ARBOR ASSAYS™
Interactive Assay Solutions™

MULTI SPECIES

MOST SENSITIVE

DetectX®

Prostaglandin E_2
Enzyme Immunoassay Kit

1 or 5 Strip Plates Catalog Numbers K051-H1/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 K051-H 231220

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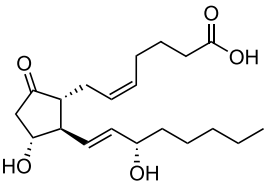
BACKGROUND



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].

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2. Vane, J. R. (1971). Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature New Biology*, *231*(25), 232–235.
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4. Higgs, G.A., et al. (1979). Microcirculatory effects of prostacyclin (PGI2) in the hamster cheek pouch. *Microvascular Research, 18*(2), 245-254.
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7. Richardson, P. D. & Withrington, P. G. (1976). The vasodilator actions of isoprenaline, histamine, prostaglandin E_2, glucagon and secretin on the hepatic arterial vascular bed of the dog. *British Journal of Pharmacolology, 57*(4), 581-588.
8. Hayaishi, O. (1988). Sleep-wake regulation by prostaglandins D_2 and E_2. *Journal of Biological Chemistry, 263*(29), 14593-14596
9. Kuno, S., et al. (1986). Prostaglandin E_2, a seminal constituent, facilitates the replication of acquired immune deficiency syndrome virus in vitro. *Proceedings of the National Academy of Sciences of the United States of America, 83*(10), 3487-3490.
10. Bareis, D. L., et al. (1983). Bradykinin stimulates phospholipid methylation, calcium influx, prostaglandin formation, and cAMP accumulation in human fibroblasts. *Proceedings of the National Academy of Sciences of the United States of America, 80*(9), 2514-2518.
11. Raisz, L. G., et al. (1977). Effect of prostaglandin endoperoxides and metabolites on bone resorption in vitro. *Nature, 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, cells, tissue, and tissue culture media samples. This EIA kit allows for the widest variations in sample size, sensitivity and assay timing of any PGE_2 kit. The protocol variations are outlined on page 5.

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 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 450 nm 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	Catalog No.
Urinary Creatinine Detection Kit	K002-H1/H5
2',3'-Cyclic GAMP ELISA Kits	K067-H1/H5
2',3'-Cyclic GAMP ELISA Kit (384-Well Plate)	K067-H1D
Allopregnanolone ELISA Kits	K061-H1/H5
Arg^8-Vasopressin (AVP) ELISA Kits	K049-H1/H5
Atrial Natriuretic Peptide (ANP) ELISA Kits - Improved	K071-H1/H5
Cyclic GMP Direct ELISA Kits - Improved Sensitivity	K065-H1/H5
Endothelin-1 (ET-1) ELISA Kit	K045-H1
Nitric Oxide (NO) Colorimetric Detection Kit	K023-H1
Protein Kinase A (PKA) Colorimetric Activity Kit	K027-H1

ASSAY FORMAT OPTIONS

Multi-Format Assay

This Prostaglandin E_2 (PGE_2) Immunoassay kit uses a mouse monoclonal antibody that allows for an exceptional wide range of PGE_2 concentrations to be measured. By varying the volume of sample used in the assay PGE_2 concentrations from 1,000 pg/mL to below 2 pg/mL can be determined. This allows the most sensitive detection of PGE_2 to be measured in any sample.

The monoclonal antibody, clone 3H10, displays extremely fast kinetics for binding PGE_2 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 PGE_2 assay to be run to fit your workflow.

REGULAR FORMAT

For samples with PGE_2 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 PGE_2 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 PGE_2 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
Each are coated with goat anti-mouse IgG.

Kit K051-H1 **or** -H5	1 **or** 5 Each	Catalog Number X012-1EA, 1 x 8 Strip Well

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

Kit K051-H1 **or** -H5	3 mL **or** 13 mL	Catalog Number C178-3ML **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 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 PGE_2 Standard and PGE_2 Conjugate. These must be stored at ≤ -20°C.**

The PGE_2 Conjugate will lose about 40% of its signal when stored at -20°C. **The conjugate can be stored at -80°C** to reduce loss of signal up to the expiration date on the kit label. No change in %B/B0 will be seen for standards or samples. The frozen PGE_2 Conjugate should be aliquotted into smaller volumes prior to refreezing to avoid multiple freeze-thaw cycles.

