ARBOR ASSAYS

DetectX®

Prostaglandin E_2

Enzyme Immunoassay Kit

1 Plate Kit	Catalog Number K018-H1
5 Plate Kit	Catalog Number K018-H5

SPECIES INDEPENDENT

Sample Types Validated:

Saliva, Urine, Serum, EDTA and Heparin Plasma and Tissue Culture Media

Please read this insert completely prior to using the product.

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

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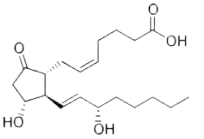
Background



Prostaglandin E_2

Eicosanoid signal transduction pathways are highly conserved and are involved in a number of physiological processes. Prostaglandins are synthesized from arachidonic acid by cyclooxygenase (COX)-1 or -2, which convert the acid into PGH_2. This is further processed by cytosolic or microsomal prostaglandin synthases to become PGE_2 or one of several other prostanoids[1-3]. Prostacyclin is the major cyclooxygenase product in blood vessel walls and it is present in inflammatory fluids in similar concentrations to PGE_2. Prostacyclin is a potent vasodilator and is more potent than PGE_2 in producing hyperalgesia[4]. PGE_2 is produced by a wide variety of tissues[5-14] and in several pathological conditions, including inflammation, arthritis, fever, tissue injury, endometriosis, and a variety of cancers[5,6].

Other biological actions of PGE_2 include vasodilation, modulation of sleep/wake cycles, and facilitation of human immunodeficiency virus replication. It elevates cAMP levels, stimulates bone resorption, and has thermoregulatory effects. It has been shown to be a regulator of sodium excretion and renal hemodynamics[7-12].

1. Moncada, S., Ferriera, SH. & Vane, JR. (1979). "Pain and inflammatory mediators." In Anti-Inflammatory Drugs. Handbook of Experimental Pharmacology, 50/II. Pp. 588-616. Vane, J.R. & Ferreira, S.H. Berlin, New York: Springer.
2. Vane, JR. (1971). "Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs." Nature, 231, 232-235.3.
3. Willis, AL. (1969). "Release of histamine, kinin and prostaglandins during carrageenin-induced inflammation in the rat." In Prostaglandins, Peptides and Amines. Pp. 31-38. Ed. Mantegazza, P. & Horton, E.W. London: Academic Press.
4. Higgs, GA., Cardinal, DC., Moncada, S. & Vane, JR. (1979). "Microcirculatory effects of prostacyclin (PGI2) in the hamster cheek pouch." Microvascular Res., 18, 245-254.
5. Kargman, S. et al. "Mechanism of selective inhibition of human prostaglandin G/H synthase-1 and -2 in intact cells" (1996) Biochem Pharmacol. 52(7):1113-25
6. Thun MJ, Namboodiri MM, Heath CW Jr. "Aspirin use and reduced risk of fatal colon cancer." New Engl. J. Med. 1991; 325: 1593–6.
7. Richardson PD and Withrington PG, , "The vasodilator actions of isoprenaline, histamine, prostaglandin E_2, glucagon and secretin on the hepatic arterial vascular bed of the dog." Brit. J. Pharmacol., (1976) 57: 581-588.
8. O. Hayaishi, "Sleep-Wake Regulation by Prostaglandins D_2 and E_2." J. Biol. Chem., (1988) 263: 14593-14596.
9. S. Kuno, et al., "Prostaglandin E_2, a seminal constituent, facilitates the replication of acquired immune deficiency syndrome virus in vitro. "Proc. Natl. Acad. Sci., USA, (1986) 83: 3487-3490.
10. D.L. Bareis, et al., "Bradykinin stimulates phospholipid methylation, calcium influx, prostaglandin formation, and cAMP accumulation in human fibroblasts". Proc. Natl. Acad. Sci., USA, (1983) 80: 2514-2518.
11. L.G. Raisz, et al., "Effect of prostaglandin endoperoxides and metabolites on bone resorption in vitro." Nature, (1977) 267: 532-534.
12. C.R. Long, Kinoshita Y, Knox FG., "Prostaglandin E_2 induced changes in renal blood flow, renal interstitial hydrostatic pressure and sodium excretion in the rat." Prostaglandins, (1990) 40: 591-601.

