

ARBOR ASSAYS™
Interactive Assay Solutions™



DetectX[®]

PROSTAGLANDIN E₂ Enzyme Immunoassay Kit

1 Plate Kit Catalog Number K051-H1

5 Plate Kit Catalog Number K051-H5

Species Independent

Multi-Format Kit

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   

WEB INSERT 171005

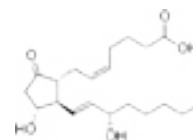
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BACKGROUND

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



Prostaglandin E₂

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}.

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.

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2. Vane, JR. (1971). "Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs." *Nature*, 231, 232-235.3.
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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.
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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.
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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.

ASSAY PRINCIPLE

The DetectX® Prostaglandin E₂ (PGE₂) Immunoassay kit is designed to quantitatively measure PGE₂ present in serum, plasma, urine, saliva, cells, tissue, and tissue culture media samples. This EIA kit allows for the widest variations in sample size, sensitivity and assay timing of any PGE₂ kit. The protocol variations are outlined on page 5.

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 incubation, 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 450 nm 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	Catalog No.
Urinary Creatinine Detection Kit (2 or 10 Plate)	K002-H1/H5
Cortisol EIA Kits (Strip Wells)	K003-H1/H5
Cortisol EIA Kits (Whole Plate)	K003-H1W/H5W
Corticosterone EIA Kits	K014-H1/H5
Cortisone CLIA Kits	K017-C1/C5



ASSAY FORMAT OPTIONS

Multi-Format Assay

This Prostaglandin E₂ (PGE₂) Immunoassay kit uses a mouse monoclonal antibody that allows for an exceptional wide range of PGE₂ concentrations to be measured. By varying the volume of sample used in the assay PGE₂ concentrations from 1,000 pg/mL to below 2 pg/mL can be determined. This allows the most sensitive detection of PGE₂ to be measured in any sample.

The monoclonal antibody, clone 3H10, displays extremely fast kinetics for binding PGE₂ and incubation for 2 hours or overnight yields identical binding curves (OD variation will be seen between 2 hour and overnight incubation but %B/B0 curves will be very similar). This lack of sensitivity to the time of incubation allows any format of the PGE₂ assay to be run to fit your workflow.

REGULAR FORMAT

For samples with PGE₂ concentrations from 500 to 3.9 pg/mL

The **Regular Format** uses 50 µL of sample or standard to give results in 2.5 hours.

LOW SAMPLE VOLUME FORMAT

For samples with PGE₂ concentrations from 1,000 to 15.6 pg/mL

The **Low Sample Volume Format** uses 25 µL of sample or standard for results in 2.5 hours, but uses lower sample volumes.

HIGH SENSITIVITY FORMAT

For samples with PGE₂ concentrations from 500 to 1.95 pg/mL

The **High Sensitivity Format** uses 100 µL of sample or standard gives results in 2.5 hours, but is the highest sensitivity kit of any type available.

SUPPLIED COMPONENTS

Coated Clear 96 Well Plates

A clear plastic microtiter plate(s) coated with goat anti-mouse IgG.
Kit K051-H1 **or** -H5 1 **or** 5 Each Catalog Number X012-1EA

Prostaglandin E₂ Standard **Must be stored at -20°C.**

Prostaglandin E₂ at 20,000 pg/mL in a special stabilizing solution.
Kit K051-H1 **or** -H5 70 µL **or** 350 µL Catalog Number C057-70UL **or** -350UL

DetectX[®] Prostaglandin E₂ Antibody

A mouse monoclonal antibody specific for Prostaglandin E₂.
Kit K051-H1 **or** -H5 3 mL **or** 13 mL Catalog Number C178-3ML **or** -13ML

DetectX[®] Prostaglandin E₂ Conjugate **Must be stored at -20°C.**

A Prostaglandin E₂-peroxidase conjugate in a special stabilizing solution.
Kit K051-H1 **or** -H5 3 mL **or** 13 mL Catalog Number C179-3ML **or** -13ML

Assay Buffer Concentrate

A 5X concentrate that must be diluted with deionized or distilled water.
Kit K051-H1 **or** -H5 28 mL **or** 55 mL Catalog Number X067-28ML **or** -55ML

Wash Buffer Concentrate

A 20X concentrate that should be diluted with deionized or distilled water.
Kit K051-H1 **or** -H5 30 mL **or** 125 mL Catalog Number X007-30ML **or** -125ML

