Sample Recovery (Contd)

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 for details.

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 E2 Standard provided, is supplied in ethanolic buffer at a pH optimized to maintain PGE2 integrity. Care should be taken handling this material because of the known and unknown effects of prostaglandins.

The Control Serum , if supplied, has been prepared from human sera shown to be negative for HBsAg and HIV antibodies. Nevertheless, such tests are unable to prove the complete absence of viruses; therefore, sera should be handled with appropriate precautions.

Applicable MSDS, if not already on file, for the following reagents can be obtained from ADI or the web site for Prolcin-300 (0.1% v/v in standards, and assay buffers).
http://4adi.com/commerce/info/showpage.jsp?page_id=1060&category_id=2430&visit=10

References: 1. K. Green, et al., Anal. Biochem, (1973) 54: 434; 2. J. Frolich, et al., J. Clin. Invest., (1975) 55: 763; 3. J.E. Shaw & P.W. Ramwell, Meth. Biochem. Anal., (1969) 17: 325; 4. K. Green, et al., Adv. Prostaglandin & Thromboxane Res., (1978) 5: 15.

Instruction Manual No. M-100-560-PG2

Prostaglandin E2 (PGE2) ELISA KIT

Cat# 100-560-PG2, 96 Tests

For Quantitative Determination of Prostaglandin E2 (PGE2)
In Human Serum or other biological fluids

For In Vitro Research Use Only

ALPHA DIAGNOSTIC INTERNATIONAL

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Toll Free (800) 786-5777
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Web Site: www.4adi.com

Please consult the manual supplied with the kit for any lot specific change.

PROSTAGLANDIN E2 (PGE2) ELISA KIT# 100-560-PG2

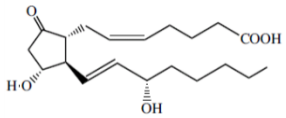
Kit Contents: (reagents for 96 tests)

Components	#
Anti-mouse IgG Coated Microwell strip plate (96 wells), #100561	1 Plate
PGE2 Std. A (50,000 pg/ml), 0.5 ml; additional standards are made from this stock, # 100562	1 vial
PGE2 Antibody, 5 ml, Yellow Solution, # 100563	1 bottle
PGE2 Assay Buffer, 27 ml, # 100564	1 bottle
PGE2 Conjugate, 5 ml, Blue Solution, #100565	1 bottle
Wash Buffer Conc. (20X), 27 ml (dilute with water), # 100560-WB	1 bottle
pNpp substrate Solution ; 20 ml, # 100560-SS	1 bottle
Stop solution, 5 ml, # 100560-ST	1 bottle
Plate cover & Plate sheet	1 each
Complete Instruction Manual, M-100-560-PG2	1
Note: 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 arrival.** Samples required extraction and additional material requited (see page 2).	

Intended Use

ADI's PGE2 ELISA kit is a competitive ELISA for the measurement of PGE2 in human serum, urine and saliva, tissue culture media. This kit requires samples to be extracted or process before testing (see page 3). **For research use only (RUO)**, not for diagnosis, cure or prevention of the disease.

Introduction



Prostaglandin E2 is formed in a variety of cells from PGH2, which itself is synthesized from arachidonic acid by the enzyme prostaglandin synthetase3-6 . PGE2 has been shown to have a number of biological actions, including vasodilation7 , both anti- and proinflammatory action8,9, modulation of sleep/wake cycles10, and facilitation of the replication of human immunodeficiency virus11. It elevates cAMP levels12, stimulates bone resorption13, and has thermoregulatory effects. It has been shown to be a regulator of sodium excretion and renal hemodynamicsmicropenis.

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.

Sensitivity
Sensitivity was calculated by determining the average optical density bound for sixteen (16) wells run as Bo, and comparing to the average optical density for sixteen (16) wells run with Standard #7. The detection limit was determined as the concentration of PGE2 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 = $\frac{0.022}{0.064}$ x 39.1 pg/mL **= 13.4 pg/mL**

Linearity

A sample containing 50,000 pg/mL PGE2 was diluted 6 times 1:2 in the kit Assay Buffer and measured in the assay. The data was plotted graphically as actual PGE2 concentration versus measured PGE2 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 PGE2 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 PGE2 in multiple assays (n=8).

The precision numbers listed below represent the percent coefficient of variation for the concentrations of PGE2 determined in these assays as calculated by a 4 parameter logistic curve fitting program.

Intra-assay
Low (116 pg/ml), CV% 8.9 Medium (492 pg/ml) CV% 5.8 High (2416 pg/ml) CV% 17.5

Inter-assay
Low (111 pg/ml), CV% 3.0 Medium (419 pg/ml) CV% 5.1 High (1902 pg/ml) CV% 3.9

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 39 pg/mL. These samples were then measured in the PGE2 assay, and the measured PGE2 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.

PGE2 (100%), PGE1 (70%), PGE3 (16.3), PGF1a (1.4%), PGF2a (0.7%), 6-keto-pGF1a (0.6%).
PGA2 0.1%, PGB1 0.1%, 13,14-dihydro-15-keto-PGF2a <0.1%, 6,15-keto-13,14-dihydro-PGF1a <0.1%, Thromboxane B2 <0.1%, 2-Arachidonoylglycerol <0.1%, Anandamide <0.1%, PGD2 <0.1%
Arachadonic Acid <0.1%

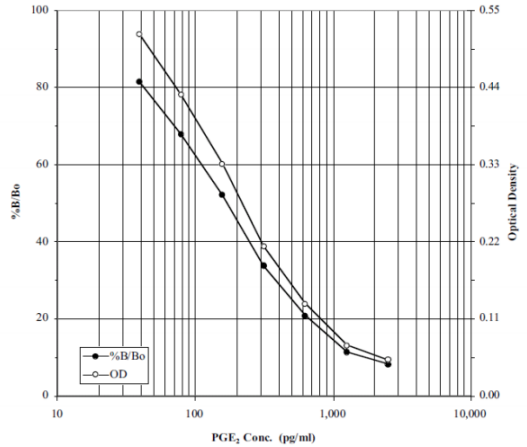
Sample Recoveries
Please refer to pages 2 and 3 for Sample Handling recommendations and Standard preparation.

