tris buffered saline containing detergents.
6. **Prostaglandin E_2 Standard, 0.5 mL**
   A solution of 50,000 pg/mL PGE_2.
7. **pNpp Substrate, 20 mL**
   A solution of p-nitrophenyl phosphate in buffer. Ready to use.
8. **Stop Solution, 5 mL**
   A solution of trisodium phosphate in water. Keep tightly capped. Caution: **Caustic.**
9. **PGE_2 Assay Layout Sheet, 1 each**
10. **Plate Sealer, 1 each**

Storage

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

Materials Needed but Not Supplied

1. Deionized or distilled water.
2. Precision pipets for volumes between 5 µL and 1,000 µL.
3. Repeater pipets for dispensing 50 µL and 200 µL.
4. Disposable beaker for diluting buffer concentrates.
5. Graduated cylinders.
6. A microplate shaker.
7. Absorbent paper for blotting.
8. Microplate reader able to read at 405 nm, preferably with correction between 570 and 590 nm.

Sample Handling

The PGE_2 ELISA kit is compatible with PGE_2 samples in a wide range of matrices after dilution in Assay Buffer. Please refer to the Sample Recovery recommendations on page 8 for details of suggested dilutions. However, the end user **must verify** that the recommended dilutions are appropriate for their samples. **Samples containing mouse IgG may interfere with the assay.**

Samples in most 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 PGE_2 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 PGE_2 present and extraction may be necessary for accurate measurement. A suitable extraction procedure is outlined below:

Materials Needed

1. PGE_2 Standard to allow extraction efficiency 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°C for 15 minutes. Centrifuge samples in a microcentrifuge for 2 minutes to remove any precipitate.
2. Prepare the C_{18} reverse phase column by washing with 10mL of ethanol followed by 10mL 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 sample. Vortex well, then allow to sit for five minutes at room temperature. 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 15-18 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 the 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. PGE_2 Standard

Allow the 50,000 pg/mL PGE_2 standard solution to warm to room temperature. Label seven 12 x 75 mm glass tubes #1 through #7. Pipet 1 mL of standard diluent (Assay Buffer or Tissue Culture Media) into tube #1. Pipet 500 µL of standard diluent into tubes #2 through #7. Remove 50 µL of diluent from tube #1. Add 50 µL of the 50,000 pg/mL standard to tube #1. Vortex thoroughly. Add 500 µL of tube #1 to tube #2 and vortex thoroughly. Add 500 µL of tube #2 to tube #3 and vortex. Continue this for tubes #4 through #7.

The concentration of PGE_2 in tubes #1 through #7 will be 2,500, 1,250, 625, 313, 156, 78.1 and 39.1 pg/mL, respectively. See PGE_2 Assay Layout Sheet for dilution details. STORE STANDARD AT -20°C, avoid repeated freeze-thaws.

Diluted standards should be used within 60 minutes of preparation.

2. PGE2 Conjugate
Allow the conjugate to warm to room temperature. Any unused conjugate should be aliquoted and re-frozen at or below -20 °C.

3. Wash Buffer
Prepare the Wash Buffer by diluting 5 mL of the supplied concentrate with 95 mL of deionized water. This can be stored at room temperature until the kit expiration date, or for 3 months, whichever is earlier.

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 ziplock. 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 #7 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 Total Activity (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 all wells to stop the reaction. 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 PGE_2 in the samples. It is recommended that the data be handled by an immunoassay software package utilizing a 4-parameter logistic curve fitting program.

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:

   $$\text{Percent Bound} = \frac{\text{Net OD}}{\text{Net Bo OD}} \times 100$$

3. Plot both the Percent Bound and the Net OD versus Concentration of PGE_2 for the standards. Sample concentrations may be calculated off Net OD values using the desired curve fitting.

Make sure to multiply sample concentrations calculated off the curve by the dilution factor used during sample preparation to get starting sample concentration.

Samples that read at concentrations outside of the standard curve range will need to be re-analyzed using a different dilution.

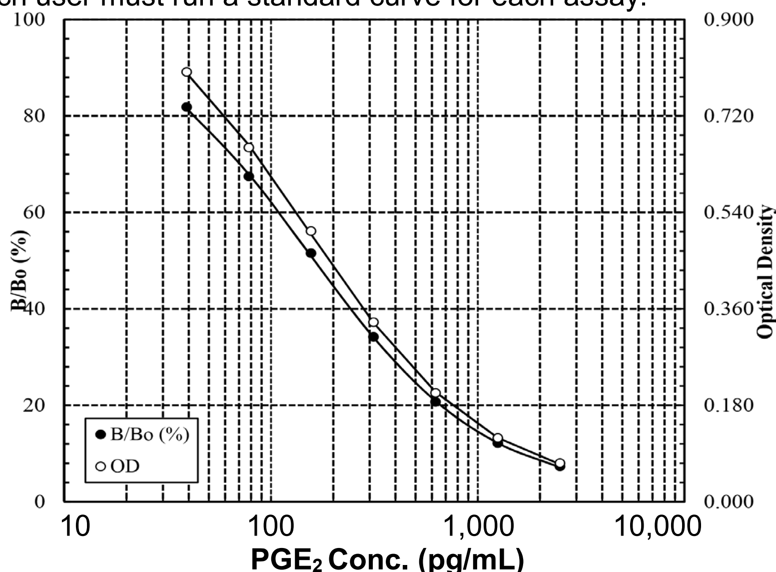
Typical Results

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

Sample	Mean OD (-Blank)	Average Net OD	Percent Bound	Percent **(pg/mL)**
Blank OD	(0.087)			
TA	1.220			
NSB	0.002	0.000	0.00%	
Bo	0.982	0.980	100%	**0**
S1	0.074	0.072	7.3%	**2,500**
S2	0.121	0.119	12.1%	**1,250**
S3	0.205	0.203	20.7%	**625**
S4	0.337	0.335	34.2%	**313**
S5	0.507	0.505	51.5%	**156**
S6	0.663	0.661	67.4%	**78.1**
S7	0.804	0.802	81.8%	**39.1**
Unknown 1	0.299	0.297	30.3%	**375.3**
Unknown 2	0.610	0.608	62.0%	**100.7**