Assay Principle

The DetectX® Prostaglandin E_2 (PGE_2) Immunoassay kit is designed to quantitatively measure PGE_2 present in serum, plasma, urine, saliva and tissue culture media samples. Please read the complete kit insert before performing this assay. A PGE_2 standard is provided to generate a standard curve for the assay and all samples should be read off the standard curve. Standards or diluted samples are pipetted into a clear microtiter plate coated with an antibody to capture mouse IgG. A PGE_2-peroxidase conjugate is added to the standards and samples in the wells. The binding reaction is initiated by the addition of a monoclonal antibody to PGE_2 to each well. After a 2 hour incubation, the plate is washed and substrate is added. The substrate reacts with the bound PGE_2-peroxidase conjugate. After a short incubation, the reaction is stopped and the intensity of the generated color is detected in a microtiter plate reader capable of measuring 450nm wavelength. The concentration of the PGE_2 in the sample is calculated, after making suitable correction for the dilution of the sample, using software available with most plate readers.

Related Products

KITS

Urinary Creatinine Detection Kit (2 Plate)	Catalog Number K002-H1
Urinary Creatinine Detection Kit (10 Plate)	Catalog Number K002-H5
Cortisol Enzyme Immunoassay Kits (Strip Wells)	Catalog Number K003-H1/H5
Cortisol Enzyme Immunoassay Kits (Whole Plate)	Catalog Number K003-H1W/H5W
Corticosterone Enzyme Immunoassay Kits	Catalog Number K014-H1/H5
Cortisone Enzyme Immunoassay Kits	Catalog Number K017-H1/H5

Supplied Components

Coated Clear 96 Well Plates
A clear plastic microtiter plate(s) coated with goat anti-mouse IgG.

Kit K018-H1 **OR** -H5	1 **OR** 5 Each	Catalog Number X012-1EA

Prostaglandin E_2 Standard **Must be stored at -20°C.**
Prostaglandin E_2 at 20,000 pg/mL in a special stabilizing solution.

Kit K018-H1 **OR** -H5	70 **OR** 350 µL	Catalog Number C057-70UL **OR** -350UL

DetectX® Prostaglandin E_2 Antibody
A mouse monoclonal antibody specific for Prostaglandin E_2.

Kit K018-H1 **OR** -H5	3 mL **OR** 13 mL	Catalog Number C058-3ML **OR** -13ML

DetectX® Prostaglandin E_2 Conjugate **Must be stored at -20°C.**
A Prostaglandin E_2-peroxidase conjugate in a special stabilizing solution.

Kit K018-H1 **OR** -H5	3 mL **OR** 13 mL	Catalog Number C060-3ML **OR** -13ML

Assay Buffer (or Concentrate)
One plate kit uses a ready-to-use Assay Buffer. Five plate kit uses a 5X concentrate that should be diluted with deionized or distilled water.

Kit K018-H1	28 mL	Catalog Number X066-28ML
Kit K018-H5	28 mL (Conc)	Catalog Number X067-28ML

Wash Buffer Concentrate
A 20X concentrate that should be diluted with deionized or distilled water.

Kit K018-H1 **OR** -H5	30 mL **OR** 125 mL	Catalog Number X007-30ML **OR** -125ML

TMB Substrate

Kit K018-H1 **OR** -H5	11 mL **OR** 55 mL	Catalog Number X019-11ML **OR** -55ML

Stop Solution
A 1M solution of hydrochloric acid. CAUSTIC.

Kit K018-H1 **OR** -H5	5 mL **OR** 25 mL	Catalog Number X020-5ML **OR** -25ML

Plate Sealer

Kit K018-H1 **OR** -H5	1 **OR** 5 Each	Catalog Number X002-1EA

Storage Instructions

The unopened kit should be stored at -20°C.
Once opened the kit can be stored at 4°C up to the expiration date on the kit label, **except for the PGE$_2$ Standard and PGE$_2$ Conjugate. These must be stored at -20°C.** The frozen PGE$_2$ Conjugate can be freeze-thawed multiple times.