TMB Substrate

Kit K051-H1 **or** -H5 11 mL **or** 55 mL Catalog Number X019-11ML **or** -55ML

Stop Solution

A 1M solution of hydrochloric acid. **CAUSTIC.**
Kit K051-H1 **or** -H5 5 mL **or** 25 mL Catalog Number X020-5ML **or** -25ML

Plate Sealer

Kit K051-H1 **or** -H5 1 **or** 5 Each Catalog Number X002-1EA

STORAGE INSTRUCTIONS

The unopened kit must 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 PGE₂ Standard and PGE₂ Conjugate. These must be stored at -20°C.**

The PGE₂ Conjugate will lose about 40% of its signal when stored at -20°C. No change in %B/B₀ will be seen for standards or samples. It can be stored at -80°C without loss of signal up to the expiration date on the kit label. The frozen PGE₂ 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

Prostaglandin E₂ (PGE₂) is identical across all species and we expect this kit may measure PGE₂ from sources other than human. The end user should evaluate recoveries of PGE₂ in other samples being tested. 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 particulates should be centrifuged prior to use. Severely hemolyzed samples should not be used in this kit. All samples with high lipid content may interfere with the measurement of PGE₂ and may be extracted as described below. An online resource for the extraction of bioactive lipids can be found at: <http://pubs.acs.org/doi/abs/10.1021/ac1015563>.

SAMPLE VALUES

The normal range for human serum PGE₂ is 25-1,000 pg/mL and mouse serum PGE₂ is typically ≥ 100-450 pg/mL. Dilutions for samples will have to be adjusted for the sample type and expected PGE₂ levels before and after any treatment. This kit can determine PGE₂ between 2-1,000 pg/mL. A minimum dilution of 1:10 for human and 1:20 for mouse samples (as described in "SAMPLE PREPARATION") must be made to ensure linearity of response for serum or plasma samples. Normal 24-hour urine PGE₂ levels are between 400-620 ng/24 hours for most species.

SAMPLE PREPARATION

Serum and Plasma Samples

Serum and plasma samples should be treated immediately with a COX inhibitor such as indomethacin and diluted ≥ 1:10 with the supplied diluted Assay Buffer prior running in the assay. **Mouse serum and plasma samples** must be diluted ≥ 1:20 with the supplied diluted Assay Buffer prior running in the assay to minimize any interference of mouse IgG on the assay. Typical normal mouse PGE₂ serum levels are ≥ 100-450 ng/mL.

Urine Samples

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

Saliva Samples

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

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 website at: www.arborassays.com/resources/#protocols. The ethanol concentration in the final diluted Assay Buffer dilution added to the well should be < 5%.

Use all samples within 2 hours of preparation.



ASSAY 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 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 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.

Wash Buffer

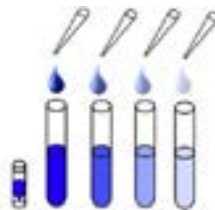
Dilute Wash Buffer Concentrate 1:20 by adding one part of the concentrate to nineteen parts of deionized water. Once diluted this is stable at room temperature for 3 months.

Watch videos on sample preparation and setting up an assay on our website at:

www.arborassays.com/resources/#videos

STANDARD PREPARATION - REGULAR FORMAT

Label test tubes as #1 through #8. Pipet 390 μL of Assay Buffer into tube #1 and 200 μL into tubes #2 to #8. **The Prostaglandin E₂ stock solution contains an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery.** Carefully add 10 μL of the Prostaglandin E₂ stock solution to tube #1 and vortex completely. Take 200 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 #8. The concentration of Prostaglandin E₂ in tubes 1 through 8 will be 500, 250, 125, 62.5, 31.25, 15.625, 7.813 and 3.906 pg/mL .



	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7	Std 8
Assay Buffer (μL)	390	200	200	200	200	200	200	200
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7
Vol of Addition (μL)	10	200	200	200	200	200	200	200
Final Conc (pg/mL)	500	250	125	62.5	31.25	15.625	7.813	3.906

Use all Standards within 2 hours of preparation.

ASSAY PROTOCOL - REGULAR FORMAT

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 50 µL of samples or standards into wells in the plate.
3. Pipet 75 µL of Assay Buffer into the non-specific binding (NSB) wells.
4. Pipet 50 µL of Assay Buffer into the maximum binding (BO or Zero standard) wells.
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 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.
8. Incubation Options:
EITHER:
8a. Shake at room temperature for 2 hours. If the plate is not shaken signals bound will be approximately 40% lower.