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 E_2 (PGE_2) is identical across all species and we expect this kit may measure PGE_2 from sources other than human. The end user should evaluate recoveries of PGE_2 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 PGE_2 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 PGE_2 is 25-1,000 pg/mL and mouse serum PGE_2 is typically ≥ 100-450 pg/mL. Dilutions for samples will have to be adjusted for the sample type and expected PGE_2 levels before and after any treatment. This kit can determine PGE_2 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 PGE_2 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 ≥ 10-fold with 1X Assay Buffer prior running in the assay. **Mouse serum and plasma samples** must be diluted ≥ 20-fold with the 1X 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 ≥ 100-450 ng/mL.

Urine Samples

Urine samples should be diluted ≥ 8-fold with 1X Assay Buffer prior running in the assay.

Saliva Samples

Saliva samples should be diluted ≥ 2-fold with 1X 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 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: 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 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 Assay Buffer Concentrate 1:5 by adding one part of the concentrate to four parts of deionized water. The 1X Assay Buffer is stable at 4°C for 3 months.

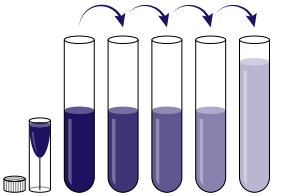
Wash Buffer

Dilute Wash Buffer Concentrate 1:20 by adding one part of the concentrate to nineteen parts of deionized water. The 1X Wash Buffer 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 1X Assay Buffer into tube #1 and 200 µL into tubes #2 to #8. **The Prostaglandin E_2 stock solution contains an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery.** Carefully add 10 µL of the Prostaglandin E_2 stock solution to tube #1 and vortex completely. Take 200 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 #8. The concentration of Postaglandin E_2 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
1X 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.
2. 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.

   Pipet standards or samples down the plate strip columns (A to H) to ensure maximum use of the strip wells.
3. Pipet 50 µL of samples or standards into wells in the plate.
4. Pipet 75 µL of 1X Assay Buffer into the non-specific binding (NSB) wells.
5. Pipet 50 µL of 1X Assay Buffer into the maximum binding (B0 or Zero standard) wells.
6. Add 25 µL of the DetectX® Prostaglandin E_2 Conjugate to each well using a repeater pipet.
7. Add 25 µL of the DetectX® Prostaglandin E_2 Antibody to each well, **except the NSB wells**, using a repeater pipet.
8. Gently tap the sides of the plate to ensure adequate mixing of the reagents. Cover the plate with the plate sealer.
9. Incubation Options:

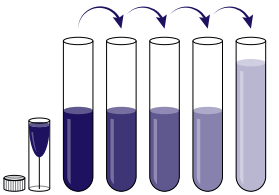
   EITHER:
   8a. Shake at room temperature for 2 hours. We recommend shaking at around 700–900 rpm. If the plate is not shaken, signals bound will be approximately 40% lower.

   OR:
   8b. Shake the plate in a plate shaker at room temperature for 15 minutes to ensure adequate mixing of the reagents. We recommend shaking at around 700–900 rpm. Incubate at 4°C for 16-18 hours.
10. 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.**
11. Aspirate the plate and wash each well 4 times with 300 µL 1X Wash Buffer. Tap the plate dry on clean absorbent towels.
12. Add 100 µL of TMB Substrate to each well, using a repeater pipet.
13. Incubate the plate at room temperature for 30 minutes without shaking.
14. Add 50 µL of the Stop Solution to each well, using a repeater pipet.
15. Read the optical density generated from each well in a plate reader capable of reading at 450 nm.
16. Use the plate reader's built-in 4PLC software capabilities to calculate prostaglandin E_2 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 1X Assay Buffer into tube #1 and 200 µL into tubes #2 to #7. **The Prostaglandin E_2 stock solution contains an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery.** Carefully add 20 µL of the Prostaglandin E_2 stock solution to tube #1 and vortex completely. Take 200 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 #7. The concentration of Prostaglandin E_2 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
1X 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.
2. 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.