Other Materials Required

Distilled or deionized water.

Repeater pipet, such as an Eppendorf repeater, with disposable tips to accurately dispense 25, 50 and 100 µL.

A microplate shaker.

Colorimetric 96 well microplate reader capable of reading optical density at 450 nm.

Software for converting raw relative optical density readings from the plate reader and carrying out four parameter logistic curve (4PLC) fitting. Contact your plate reader manufacturer for details.

Precautions

As with all such products, this kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

The antibody coated plate needs to be stored desiccated. The silica gel pack included in the foil ziploc bag will keep the plate dry. The silica gel pack will turn from blue to pink if the ziploc has not been closed properly.

This kit utilizes a peroxidase-based readout system. Buffers, including other manufacturers Wash Buffers, containing sodium azide will inhibit color production from the enzyme. Make sure **all** buffers used for samples are **azide free**. Ensure that any plate washing system is rinsed well with deionized water prior to using the supplied Wash Buffer as prepared on Page 8.

The Stop Solution is acid. The solution should not come in contact with skin or eyes. Take appropriate precautions when handling this reagent.

Sample Types

This assay has been validated for saliva, urine, serum, EDTA and heparin plasma samples and for tissue culture samples. A general cyclooxygenase inhibitor, such as meclofenamic acid or indomethacin at 15 µM should be added immediately after collection of any biological samples, such as serum and plasma. All samples should be frozen rapidly in dry ice/ethanol and **stored at -80°C**.

Samples containing visible particulate should be centrifuged prior to using. Severely hemolyzed samples should not be used in this kit. All samples containing lipids may interfere with the measurement of PGE_2. Samples containing high lipid content may be extracted as described below. A useful online resource for the extraction of bioactive lipids can be found at: http://lipidlibrary.aocs.org/topics/spe_alm/index.htm#ext .

Prostaglandin E_2 is identical across all species and we expect this kit may measure prostaglandin E_2 from sources other than human. The end user should evaluate recoveries of prostaglandin E_2 in other samples being tested.

Sample Preparation

Serum and Plasma Samples

Serum and plasma samples should be diluted ≥ 1:10 with the supplied Assay Buffer prior running in the assay. **Mouse serum and plasma samples** need to be diluted ≥ 1:20 with the supplied Assay Buffer prior running in the assay to minimize any interference of mouse IgG on the assay. Typical normal mouse PGE_2 serum levels are 45-150 ng/mL.

Urine Samples

Urine samples should be diluted ≥ 1:8 with the supplied Assay Buffer prior running in the assay.

Saliva Samples

Saliva samples should be diluted ≥ 1:2 with the supplied Assay Buffer prior running in the assay. See our Saliva Sample Handling Instructions at http://www.arborassays.com/documents/.

Tissue Culture Media

For measuring prostaglandin E_2 in tissue culture media (TCM), samples should be read off a standard curve generated in TCM. Samples may need to be diluted further in TCM. We have validated the assay using RPMI-1640.

Extracted Samples

We have a detailed Extraction Protocol available on our website at: http://www.ArborAssays.com/resources/lit.asp. The ethanol concentration in the final Assay Buffer dilution added to the well should be <5%.

Use all samples within 2 hours of preparation.

Reagent Preparation

Allow the kit reagents to thaw and come to room temperature for 30-60 minutes. We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine prostaglandin E_2 concentrations. Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit.

Assay Buffer (Dilute ONLY for the Five Plate Kit, K018-H5)

For the Five Plate Kit, K018-HX5, prepare the Assay Buffer by diluting the Assay Buffer Concentrate 1:5 by adding one part of the concentrate to four parts of deionized water. Once diluted this is stable at 4°C for 3 months. **Do not** dilute the Assay Buffer in the One Plate Kit, K018-H1.