OR:
8b. Shake the plate in a plate shaker at room temperature for 15 minutes to ensure adequate mixing of the reagents. Incubate at 4°C for 16-18 hours.
9. If using Option 8b., the following day remove the TMB Substrate from the refrigerator and allow to come to room temperature for at least 30 minutes. **Addition of cold Substrate will cause depressed signal.**
10. Aspirate the plate and wash each well 4 times with 300 µL wash buffer. Tap the plate dry on clean absorbent towels.
11. Add 100 µL of TMB Substrate to each well, using a repeater pipet.
12. Incubate the plate at room temperature for 30 minutes without shaking.
13. Add 50 µL of the Stop Solution to each well, using a repeater pipet.
14. Read the optical density generated from each well in a plate reader capable of reading at 450 nm.
15. Use the plate reader's built-in 4PLC software capabilities to calculate prostaglandin E₂ concentration for each sample.

NOTE: If you are using only part of a strip well plate, at the end of the assay throw away the used wells and retain the plate frame for use with the remaining unused wells.

STANDARD PREPARATION - LOW SAMPLE VOLUME FORMAT

Label test tubes as #1 through #7. Pipet 380 μL of Assay Buffer into tube #1 and 200 μL into tubes #2 to #7. **The Prostaglandin E₂ stock solution contains an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery.** Carefully add 20 μL of the Prostaglandin E₂ stock solution to tube #1 and vortex completely. Take 200 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 #7. The concentration of Prostaglandin E₂ in tubes 1 through 7 will be 1,000, 500, 250, 125, 62.5, 31.25, and 15.625 pg/mL .



	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7
Assay Buffer (μL)	380	200	200	200	200	200	200
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Vol of Addition (μL)	20	200	200	200	200	200	200
Final Conc (pg/mL)	1,000	500	250	125	62.5	31.25	15.625

Use all Standards within 2 hours of preparation.

ASSAY PROTOCOL - LOW SAMPLE VOLUME FORMAT

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 25 µL of samples or standards into wells in the plate.
3. Pipet 50 µL of Assay Buffer into the non-specific binding (NSB) wells.
4. Pipet 25 µL of Assay Buffer into the maximum binding (B0 or Zero standard) wells.
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 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.
8. Incubation Options
EITHER:
8a. Shake at room temperature for 2 hours. If the plate is not shaken signals bound will be approximately 40% lower.

OR:
8b. Shake the plate in a plate shaker at room temperature for 15 minutes to ensure adequate mixing of the reagents. Incubate at 4°C for 16-18 hours.
9. If using Option 8b., the following day remove the TMB Substrate from the refrigerator and allow to come to room temperature for at least 30 minutes. **Addition of cold Substrate will cause depressed signal.**
10. Aspirate the plate and wash each well 4 times with 300 µL wash buffer. Tap the plate dry on clean absorbent towels.
11. Add 100 µL of TMB Substrate to each well, using a repeater pipet.
12. Incubate the plate at room temperature for 30 minutes without shaking.
13. Add 50 µL of the Stop Solution to each well, using a repeater pipet.
14. Read the optical density generated from each well in a plate reader capable of reading at 450 nm.
15. Use the plate reader's built-in 4PLC software capabilities to calculate prostaglandin E₂ concentration for each sample.

NOTE: If you are using only part of a strip well plate, at the end of the assay throw away the used wells and retain the plate frame for use with the remaining unused wells.

STANDARD PREPARATION - HIGH SENSITIVITY FORMAT

Label test tubes as #1 through #9. Pipet 585 μL of Assay Buffer into tube #1 and 300 μL into tubes #2 to #9. **The Prostaglandin E₂ stock solution contains an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery.** Carefully add 15 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 #9. The concentration of Prostaglandin E₂ in tubes 1 through 9 will be 500, 250, 125, 62.5, 31.25, 15.625, 7.813, 3.906, and 1.953 pg/mL .



	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7	Std 8	Std 9
Assay Buffer (μL)	585	300	300	300	300	300	300	300	300
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7	Std 8
Vol of Addition (μL)	15	300	300	300	300	300	300	300	300
Final Conc (pg/mL)	500	250	125	62.5	31.25	15.625	7.813	3.906	1.953

Use all Standards within 2 hours of preparation.