   Pipet standards or samples down the plate strip columns (A to H) to ensure maximum use of the strip wells.
3. Pipet 25 µL of samples or standards into wells in the plate.
4. Pipet 50 µL of 1X Assay Buffer into the non-specific binding (NSB) wells.
5. Pipet 25 µL of 1X Assay Buffer into the maximum binding (B0 or Zero standard) wells.
6. Add 25 µL of the DetectX® Prostaglandin E_2 Conjugate to each well using a repeater pipet.
7. Add 25 µL of the DetectX® Prostaglandin E_2 Antibody to each well, **except the NSB wells**, using a repeater pipet.
8. Gently tap the sides of the plate to ensure adequate mixing of the reagents. Cover the plate with the plate sealer.
9. Incubation Options:

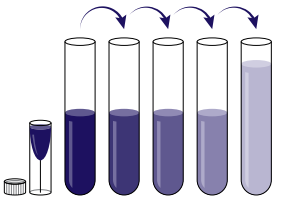
   EITHER:
   8a. Shake at room temperature for 2 hours. We recommend shaking at around 700–900 rpm. If the plate is not shaken, signals bound will be approximately 40% lower.

   OR:
   8b. Shake the plate in a plate shaker at room temperature for 15 minutes to ensure adequate mixing of the reagents. We recommend shaking at around 700–900 rpm. Incubate at 4°C for 16-18 hours.
10. 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.**
11. Aspirate the plate and wash each well 4 times with 300 µL 1X Wash Buffer. Tap the plate dry on clean absorbent towels.
12. Add 100 µL of TMB Substrate to each well, using a repeater pipet.
13. Incubate the plate at room temperature for 30 minutes without shaking.
14. Add 50 µL of the Stop Solution to each well, using a repeater pipet.
15. Read the optical density generated from each well in a plate reader capable of reading at 450 nm.
16. Use the plate reader's built-in 4PLC software capabilities to calculate prostaglandin E_2 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 1X Assay Buffer into tube #1 and 300 µL into tubes #2 to #9. **The Prostaglandin E_2 stock solution contains an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery.** Carefully add 15 µL of the Prostaglandin E_2 stock solution to tube #1 and vortex completely. Take 300 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 #9. The concentration of Prostaglandin E_2 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
1X 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.
2. 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.

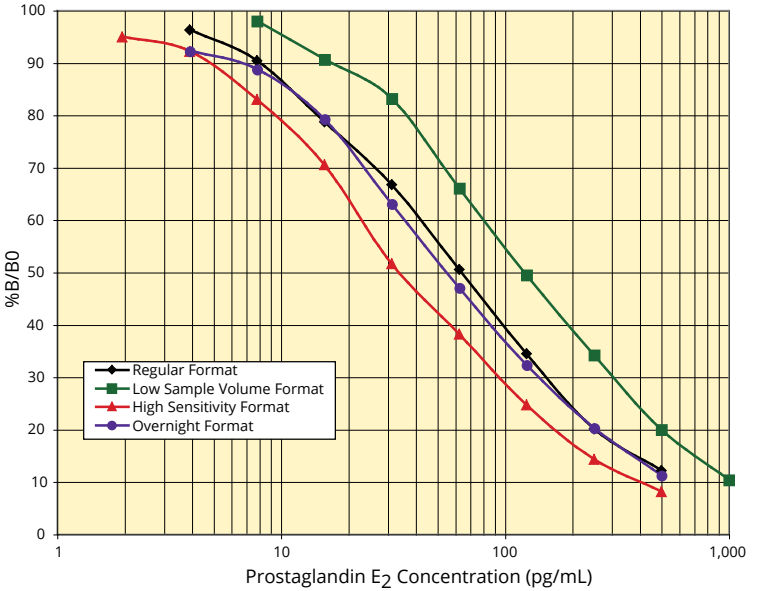
   Pipet standards or samples down the plate strip columns (A to H) to ensure maximum use of the strip wells.
3. Pipet 100 µL of samples or standards into wells in the plate.
4. Pipet 125 µL of 1X Assay Buffer into the non-specific binding (NSB) wells.
5. Pipet 100 µL of 1X Assay Buffer into the maximum binding (B0 or Zero standard) wells.
6. Add 25 µL of the DetectX® Prostaglandin E_2 Conjugate to each well using a repeater pipet.
7. Add 25 µL of the DetectX® Prostaglandin E_2 Antibody to each well, **except the NSB wells**, using a repeater pipet.
8. Gently tap the sides of the plate to ensure adequate mixing of the reagents. Cover the plate with the plate sealer.
9. Incubation Options:

