



Correlate-CLIA™

High Sensitivity Prostaglandin E₂ Chemiluminescence Enzyme Immunoassay Kit

Catalog No. 910-001

96 Well Kit

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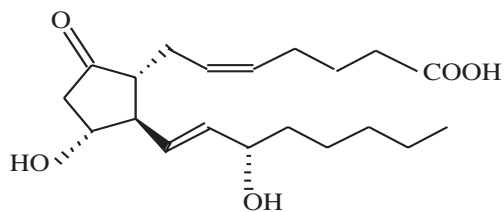
Description

Assay Designs' Correlate-CLIA™ Prostaglandin E₂ kit is a competitive immunoassay for the quantitative sensitive 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 standard or sample 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 chemiluminescent substrate is added. The substrate reacts with the bound alkaline phosphatase conjugate to produce light emission at approximately 530nm. The intensity of the emitted light is inversely proportional to the concentration of PGE₂ in either standards or samples. The measured chemiluminescence is used to calculate the concentration of PGE₂. For further explanation of the principles and practice of immunoassays please see the excellent books by Chard¹ or Tijssen².

Introduction

Prostaglandin E₂ (PGE₂) 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 action^{8,9}, modulation of sleep/wake cycles¹⁰, and facilitation of the replication of human immunodeficiency virus¹¹. It elevates cAMP levels¹² and stimulates bone resorption¹³, and has thermoregulatory effects. It has been shown to be a regulator of sodium excretion and renal hemodynamics¹⁴.

Prostaglandin E₂



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. 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 high concentrations of chelators, such as EDTA and EGTA. Please contact Assay Designs for further information.
3. 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.
4. The Prostaglandin E₂ Standard provided, Catalog No. 80-0004, 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.

Materials Supplied

1. **Goat anti-mouse Microtiter Plate, One Plate of 96 Wells, Catalog No. 80-0035**
A white plate using break-apart strips coated with goat antibody specific to mouse IgG.
2. **PGE₂ CLIA Conjugate, 6 mL, Catalog No. 80-0013**
A blue solution of alkaline phosphatase conjugated with PGE₂.
3. **PGE₂ CLIA Antibody, 6 mL, Catalog No. 80-0014**
A yellow solution of a monoclonal antibody to PGE₂.
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 E₂ Standard, 0.5 mL, Catalog No. 80-0004**
A solution of 50,000 pg/mL PGE₂.
7. **Lumiphos 530™ CLIA Substrate*, 21 mL, Catalog No. 80-0134**
Alkaline Phosphatase substrate in diethanolamine buffer at pH 9.5, containing fluorescent enhancers, with sodium azide as preservative.
8. **PGE₂ Plate CLIA Assay Layout Sheet, 1 each, Catalog No. 30-0039**
9. **Plate Sealer, 1 each, Catalog No. 30-0012**

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.

Materials Needed but Not Supplied

1. Deionized or distilled water. No difference is seen in assay results with 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 beakers for diluting buffer concentrates.
5. Graduated cylinders.
6. A micro plate shaker.
7. Adsorbent paper for blotting.
8. Plate luminometer, such as an EG&G Wallac LB 96P, capable of reading glow chemiluminescence. Some radiation counters may be suitable. Please refer to the counter instruction manual for recommendations on suitability for chemiluminescence measurements.

*Lumiphos 530 is the trademark of Lumigen Inc., Southfield, MI, USA and supplied under US patents 4,857,652; 4,983,779; 4,959,182; 5,004,565; 4,962,192, & 5,386,017; European patents 254051B1 & 352713B1; Japanese patent 5-45590; Australian patent 603,736; Korean patent 69,259 and Taiwanese patent 46,563.

Sample Handling

Assay Designs' Correlate-CLIA™ is compatible with PGE₂ samples in a wide range of matrices. Samples diluted sufficiently into Assay Buffer can be read directly from the standard curve. Please refer to the Sample Recovery recommendations on page 11 for details of suggested dilutions. **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. Most samples may be used in the assay directly by dilution in the range of 1:10 in Assay Buffer.