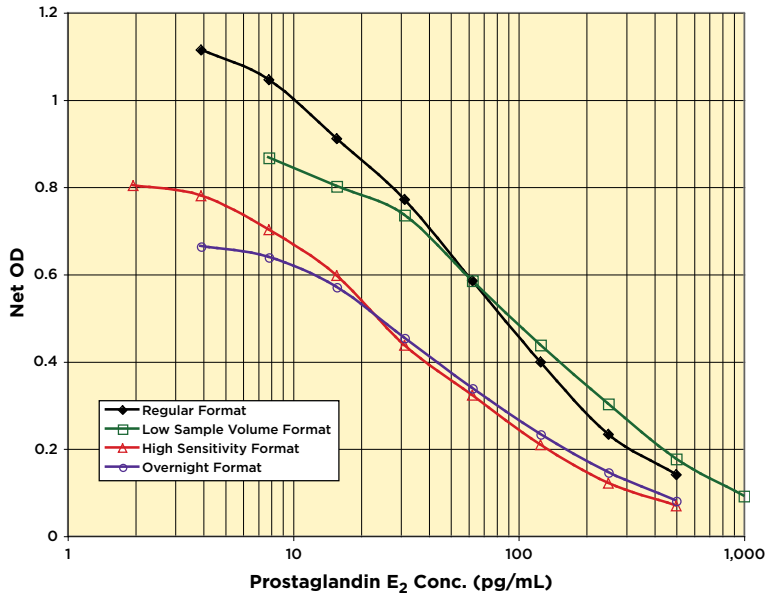
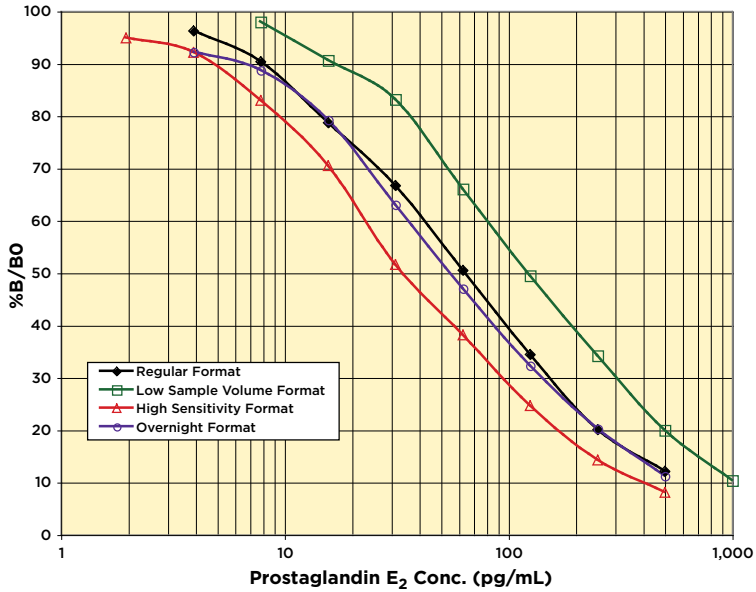
ASSAY PROTOCOL - HIGH SENSITIVITY FORMAT

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 the maximum binding (BO or Zero standard) wells.
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 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.
8. Incubation Options
EITHER:
8a. Shake at room temperature for 2 hours. If the plate is not shaken signals bound will be approximately 40% lower.

OR:
8b. Shake the plate in a plate shaker at room temperature for 15 minutes to ensure adequate mixing of the reagents. Incubate at 4°C for 16-18 hours.
9. If using Option 8b., the following day remove the TMB Substrate from the refrigerator and allow to come to room temperature for at least 30 minutes. **Addition of cold Substrate will cause depressed signal.**
10. Aspirate the plate and wash each well 4 times with 300 µL wash buffer. Tap the plate dry on clean absorbent towels.
11. Add 100 µL of TMB Substrate to each well, using a repeater pipet.
12. Incubate the plate at room temperature for 30 minutes without shaking.
13. Add 50 µL of the Stop Solution to each well, using a repeater pipet.
14. Read the optical density generated from each well in a plate reader capable of reading at 450 nm.
15. Use the plate reader's built-in 4PLC software capabilities to calculate prostaglandin E₂ concentration for each sample.

NOTE: If you are using only part of a strip well plate, at the end of the assay throw away the used wells and retain the plate frame for use with the remaining unused wells.

COMPARATIVE TYPICAL DATA - ALL FORMAT OPTIONS



Overnight Data is from the Regular Format.