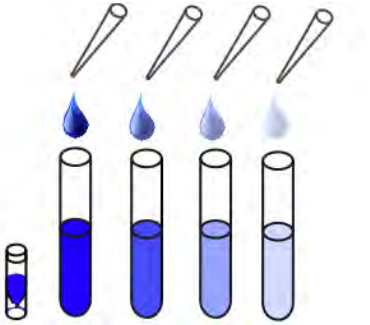
Wash Buffer

Dilute Wash Buffer Concentrate 1:20 by adding one part of the concentrate to nineteen parts of deionized water. Once diluted this is stable at room temperature for 3 months.

Standard Preparation

Label six test tubes as #1 through #6. Pipet 475 µL of Assay Buffer into tube #1 and 250 µL into tubes #2 to #6. **The Prostaglandin E_2 stock solution contains an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery.** Carefully add 25 µL of the Prostaglandin E_2 stock solution to tube #1 and vortex completely. Take 250 µL of the Prostaglandin E_2 solution in tube #1 and add it to tube #2 and vortex completely. Repeat the serial dilutions for tubes #3 through #6. The concentration of Prostaglandin E_2 in tubes 1 through 6 will be 1,000, 500, 250, 125, 62.5, and 31.25 pg/mL.

Use all Standards within 2 hours of preparation.



	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Assay Buffer (µL)	**475**	250	250	250	250	250
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5
Vol of Addition (µL)	**25**	250	250	250	250	250
Final Conc (pg/mL)	1,000	500	250	125	62.5	31.25

Assay Protocol

1. Use the plate layout sheet on the back page to aid in proper sample and standard identification. Determine the number of wells to be used and return unused wells to the foil pouch with desiccant. Seal the ziploc plate bag and store at 4˚C.

2. Pipet 100 µL of samples or standards into wells in the plate.

3. Pipet 125 µL of Assay Buffer into the non-specific binding (NSB) wells.

4. Pipet 100 µL of Assay Buffer into wells to act as maximum binding wells (Bo or 0 pg/mL).

5. Add 25 µL of the DetectX® Prostaglandin E_2 Conjugate to each well using a repeater pipet.

6. Add 25 µL of the DetectX® Prostaglandin E_2 Antibody to each well, **except the NSB wells**, using a repeater pipet.

7. Gently tap the sides of the plate to ensure adequate mixing of the reagents. Cover the plate with the plate sealer and shake at room temperature for 2 hours. If the plate is not shaken signals bound will be approximately 40% lower.

8. Aspirate the plate and wash each well 4 times with 300 µL wash buffer. Tap the plate dry on clean absorbent towels.

9. Add 100 µL of TMB Substrate to each well, using a repeater pipet.

10. Incubate the plate at room temperature for 30 minutes without shaking.

11. Add 50 µL of the Stop Solution to each well, using a repeater pipet.

12. Read the optical density generated from each well in a plate reader capable of reading at 450 nm.

13. Use the plate reader's built-in 4PLC software capabilities to calculate prostaglandin E_2 concentration for each sample.

Calculation of Results

Average the duplicate OD readings for each standard and sample. Create a standard curve by reducing the data using the 4PLC fitting routine on the plate reader, after subtracting the mean OD's for the NSB. The sample concentrations obtained, calculated from the %B/B0 curve, should be multiplied by the dilution factor to obtain neat sample values.

Or use the online tool from http://www.myassays.com/arbor-assays-pge2-enzyme-immunoassay-kit.assay to calculate the data.

*The MyAssays logo is a registered trademark of MyAssays Ltd.