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 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 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 reagents from different lot numbers or use reagents beyond the 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 CLIA 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.**
10. **The chemiluminescent signal generated is read after 60 minutes. The signal is still being generated and the wells must be read in the order in which the substrate was added. If you do not have a luminometer that will precisely time the substrate incubation the following protocol must be followed. We suggest adding the substrate at 10 second intervals between wells and reading the generated chemiluminescence for 2 seconds at 10 second intervals for consistency. If luminometer injection is not used, we suggest using a repeater type syringe, such as an Eppendorf™ Repeater™ Pipette, Catalog Number 2226000-6 and a 5 mL repeater Combipip™ set for delivery of 200 µL. Use the repeater to add substrate to the wells in the order in which they will be read. Please note the order that plate luminometers read wells and ensure substrate addition follows this sequence.**

Reagent Preparation

1. PGE₂ Standard

Allow the 50,000 pg/mL PGE₂ Standard solution to warm to room temperature. Label eight 12 x 75 mm tubes #1 through #8. Pipet 980 µL of standard diluent (Assay Buffer or Tissue Culture Media) into tube #1. Pipet 500 µL of standard diluent (Assay Buffer or Tissue Culture Media) into tubes #2 - #8. Add 20 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 #8.

The concentration of PGE₂ in tubes #1 through #8 will be 1,000, 500, 250, 125, 62.5, 31.25, 15.63 and 7.81 pg/mL respectively. See PGE₂ Plate CLIA Assay Layout Sheet for dilution details.

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 strips with the desiccant back into the pouch and seal the ziploc. Store unused strips 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) wells.
3. Pipet 100 µL of Standards #1 through #8 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 the blue Conjugate into each well, except the Total Activity (TA) and Blank wells.
7. Pipet 50 µL of the 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.
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 dry 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 CLIA Substrate solution to every well. Incubate at room temperature for 60 minutes with shaking. **Note: Refer to substrate addition timing and sequence on page 5**
13. Read each well for 2 seconds each on a suitable luminometer.

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 Relative Light Units (RLU) bound for each standard and sample by subtracting the average Blank RLU from the average RLU's bound.

$$\text{Average Net RLU} = \text{Average Bound RLU} - \text{Average NSB RLU}$$

2. Calculate the binding of each pair of standard wells as a percentage of the maximum binding wells (Bo), using the following formula:

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

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.

