



ARBOR
ASSAYS

DetectX[®]
High Sensitivity
Prostaglandin E₂
Enzyme Immunoassay Kit

1 Plate Kit
5 Plate Kit

Catalog Number K018-HX1
Catalog Number K018-HX5

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.

www.ArborAssays.com

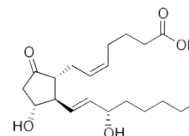
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Eicosanoid signal transduction pathways are highly conserved and are involved in a number of physiological processes. Prostaglandins are synthesized from arachidonic acid by cyclooxygenase (COX)-1 or -2, which convert the acid into PGH₂. This is further processed by cytosolic or microsomal prostaglandin synthases to become PGE₂ or one of several other prostanoids¹⁻³. Prostacyclin is the major cyclooxygenase product in blood vessel walls and it is present in inflammatory fluids in similar concentrations to PGE₂. Prostacyclin is a potent vasodilator and is more potent than PGE₂ in producing hyperalgesia⁴. PGE₂ is produced by a wide variety of tissues⁵⁻¹⁴ and in several pathological conditions, including inflammation, arthritis, fever, tissue injury, endometriosis, and a variety of cancers^{5,6}.



Prostaglandin E₂

Other biological actions of PGE₂ 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 hemodynamics⁷⁻¹².

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 E₂, 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 D₂ and E₂." *J. Biol. Chem.*, (1988) 263: 14593-14596.
9. S. Kuno, et al., "Prostaglandin E₂, 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 E₂ induced changes in renal blood flow, renal interstitial hydrostatic pressure and sodium excretion in the rat." *Prostaglandins*, (1990) 40: 591-601.

The DetectX[®] Prostaglandin E₂ (PGE₂) **HIGH SENSITIVITY** Immunoassay kit is designed to quantitatively measure PGE₂ present in serum, plasma, urine, saliva and tissue culture media samples. Please read the complete kit insert before performing this assay. A PGE₂ 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 PGE₂-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 PGE₂ to each well. After an overnight incubation at 4°C, the plate is washed and substrate is added. The substrate reacts with the bound PGE₂-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 PGE₂ 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
Prostaglandin E ₂ Enzyme Immunoassay Kits	Catalog Number K018-H1/H5

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SUPPLIED COMPONENTS

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Coated Clear 96 Well Plates

Clear plastic microtiter plate(s) coated with goat anti-mouse IgG.

Kit K018-HX1 **OR** -HX5 1 **OR** 5 Each Catalog Number X012-1EA

Prostaglandin E₂ Standard

Prostaglandin E₂ at 20,000 pg/mL in a special stabilizing solution.

Kit K018-HX1 **OR** -HX5 70 **OR** 350 μ L Catalog Number C057-70UL **OR** -350UL

DetectX[®] Prostaglandin E₂ High Sensitivity Antibody

A mouse monoclonal antibody specific for Prostaglandin E₂.

Kit K018-HX1 **OR** -HX5 3 mL **OR** 13 mL Catalog Number C059-3ML **OR** -13ML

DetectX[®] Prostaglandin E₂ Conjugate

A Prostaglandin E₂-peroxidase conjugate in a special stabilizing solution.

Kit K018-HX1 **OR** -HX5 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-HX1 28 mL Catalog Number X066-28ML
Kit K018-HX5 28 mL (Conc) Catalog Number X067-28ML

Wash Buffer Concentrate

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

Kit K018-HX1 **OR** -HX5 30 mL **OR** 125 mL Catalog Number X007-30ML **OR** -125ML

TMB Substrate

Kit K018-HX1 **OR** -HX5 11mL **OR** 55 mL Catalog Number X019-11ML **OR** -55ML

Stop Solution

A 1M solution of hydrochloric acid. CAUSTIC.

Kit K018-HX1 **OR** -HX5 5 mL **OR** 25 mL Catalog Number X020-5ML **OR** -25ML

Plate Sealer

Kit K018-HX1 **OR** -HX5 1 **OR** 5 Each Catalog Number X002-1EA

STORAGE INSTRUCTIONS

This kit should be stored at -20°C until the expiration date of the kit.

Once opened the kit can be stored at 4°C up to the expiration date on the kit label, **except for the PGE₂ Conjugate which must be stored at -20°C.** The frozen PGE₂ Conjugate can be freeze-thawed multiple times.