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

Or use the online tool from MyAssays to calculate the data:

www.myassays.com/arbora-assays-pge2-enzyme-immunoassay-kit-k051-h.assay



TYPICAL DATA - 2 HOUR REGULAR FORMAT

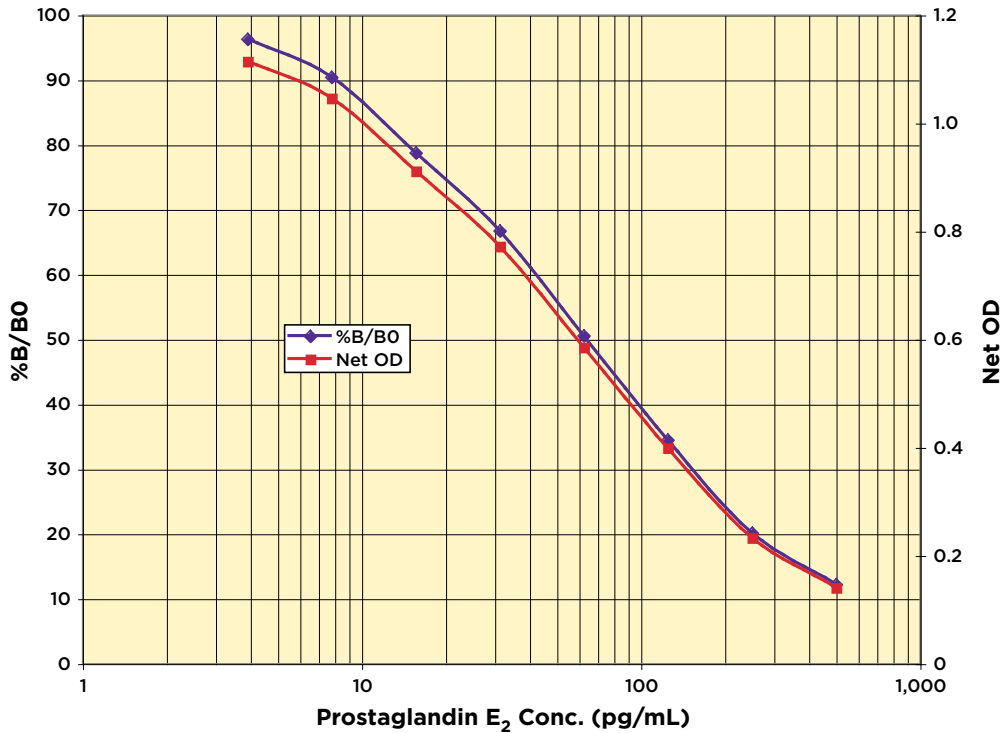
Sample	Mean OD	Net OD	% B/BO	PGE ₂ Conc. (pg/mL)
NSB	0.061	0	-	-
Standard 1	0.202	0.141	12.19	500
Standard 2	0.294	0.233	20.14	250
Standard 3	0.460	0.399	34.49	125
Standard 4	0.646	0.585	50.56	62.5
Standard 5	0.833	0.772	66.72	31.25
Standard 6	0.972	0.911	78.74	15.625
Standard 7	1.107	1.046	90.41	7.813
Standard 8	1.175	1.114	96.28	3.906
BO	1.218	1.157	100	0
Sample 1	0.464	0.403	34.83	121.8
Sample 2	1.030	0.969	83.75	12.28

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.

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

Typical Standard Curve - 2 Hour Regular Format



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

VALIDATION DATA

Generated in 2 Hour Regular Format.

Sensitivity and Limit of Detection

Sensitivity was calculated by comparing the OD's for nineteen wells run for each of the BO and standard #8. The detection limit was determined at two (2) standard deviations from the BO along the standard curve.

Sensitivity was determined as 3.07 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 3.25 pg/mL.

We expect the High Sensitivity Format to give enhanced Sensitivity and LoD.



Linearity

Linearity was determined in human plasma and urine samples by taking two diluted samples with known PGE₂ concentrations. A plasma sample with a high PGE₂ concentration of 216.4 pg/mL was mixed with one with a lower value of 42.5 pg/mL. A urine sample with a high PGE₂ concentration of 32.6 pg/mL was mixed with one with a lower value of 8.6 pg/mL. They were mixed in the ratios given below. The measured concentrations were compared to the expected values based on the ratios used.

