Datasheet for ABIN2866584

PGE2 ELISA Kit

24 Publications

Go to Product page

Overview

Quantity:	96 tests
Target:	PGE2
Reactivity:	Various Species, Human, Mouse
Method Type:	Sandwich ELISA
Application:	ELISA

Product Details

Purpose:	The DetectX® Prostaglandin E (PGE) Immunoassay kit is designed to quantitatively measure 2 2 PGE present in serum, plasma, urine, saliva, cells, tissue, and tissue culture media samples.
Brand:	DetectX®
Sample Type:	Saliva, Urine, Serum, Plasma (EDTA), Plasma (heparin), Tissue Culture Medium
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Cross-Reactivity (Details):	Cross reactivity (%) Prostaglandin E 100 % 2 Prostaglandin E 27.28 % 1 Prostaglandin F 2a 0.33 % Thromboxane B < 0.02 % 2 6-keto-Prostaglandin F < 0.02 % 1a 15-keto-Prostaglandin E < 0.02 % 1 16,16-dimethyl-Prostaglandin E < 0.02 % 2 Arachidonic Acid < 0.02 %
Components:	Coated Clear 96 Well Plates A clear plastic microtiter plate(s) coated with goat anti-mouse IgG. 1 or 5 Each Prostaglandin E2 standard Must be stored at -20°C. Prostaglandin E2 at 20,000 pg/mL in a special stabilizing solution. 70 µL or 350 µL detectX® Prostaglandin E2 Antibody A mouse monoclonal antibody specific for Prostaglandin

Product Details

	E2. 3 mL or 13 mL DetectX® Prostaglandin E2 Conjugate Must be stored at -20°C. A Prostaglandin E2-peroxidase conjugate in a special stabilizing solution. 3 mL or 13 mL Assay buffer Concentrate A 5X concentrate that must be diluted with deionized or distilled water. 28 mL or 55 mL Wash buffer Concentrate A 20X concentrate that should be diluted with deionized or distilled water. 30 mL or 125 mL TMB substrate 11 mL or 55 mL Stop solution A 1M solution of hydrochloric acid. CAUstiC. 5 mL or 25 mL Plate sealer 1 or 5 each
Material not included:	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.

Target Details

Target:	PGE2
Alternative Name:	Prostaglandin E2 (PGE2 Products)
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 PGH . This is further processed by cytosolic or microsomal prostaglandin 2 synthases to become PGE or one of several other prostanoids1-3. Prostacyclin is 2 the major cyclooxygenase product in blood vessel walls and it is present in Prostaglandin E 2 inflammatory fluids in similar concentrations to PGE . Prostacyclin is a potent vasodilator and 2 is more potent than PGE in producing hyperalgesia4. PGE is produced by a wide variety of 2 2 tissues5-14 and in several pathological conditions, including inflammation, arthritis, fever, tissue injury, endometriosis, and a variety of cancers5,6. Other biological actions of PGE include vasodilation, modulation of sleep/wake cycles, and 2 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

Application Details

Application Notes:	Prostaglandin E (PGE) is identical across all species and we expect this kit may measure PGE from sources other than human. The end user should evaluate recoveries of PGE in other samples being 2 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 and may be extracted as described below. The normal reference range for serum Prostaglandin E (containing COX inhibitors) is 25-1,000 2 pg/mL. Typical normal mouse PGE serum levels are 45-150 ng/mL. Normal 24-hour urine PGE 2 2 levels are between 400-620 ng/24 hours.
Comment:	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
Plate:	Pre-coated
Protocol:	This 2 EIA kit allows for the widest variations in sample size, sensitivity and assay timing of any A PGE 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 -peroxidase conjugate is added to the standards and samples in 2 the wells. The binding reaction is initiated by the addition of a monoclonal antibody to PGE to 2 each well. After incubation, the plate is washed and substrate is added.

	The substrate reacts with the bound PGE -peroxidase conjugate. After a short incubation, the reaction is stopped and the 2 intensity of the generated color is detected in a microtiter plate reader capable of measuring 450nm wavelength. The concentration of the PGE in the sample is calculated, after making 2 suitable correction for the dilution of the sample, using software available with most plate readers.
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 concentrations. Ensure that all samples have reached room temperature and 2 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 ® www.ArborAssays.com 9 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 E stock solution contains 2 an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery. Carefully add 10 µL of the Prostaglandin E stock solution to tube #1 2 and vortex completely. Take 200 µL of the Prostaglandin E solution in tube 2 #1 and add it to tube #2 and vortex completely. Repeat the serial dilutions for tubes #3 through #8. The concentration of Postaglandin E in tubes 1 through 2 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. 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 E stock solution contains 2 an organic solvent.