WEB INSERT
OTHER MATERIALS REQUIRED

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Distilled or deionized water.

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

A microplate shaker.

A 4°C refrigerator.

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.

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.



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 μ M 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 PGE₂. 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 E₂ is identical across all species and we expect this kit may measure Prostaglandin E₂ from sources other than human. The end user should evaluate recoveries of Prostaglandin E₂ in other samples being tested.

SAMPLE PREPARATION

Serum and Plasma Samples

Serum and plasma samples should be diluted \geq 1:10 with the supplied Assay Buffer prior running in the assay. **Mouse serum and plasma samples** need to be diluted \geq 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 PGE₂ serum levels are 45-150 ng/mL.

Urine Samples

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

Saliva Samples

Saliva samples should be diluted \geq 1:2 with the supplied Assay Buffer prior running in the assay.

Tissue Culture Media

For measuring prostaglandin E₂ 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 web site 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.

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 E₂ 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-HX5)

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-HX1.

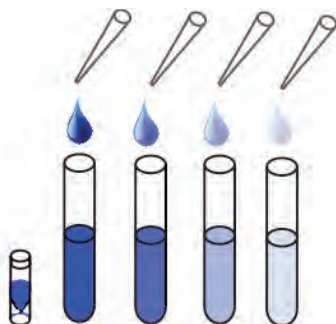
Wash Buffer

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 588 µL of Assay Buffer into tube #1 and 300 µL into tubes #2 to #6. **The Prostaglandin E₂ stock solution contains an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery.** Carefully add 12 µL of the Prostaglandin E₂ stock solution to tube #1 and vortex completely. Take 300 µL of the Prostaglandin E₂ 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 E₂ in tubes 1 through 6 will be 400, 200, 100, 50, 25, and 12.5 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)	588	300	300	300	300	300
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5
Vol of Addition (µL)	12	300	300	300	300	300
Final Conc (pg/mL)	400	200	100	50	25	12.5

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 (B0 or 0 pg/mL).
5. Add 25 µL of the DetectX® Prostaglandin E₂ Conjugate to each well using a repeater pipet.
6. Add 25 µL of the DetectX® Prostaglandin E₂ High Sensitivity Antibody to each well, **except the NSB wells**, using a repeater pipet.
7. Cover the plate with the plate sealer and shake the plate for 15 minutes at room temperature.
8. Place the covered plate in a 4°C refrigerator for 16 hours.
9. The next morning take the plate from the refrigerator and wash each well 4 times with 300 µL wash buffer. Tap the plate dry on clean absorbent towels.
10. Add **100** µL of TMB Substrate to each well, using a repeater pipet.
(Note change in TMB volume!!)
11. Incubate the plate at room temperature for 30 minutes.
12. Add 50 µL of the Stop Solution to each well, using a repeater pipet.
13. Read the optical density generated from each well in a plate reader capable of reading at 450 nm.
14. Use the plate reader's built-in 4PLC software capabilities to calculate Prostaglandin E₂ concentration for each sample.

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CALCULATION OF RESULTS

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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/BO curve, should be multiplied by the dilution factor to obtain neat sample values.