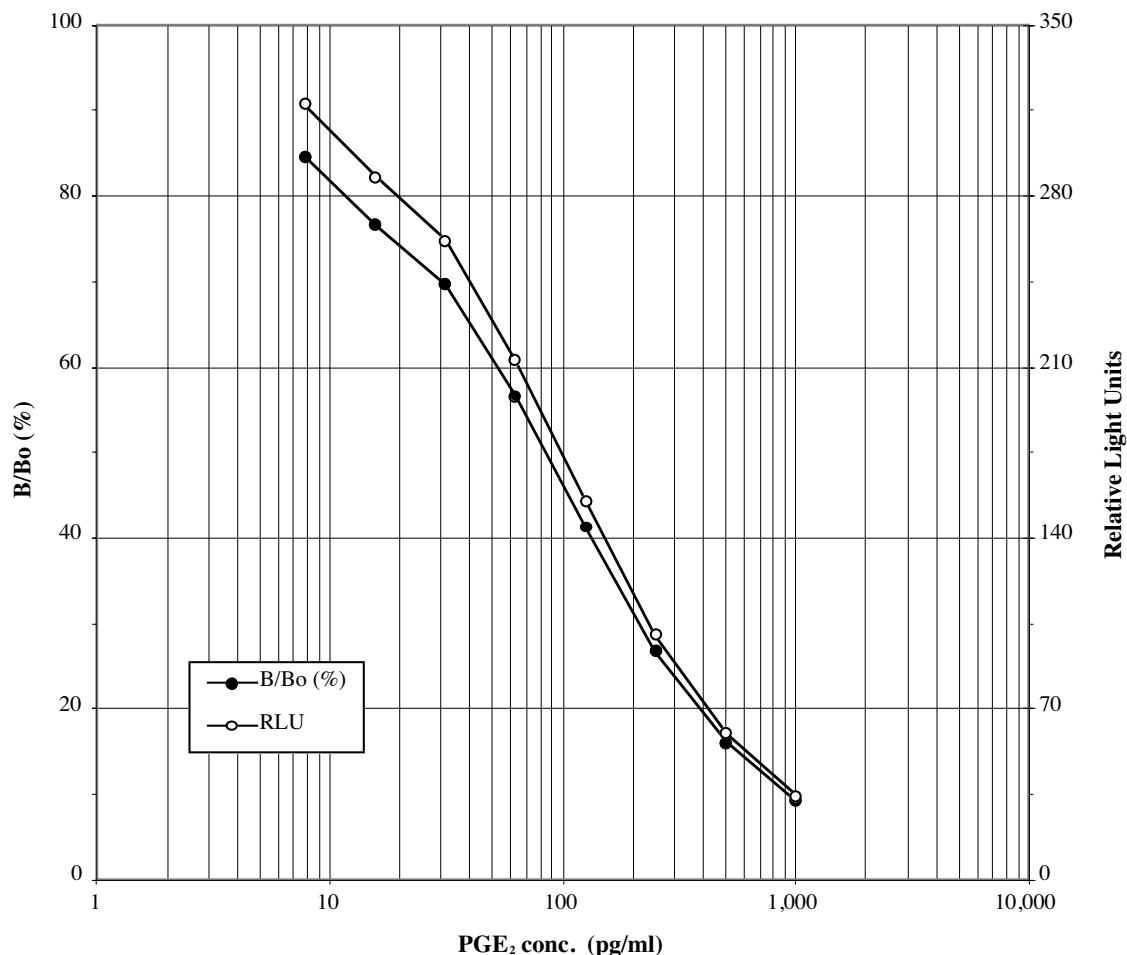
Typical Results

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

<u>Sample</u>	<u>Mean RLU (-Blank)</u>	<u>Average Net RLU</u>	<u>Percent Bound</u>	<u>PGE₂ (pg/mL)</u>
Blank RLU	2.487			
TA	2804.195			
NSB	0.066	0	0.00%	
S1	34.431	34.365	9.2%	1,000
S2	60.124	60.058	16.0%	500
S3	100.485	100.419	26.7%	250
S4	155.352	155.286	41.3%	125
S5	212.630	212.514	56.6%	62.5
S6	261.975	261.909	69.7%	31.25
S7	287.745	287.679	76.6%	15.63
S8	317.872	317.806	84.6%	7.81
Bo	375.721	375.655	100%	0
Unknown 1	56.138	56.072	14.9%	548.8
Unknown 2	199.060	198.994	53.0%	73.2

Typical Standard Curve

A typical standard curve is shown below. This curve **must not** be used to calculate PGE₂ concentrations; each user must run a standard curve for each assay.



Typical Quality Control Parameters

Total Activity Added	=	2804.195 x 10 = 28041.95
%Bo/TA	=	1.3%
Quality of Fit	=	0.999 (Calculated from 4 parameter logistic curve fit)
20% Intercept	=	328.9 pg/mL
50% Intercept	=	84.0 pg/mL
80% Intercept	=	12.9 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 Protocols¹⁹.

Sensitivity

Sensitivity was calculated in Assay Buffer by determining the average RLU signal bound for sixteen (16) wells run as Bo, and comparing to the average RLU signal for sixteen (16) wells run with Standard #8. The detection limit was determined as the concentration of PGE₂ measured at two (2) standard deviations from the zero along the standard curve.