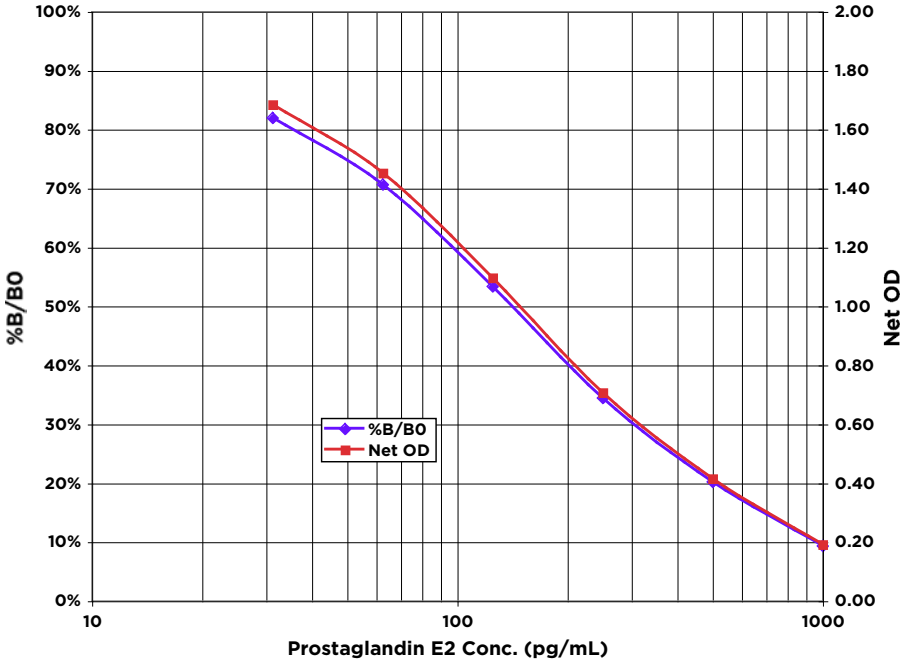
Typical Data

Sample	Mean OD	Net OD	% B/B0	PGE_2 Conc. (pg/mL)
NSB	0.101	0	-	-
Standard 1	0.293	0.192	9.3	1,000
Standard 2	0.516	0.415	20.2	500
Standard 3	0.809	0.708	34.5	250
Standard 4	1.198	1.097	53.4	125
Standard 5	1.554	1.453	70.7	62.5
Standard 6	1.786	1.685	82.0	31.25
B0	2.156	2.055	100.0	0
Sample 1	0.393	0.292	14.2	693.9
Sample 2	1.553	1.452	70.7	61.8

Always run your own standard curve for calculation of results.
Do not use this data.

Conversion Factor: 100 pg/mL of prostaglandin E_2 is equivalent to 283.7 pM.

Typical Standard Curves



Always run your own standard curves for calculation of results.
Do not use this data.

Validation Data

Sensitivity and Limit of Detection

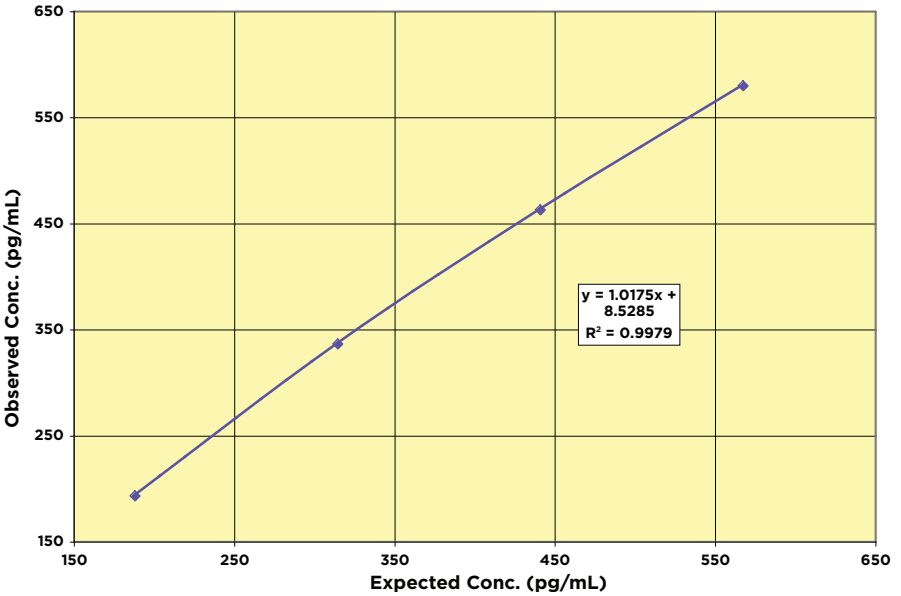
Sensitivity was calculated by comparing the OD's for nineteen wells run for each of the B0 and standard #6. The detection limit was determined at two (2) standard deviations from the B0 along the standard curve.
Sensitivity was determined as 29.1 pg/mL.