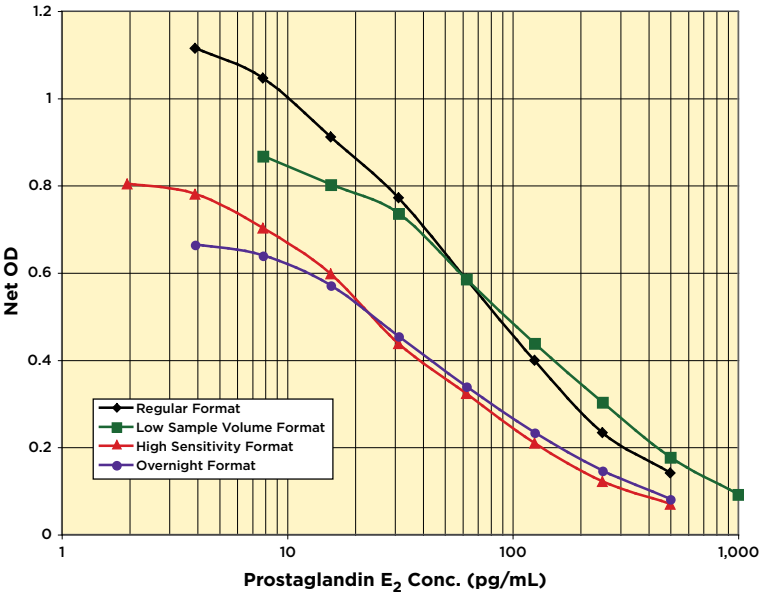
   EITHER:
   8a. Shake at room temperature for 2 hours. We recommend shaking at around 700–900 rpm. If the plate is not shaken signals bound will be approximately 40% lower.

   OR:
   8b. Shake the plate in a plate shaker at room temperature for 15 minutes to ensure adequate mixing of the reagents. We recommend shaking at around 700–900 rpm. Incubate at 4°C for 16-18 hours.
10. 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.**
11. Aspirate the plate and wash each well 4 times with 300 µL 1X Wash Buffer. Tap the plate dry on clean absorbent towels.
12. Add 100 µL of TMB Substrate to each well, using a repeater pipet.
13. Incubate the plate at room temperature for 30 minutes without shaking.
14. Add 50 µL of the Stop Solution to each well, using a repeater pipet.
15. Read the optical density generated from each well in a plate reader capable of reading at 450 nm.
16. Use the plate reader's built-in 4PLC software capabilities to calculate prostaglandin E_2 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 <u>Regular Format</u>.

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 MyAssays to calculate the data:
www.myassays.com/arbor-assays-pge2-enzyme-immunoassay-kit-k051-h.assay