Typical Standard Curve

A typical standard curve is shown below. This curve **must not** be used to calculate PGE_2 concentrations; each user must run a standard curve for each assay.



Typical Quality Control Parameters

Total Activity Added	=	1.220 x 10 = 12.20
%NSB	=	0.02%
%Bo/TA	=	8.04%
Quality of Fit (curve fit)	=	1.000 (Calculated from 4 parameter logistic fit)
20% Intercept	=	664 pg/mL
50% Intercept	=	162 pg/mL
80% Intercept	=	43 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[19].

Sensitivity

Sensitivity was calculated by determining the average optical density bound for sixteen (16) wells run as B_o and comparing to the average optical density for sixteen (16) wells run with Standard #7. The detection limit was determined as the concentration of PGE_2 measured at two (2) standard deviations from the zero along the standard curve.

Average Optical Density for the Bo = 0.565 ± 0.011 (2.0%)
Average Optical Density for Standard #7 = 0.501 ± 0.019 (3.8%)
Delta Optical Density (0-39.1 pg/mL) = 0.064

2 SD's of the Zero Standard = 2 x 0.011 = 0.022

Sensitivity = $\frac{0.022}{0.064} \times$ 39.1 pg/mL = **13.4 pg/mL**

Linearity

A sample containing 50,000 pg/mL PGE_2 was diluted 6 times 1:2 in the kit Assay Buffer and measured in the assay. The data was plotted graphically as actual PGE_2 concentration versus measured PGE_2 concentration. The line obtained had a slope of 1.1610 and a correlation coefficient of 1.000.

Precision

Intra-assay precision was determined by taking samples containing low, medium, and high concentrations of PGE_2 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 PGE_2 in multiple assays (n=8).

The precision numbers listed below represent the percent coefficient of variation for the concentrations of PGE_2 determined in these assays as calculated by a 4-parameter logistic curve fitting program

	PGE2 (pg/mL)	Intra-Assay % CV	Inter-Assay % CV
Low	116	8.9	
Medium	492	5.8	
High	2,416	17.5	
Low	111		3.0
Medium	419		5.1
High	1,902		3.9

Cross Reactivities

The cross reactivities for several 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 39 pg/mL. These samples were then measured in the PGE_2 assay, and the measured PGE_2 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
PGE_2	100%
PGE_1	70%
PGE_3	16.3%
$PGF_{1\alpha}$	1.4%
$PGF_{2\alpha}$	0.7%
6-keto-$PGF_{1\alpha}$	0.6%
PGA_2	0.1%
PGB_1	0.1%
13,14-dihydro-15-keto-$PGF_{2\alpha}$	<0.1%
6,15-keto-13,14-dihydro-$PGF_{1\alpha}$	<0.1%
Thromboxane B_2	<0.1%
2-Arachidonoylglycerol	<0.1%
Anandamide	<0.1%
PGD_2	<0.1%
Arachadonic Acid	<0.1%

Sample Recoveries

Please refer to pages 3 and 4 for Sample Handling recommendations and Standard preparation.

PGE_2 concentrations were measured in a variety of different samples including tissue culture media, human saliva, serum, and urine. PGE_2 was spiked into the undiluted samples of these matrices which were then diluted with the appropriate diluent and assayed in the kit. The following results were obtained:

Sample	**% Recovery***	**Recommended Dilution***
Tissue Culture Media	104.4	None
Human Saliva	123.3	1:10
Human Urine	108.9	1:10
Human Male Serum	126.1	1:10
Human Female Serum	113.7	1:10
Human Whole Blood	101.2	1:10

* See Sample Handling instructions on page 3 and 4 for details.

References

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PGE_2 PLATE LAYOUT:

A1 Blank	A2 Std 1	A3 Std 5	A4	A5	A6	A7	A8	A9	A10	A11	A12
B1 Blank	B2 Std 1	B3 Std 5	B4	B5	B6	B7	B8	B9	B10	B11	B12
C1 TA	C2 Std 2	C3 Std 6	C4	C5	C6	C7	C8	C9	C10	C11	C12
D1 TA	D2 Std 2	D3 Std 6	D4	D5	D6	D7	D8	D9	D10	D11	D12
E1 NSB	E2 Std 3	E3 Std 7	E4	E5	E6	E7	E8	E9	E10	E11	E12
F1 NSB	F2 Std 3	F3 Std 7	F4	F5	F6	F7	F8	F9	F10	F11	F12
G1 Bo	G2 Std 4	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
H1 Bo	H2 Std 4	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12

Kit Lot No. ________________ Exp. Date ________________ Date ________________

Tech. ________________

1st Incub.:	Start Time __________	Temp. ________	Notes:____________________________
	End Time __________	Temp. ________	___________________________________
2nd Incub.:	Start Time __________	Temp. ________	___________________________________
	End Time __________	Temp. ________	___________________________________