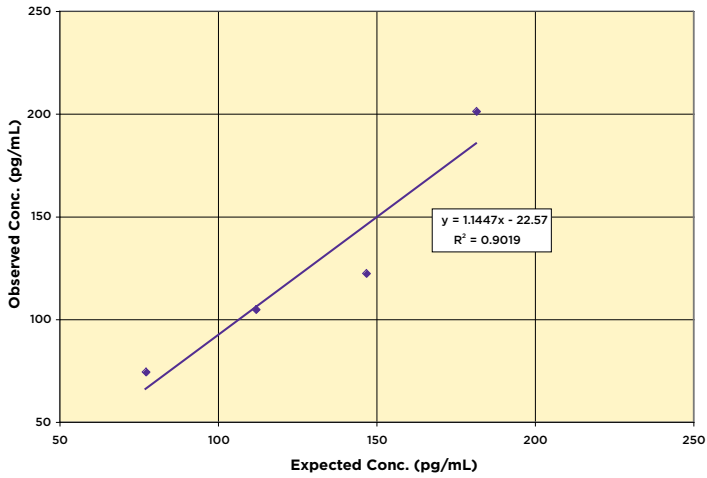
Plasma Linearity

High Sample	Low Sample	Observed Conc. (pg/mL)	Expected Conc. (pg/mL)	% Recovery
80%	20%	201.1	181.6	110.8%
60%	40%	122.3	146.8	83.3%
40%	60%	104.7	112.0	93.5%
20%	80%	74.3	77.3	96.1%
Mean Recovery				95.9%

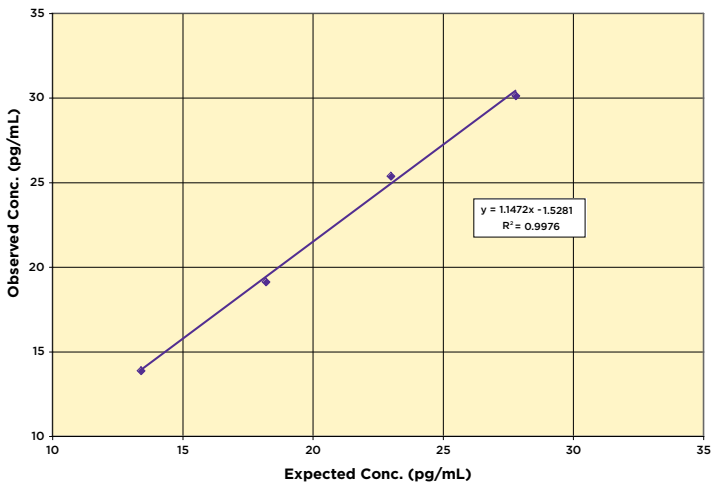
Urine Linearity

High Sample	Low Sample	Observed Conc. (pg/mL)	Expected Conc. (pg/mL)	% Recovery
80%	20%	30.1	27.8	108.3
60%	40%	25.4	23.0	110.2
40%	60%	19.1	18.2	105.0
20%	80%	13.9	13.4	103.3
Mean Recovery				106.7%

Plasma Linearity



Urine Linearity



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	11.7	12.3
2	98.6	6.3
3	131.2	4.9

Inter Assay Precision

Three human samples were diluted with Assay Buffer and run in duplicates in seventeen 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	12.3	8.8
2	100.5	8.1
3	134.7	9.8

SAMPLE VALUES

Eight human serum samples that did not contain COX inhibitors were tested in the assay. Neat sample were diluted 1:20-1:50 in Assay Buffer and adjusted values ranged from 652 to 4,170 pg/mL with an average of 2,126 pg/mL. Ten human plasma samples that did not contain COX inhibitors were tested in the assay. Neat sample were diluted 1:20-1:50 in Assay Buffer and adjusted values ranged from 219 to 4,328 pg/mL with an average of 1,717 pg/mL. Eight normal human urine samples were diluted 1:10- 1:20 in Assay Buffer and adjusted values ranged from 56.9 to 326 pg/mL with an average of 149.9 pg/mL.

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	27.28%
Prostaglandin F _{2α}	0.33%
Thromboxane B ₂	< 0.02%
6-keto-Prostaglandin F _{1α}	< 0.02%
15-keto-Prostaglandin E ₁	< 0.02%
16,16-dimethyl-Prostaglandin E ₂	< 0.02%
Arachidonic Acid	< 0.02%

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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CONTACT INFORMATION

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

Arbor Assays

1514 Eisenhower Place
Ann Arbor, Michigan 48108 USA

Phone: 734-677-1774

Fax: 734-677-6860

Web: www.ArborAssays.com

E Mail Addresses:

Info@ArborAssays.com

Orders@ArborAssays.com

Technical@ArborAssays.com

Contracts@ArborAssays.com



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