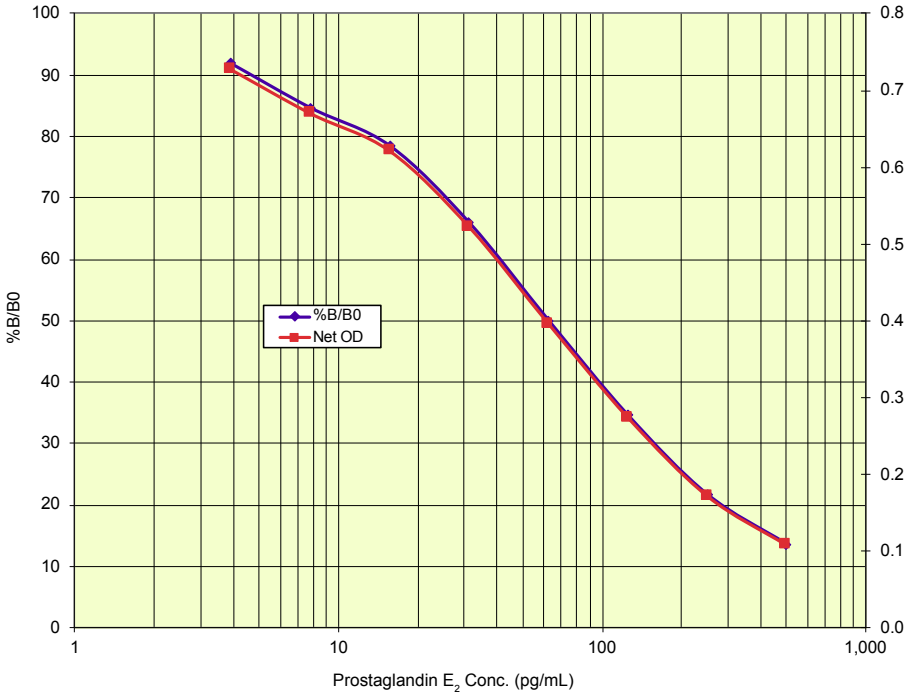
TYPICAL DATA - 2 HOUR REGULAR FORMAT

Sample	Mean OD	Net OD	% B/B0	PGE_2 Conc. (pg/mL)
NSB	0.044	0	-	-
Standard 1	0.202	0.108	13.65	500
Standard 2	0.266	0.172	21.74	250
Standard 3	0.368	0.274	34.64	125
Standard 4	0.490	0.396	50.06	62.5
Standard 5	0.616	0.522	65.99	31.25
Standard 6	0.715	0.621	78.51	15.625
Standard 7	0.764	0.670	84.70	7.813
Standard 8	0.821	0.727	91.91	3.906
B0	0.885	0.791	100	0
Sample 1	0.423	0.329	41.59	90.9
Sample 2	0.524	0.430	54.36	52.3

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 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 B0 and standard #8. The detection limit was determined at two standard deviations from the B0 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 PGE_2 concentrations. A plasma sample with a high PGE_2 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 PGE_2 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.

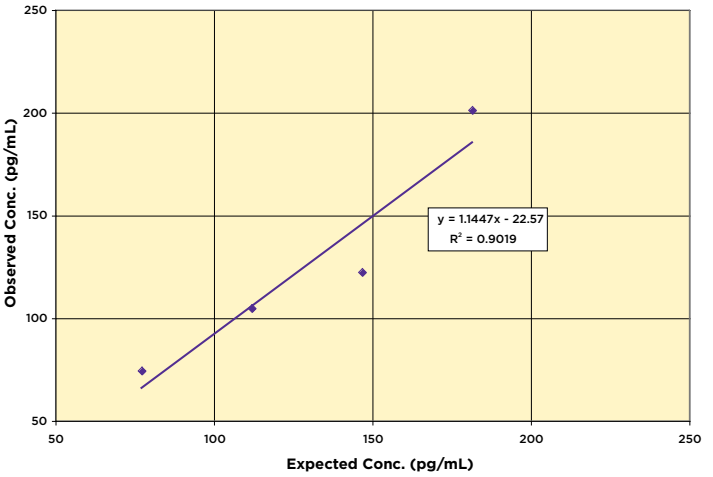
Plasma Linearity

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%**

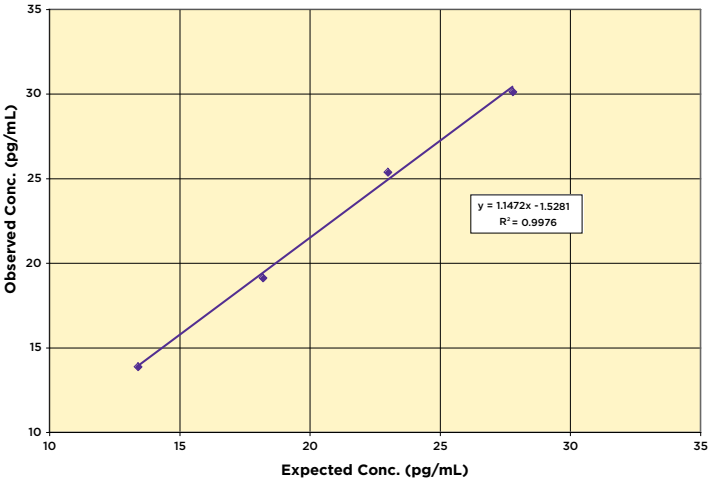
Urine Linearity

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 1X 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	11.7	12.3
2	98.6	6.3
3	131.2	4.9