PGE2 concentrations were measured in a variety of different samples including tissue culture media, human saliva, serum, and urine. PGE2 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:

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples (ng/ml)	Net Mean $A_{450\ nm}$	% Bound
Blank OD	0.079		
TA	2.81		
NSB	0.006		
Bo		0.633	100%
A1, A2	**Std. A** (2500 pg/ml)	0.052	8.2
B1, B2	**Std. B** (1250 pg/ml)	0.072	11.4
C1, C2	**Std. C** (625 pg/ml)	0.132	20.8
D1, D2	**Std. D** (313 pg/ml)	0.214	33.8
E1, E2	**Std. E** (156 pg/ml)	0.330	52.1
F1, F2	**Std. F** (78.1 pg/ml)	0.430	67.9
G1, G2	**Std. G** (39.1 pg/ml)	0.516	81.5
B1, B2	**Sample 1**	0.28	45.7

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.



*9_ADI_ELISA
A typical std. assay curve (do not use this for calculating sample values)

CALCULATION OF RESULTS

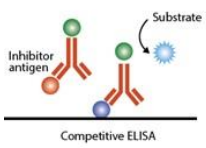
Several options are available for the calculation of the concentration of PGE2 in the samples. We recommend that the data be handled by an immunoassay software package utilizing a 4 parameter logistic curve fitting program. If data reduction software is not readily available, the concentration of PGE2 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: % Bound = Net OD x 100 Net Bo OD.

3. Using Logit-Log paper plot Percent Bound versus Concentration of PGE2 for the standards. Approximate a straight line through the points. The concentration of PGE2 in the unknowns can be determined by interpolation.

PRINCIPLE OF THE TEST



The Prostaglandin E2 (PGE2) kit is a competitive immunoassay for the determination of Prostaglandin E2 in biological fluids. Please read the complete kit insert before performing this assay. The kit uses a monoclonal antibody to PGE2 to bind, in a competitive manner, the PGE2 in the sample, standard, or an alkaline phosphatase molecule which has PGE2 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 read on a microplate reader at 405 nm. The intensity of the bound yellow color is inversely proportional to the concentration of PGE2 in either standards or samples. The measured optical density is used to calculate the concentration of PGE2.

MATERIALS AND EQUIPMENT REQUIRED 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 capable of reading at 405 nm, preferably with correction between 570 and 590 nm.

Reagent Preparation

Wash buffer (20X). Dilute with water (30 ml stock in 570 ml water). Store at 4oC.

PGE2 Standard Preparation

Stock PGE2 standard is supplied at 50 ng/ml or (50,000 pg/ml).
Prepare 2,500 pg/ml std by diluting the stock 1:20 with assay buffer

Stds	Stock std	Assay buffer	Volume	Final Std Conc
A	25 ul of 50,000 pg/ml stock	475 ul	500 ul	2,500 pg/ml
B	250 ul of Std A	250 ul	500 ul	1,250 pg/ml
C	250 ul of Std B	250 ul	500 ul	625 pg/ml
D	250 ul of Std C	250 ul	500 ul	313 pg/ml
E	250 ul of Std D	250 ul	500 ul	156 pg/ml
F	250 ul of Std E	250 ul	500 ul	78.1 pg/ml
G	250 ul of Std F	250 ul	500 ul	39.1 pg/ml

Note: When making standard dilution, allow gentle mixing for 5-10 seconds before taking a sample for serial dilution. Prepare working standards on the day of the test and store at 4oC. It must be brought to room temp before adding to the coated plates. Do not store working standards beyond the assay date. Prepare fresh standards for each run.

Additional stds of 2,500, 1,250, 625, 313, 156, 78.1 and 39.1 pg/mL are prepared by 2-fold serial dilutions. Example: for 2500 pg/ml std, dilute stock 50,

Diluted standards should be used within 60 minutes of preparation.

SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture, allow to clot, and separate the serum by centrifugation at room temperature. Do not heat inactivate the serum. If testosterone is to be measured in plasma then heparin should be used. Centrifuge for 10 min and carefully remove the plasma layer and store at 4oC. If sera cannot be immediately assayed, these could be stored at -20^{o}C for up to six months. Avoid repeated freezing and thawing of samples. No preservatives should be added to the serum.

Azide and thimerosal at concn. >0.01% interfere in this test. Therefore, samples containing high concn. Of preservative may give high values.

Sample Handling

This kit is compatible with PGE2 samples in a wide range of matrices after dilution in Assay Buffer. Please refer to the Sample Recovery recommendations on page 7 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 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 PGE2 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 PGE2 present and extraction may be necessary for accurate measurement. A suitable extraction procedure is outlined below:

Materials Needed

1. PGE2 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 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 0.5mL/minute. Wash the column with 10mL 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.

If plasma or other samples with very low concentrations of PGE2 are to be measured, we would suggest using another ADI kit available from ADI.

STORAGE AND STABILITY

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.

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).* **Dilute wash buffer (1:20) with distilled water. Prepare working standards set (see page 2).**

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 A through G** 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 1X 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.

NOTES - Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. Plate readers measure absorbance vertically. Do not touch the bottom of the wells.

Typical Quality Control Parameters

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