	Prerinse the pipet tip several times to ensure accurate delivery. Carefully add 20 µL of the Prostaglandin E stock solution to tube #1 2 and vortex completely. Take 200 µL of the Prostaglandin E solution in tube 2 #1 and add it to tube #2 and vortex completely. Repeat the serial dilutions for tubes #3 through #7. The concentration of Prostaglandin E in tubes 1 through 2 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. 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 E stock solution contains 2 an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery. Carefully add 15 µL of the Prostaglandin E stock solution to tube #1 2 and vortex completely. Take 300 µL of the Prostaglandin E solution in tube 2 #1 and add it to tube #2 and vortex completely. Repeat the serial dilutions for tubes #3 through #9. The concentration of Prostaglandin E in tubes 1 through 2 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.
Sample Preparation:	serum and Plasma samples Serum and plasma samples should be diluted ≥ 1:10 with the supplied diluted Assay Buffer prior running in the assay. Mouse serum and plasma samples need to 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 PGE serum levels are 45-150 ng/mL. 2 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.
Assay Procedure:	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 4ºC.

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 wells to act as maximum binding wells (B0 or 0 pg/mL).

5. Add 25 µL of the DetectX® Prostaglandin E Conjugate to each well using a repeater pipet. 2

6. Add 25 µL of the DetectX® Prostaglandin E Antibody to each well, except the nsb wells, 2 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:

8a. Shake at room temperature for 2 hours. If the plate is not shaken signals bound will be approximately 40 % lower.

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 E 2 concentration for each sample.

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 4ºC.

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 wells to act as maximum binding wells (B0 or 0 pg/mL).

	5. Add 25 µL of the DetectX® Prostaglandin E Conjugate to each well using a repeater pipet. 6. Add 25 µL of the DetectX® Prostaglandin E Antibody to each well, except the nsb 2 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: 8a. Shake at room temperature for 2 hours. If the plate is not shaken signals bound will be approximately 40 % lower. 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 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 MyAssays to calculate the data: assay tyPiCAl dAtA - 2 HoUr rEgUlAr ForMAt sample Mean od net od % b/b0 PgE Conc. (pg/mL) 2 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 B0 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.

Application Details

	Conversion Factor: 100 pg/mL of prostaglandin E is equivalent to 283.7 pM. typical standard Curve - 2 Hour regular Format 100 1.2 90 1.0 80 70 0.8 60 50 %B/B0 0.6 %B/B0 Net OD 40 0.4 30 20 0.2 10 0 0 1 10 100 1,000 Prostaglandin E2 Conc. (pg/mL) 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 (2) 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. %B/B0 Net OD linearity
Restrictions:	For Research Use only

Handling

Precaution of Use:	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. The Stop Solution is acid. The solution should not come in contact with skin or eyes. Take appropriate precautions when handling this reagent.
Storage:	-20 °C,-80 °C,4 °C,RT
Storage Comment:	the unopened kit must be stored at -20°C. Once opened the kit can be stored at 4°C up to the

Handling

expiration date on the kit label, except for the PgE standard and PgE Conjugate. these must be stored at -20°C. 2 2 The PGE Conjugate will lose about 40% of its signal when stored at -20°C. No change in %B/B0 2 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 PGE Conjugate can be freeze-thawed multiple times. 2

Publications

Product cited in:

García-Guerra, Canavessi, Jr, Mezera, Sartori, Kirkpatrick, Wiltbank: "Trio, a novel bovine high fecundity allele: III. Acquisition of dominance and ovulatory capacity at a smaller follicle size." in: **Biology of reproduction**, (2018) (PubMed).

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Zena, Dillon, Hunt, Navas, Bícego, Buck: "Seasonal changes in plasma concentrations of the thyroid, glucocorticoid and reproductive hormones in the tegu lizard Salvator merianae." in: **General and comparative endocrinology**, (2018) (PubMed).

Palacios-Arreola, Nava-Castro, Río-Araiza, Pérez-Sánchez, Morales-Montor: "A single neonatal administration of Bisphenol A induces higher tumour weight associated to changes in tumour microenvironment in the adulthood." in: **Scientific reports**, Vol. 7, Issue 1, pp. 10573, (2017) (PubMed).

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