Inter Assay Precision

Three human samples were diluted with 1X Assay Buffer and run in duplicates in seventeen 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	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 1X 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 1X 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 1X 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_{2a}	0.33%
Thromboxane B_2	< 0.02%
6-keto-Prostaglandin F_{1a}	< 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 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 recomendations 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.

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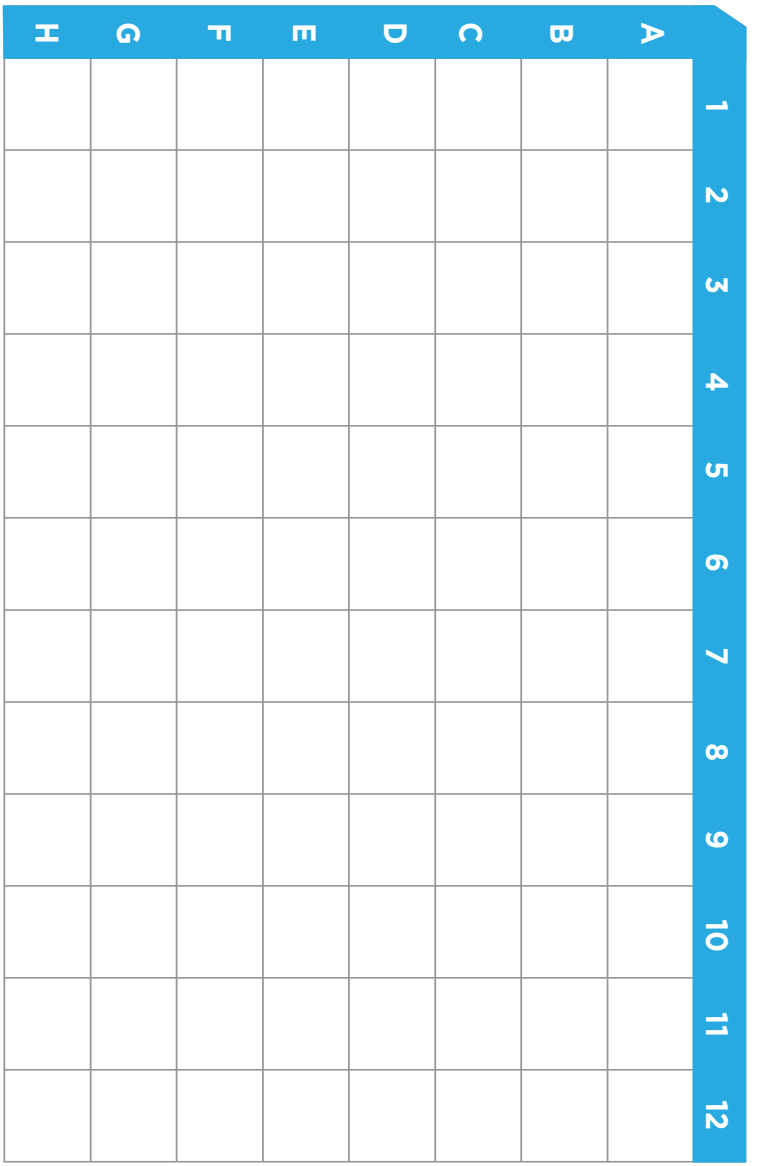
OFFICIAL SUPPLIER TO ISWE

Arbor Assays and the International Society of Wildlife Endocrinology (ISWE) signed an exclusive agreement for Arbor Assays to supply ISWE members with assay kits and reagents for wildlife conservation research.



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