The Limit of Detection for the assay was determined in a similar manner by comparing the OD's for twenty runs for each of the zero standard and a low concentration human sample.
Limit of Detection was determined as 9.86 pg/mL

Linearity

Linearity was determined by taking two serum samples, one with a low Prostaglandin E_2 level of 61.8 pg/mL and one with a higher level of 693.9 pg/mL, and mixing them in the ratios given below. The measured concentrations were compared to the expected values based on the ratios used.

Low Serum	High Serum	Observed Conc. (pg/mL)	Expected Conc. (pg/mL)	% Recovery
80%	20%	579.7	567.5	102.2
60%	40%	462.8	441.0	104.9
40%	60%	336.3	314.6	106.9
20%	80%	193.1	188.2	102.6
			Mean Recovery	**104.1%**



Intra Assay Precision

Three human samples were diluted with Assay Buffer and run in replicates of 20 in an assay. The mean and precision of the calculated Prostaglandin E_2 concentrations were:

Sample	Prostaglandin E_2 Conc. (pg/mL)	%CV
1	795.5	6.3
2	194.8	5.6
3	166.4	6.8

Inter Assay Precision - In Process

Three human samples were diluted with Assay Buffer and run in duplicates in fifteen assays run over multiple days by four operators. The mean and precision of the calculated Prostaglandin E_2 concentrations were:

Sample	Prostaglandin E_2 Conc. (pg/mL)	%CV
1	470.9	8.4
2	185.8	6.1
3	94.0	7.7

Sample Values

Eleven human serum and plasma samples that did not contain COX inhibitors that would suppress PGE_2 production were tested in the assay. Neat sample were diluted > 1:10 in Assay Buffer and values ranged from 2,007 to 11,764 pg/mL with an average for the human samples of 7,400 pg/mL. The normal reference range for serum Prostaglandin E_2 (containing COX inhibitors) is 25-200 pg/mL[13]. Five normal human urine samples were diluted > 1:8 in Assay Buffer and values ranged from 485 to 2,309 pg/mL with an average for the human samples of 1,275 pg/mL

13. Tietz, NW, In "Textbook of Clinical Chemistry", WB Saunders, 1986.

Cross Reactivity

The following cross reactants were tested in the assay and calculated at the 50% binding point.

Eicosanoid	Cross Reactivity (%)
Prostaglandin E_2	100%
Prostaglandin E_1	108.9%
Prostaglandin $F_{2\alpha}$	2.00%
Thromboxane B_2	0.30%
6-keto-Prostaglandin $F_{1\alpha}$	<0.3%
15-keto-Prostaglandin E_1	<0.3%
13,14-dihydro-15-keto-Prostaglandin $F_{2\alpha}$	<0.1%
16,16-dimethyl-Prostaglandin E_2	<0.1%
Arachidonic Acid	<0.1%

Interferents

A variety of solvents were tested as possible interfering substances in the assay. Organic solvents such as DMSO, Dimethylformamide (DMF), methanol and ethanol were tested in the assay at 0.1%. DMSO and DMF caused a 1.2% and 0.8% decrease in measured PGE_2 levels, whereas methanol and ethanol caused an increase of 2.5% and 4.6% in measured PGE_2 levels. A solvent only control should be run by the end user when appropriate.

Hemoglobin at 0.02 mg/dL caused a 1% decrease in measured PGE_2 levels.

Elevated lipids will also interfere with the measurement of PGE_2. Follow the extraction recommendations described on page 7.

Limited Warranty

Arbor Assays warrants that at the time of shipment this product is free from defects in materials and workmanship. This warranty is in lieu of any other warranty expressed or implied, including but not limited to, any implied warranty of merchantability or fitness for a particular purpose.

We must be notified of any breach of this warranty within 48 hours of receipt of the product. No claim shall be honored if we are not notified within this time period, or if the product has been stored in any way other than outlined in this publication. The sole and exclusive remedy of the customer for any liability based upon this warranty is limited to the replacement of the product, or refund of the invoice price of the goods.

Contact Information

For details concerning this kit or to order any of our products please contact us:

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