TYPICAL DATA

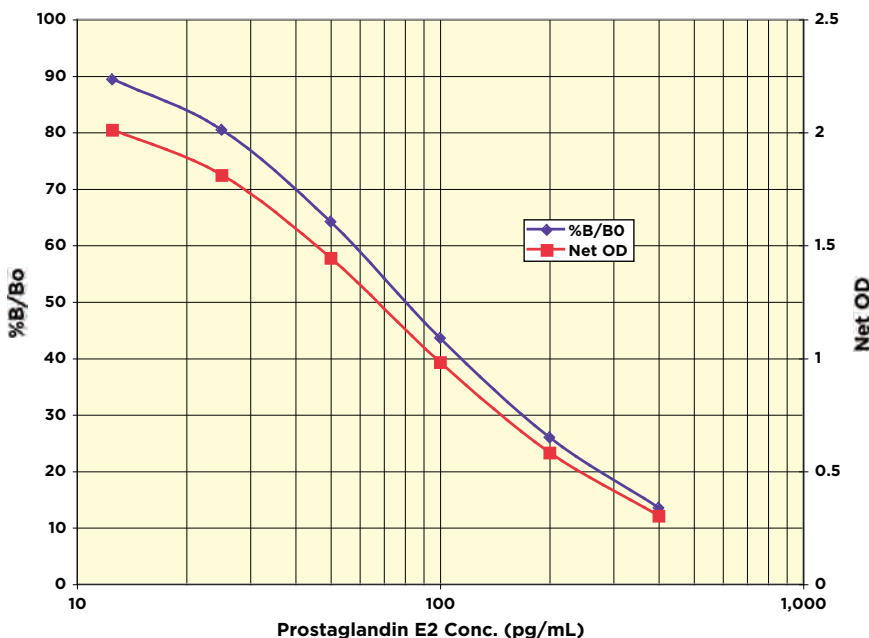
Sample	Mean OD	Net OD	% B/BO	PGE₂ Conc. (pg/mL)
NSB	0.069	0	-	-
Standard 1	0.397	0.328	12.8	400
Standard 2	0.713	0.644	25.1	200
Standard 3	1.220	1.151	44.9	100
Standard 4	1.683	1.614	63.0	50
Standard 5	2.196	2.127	83.1	25
Standard 6	2.319	2.250	87.1	12.5
BO	2.630	2.561	100.0	0
Sample 1	0.900	0.831	32.4	148.8
Sample 2	1.376	1.307	51.0	80.65

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

Conversion Factor: 100 pg/mL of prostaglandin E₂ 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 twelve 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 10.9 pg/mL.

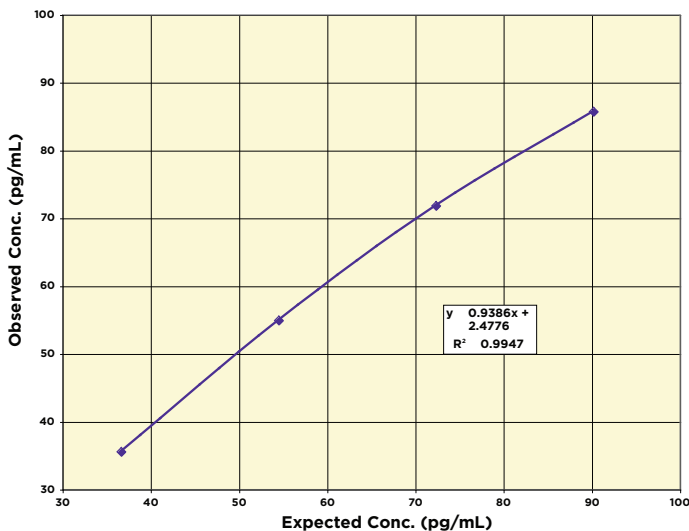
The Limit of Detection for the assay was determined in a similar manner by comparing the OD's for twenty runs for each of the zero standard and a low concentration human sample.

Limit of Detection was determined as 16.8 pg/mL

Linearity

Linearity was determined by taking two diluted human urine samples, one with a low Prostaglandin E₂ level of 18.8 pg/mL and one with a higher level of 108.0 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%	35.6	36.7	97.0
60%	40%	54.9	54.5	100.8
40%	60%	71.8	72.4	99.3
20%	80%	85.7	90.2	95.1
			Mean Recovery	98.0%



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 E₂ concentrations were:

Sample	Prostaglandin E ₂ Conc. (pg/mL)	%CV
1	134.8	5.9
2	76.2	8.7
3	166.3	7.6

Inter Assay Precision

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 E₂ concentrations were:

Sample	Prostaglandin E ₂ Conc. (pg/mL)	%CV
1	156.1	8.8
2	81.4	7.7
3	148.2	14.7

Eleven human serum and plasma samples that did not contain COX inhibitors that would suppress PGE₂ 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 E₂ (containing COX inhibitors) is 25-200 pg/mL¹³. 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 F _{2α}	2.00%
Thromboxane B ₂	0.30%
6-keto-Prostaglandin F _{1α}	<0.3%
13,14-dihydro-15-keto-Prostaglandin F _{2α}	<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 PGE₂ levels, whereas methanol and ethanol caused an increase of 2.5% and 4.6% in measured PGE₂ 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 PGE₂ levels.

Elevated lipids will also interfere with the measurement of PGE₂. Follow the extraction recommendations described on page 7.

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LIMITED WARRANTY

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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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