Average RLU for the Bo = 359,060 ± 13,190 (3.7%)
Average RLU for Standard #8 = 324,869 ± 18,271 (5.6%)

Delta RLU's (0-7.81 pg/mL) = 359,060 - 324,869 = 34,191

2 SD's of the Zero Standard = 2 x 13,190 = 26,380

Sensitivity = $\frac{26,380}{34,191} \times 7.81 \text{ pg/mL} = \mathbf{6.03 \text{ pg/mL}}$

Linearity

A sample containing 620.7 pg/mL PGE₂ was diluted 4 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 0.960 and a correlation coefficient of 0.999.

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.

	<u>PGE₂</u> (<u>pg/mL</u>)	<u>Intra-assay</u> <u>%CV</u>	<u>Inter-assay</u> <u>%CV</u>
Low	7.07	14.7	
Medium	120	4.1	
High	330	7.4	
Low	59.04		9.2
Medium	182		7.1
High	923		5.6

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 Correlate-EIA™ colorimetric 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.

<u>Compound</u>	<u>Cross Reactivity</u>	<u>Compound</u>	<u>Cross Reactivity</u>
PGE ₂	100%	PGB ₁	0.1%
PGE ₁	70%	13,14-dihydro-15-keto-PGF _{2α}	<0.1%
PGE ₃	16.3%	6,15-keto-13,14-dihydro-PGF _{1α}	<0.1%
PGF _{1α}	1.4%	Thromboxane B ₂	<0.1%
PGF _{2α}			
6-keto-PGF _{1α}	0.6%	Anandamide	<0.1%
PGA ₂	0.1%	PGD ₂	<0.1%
		Arachadonic Acid	<0.1%

Sample Recoveries

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

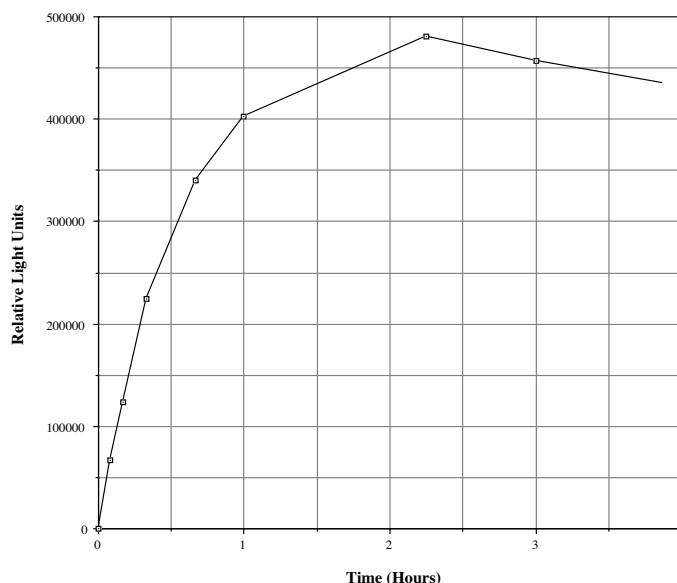
PGE₂ concentrations were measured in a variety of different samples including tissue culture media, human saliva, serum, and urine. PGE₂ was spiked into the undiluted samples of these media which were then diluted with the appropriate diluent and assayed in the Correlate-EIA™ kit. The following results were obtained:

<u>Sample</u>	<u>% Recovery*</u>	<u>Recommended Dilution*</u>
Tissue Culture Media	102.4	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	113.6	1:10

*See Sample Handling instructions on page 4 for details.

Time Course of Chemiluminescent Emission

The chemiluminescent signal generated from the reaction of the alkaline phosphatase conjugate and the CLIA substrate is a kinetic reaction that reaches a maximum light output after approximately 4 hours. The chemiluminescent emission will last for several hours. The data is presented below.



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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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