Human PGE2 Enzyme Immunoassay High Sensitivity Kit

CATALOG NO: IRAAKT2530 **LOT NO**: SAMPLE

INTENDED USE

The Prostaglandin E2 (PGE2) high sensitivity Immunoassay (CLIA) kit is designed to quantitatively measure very low concentrations of PGE2 present in serum, plasma, urine, saliva and tissue culture media samples.

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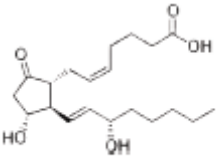
BACKGROUND

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 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].



Prostaglandin E_2

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 hemodynamics[7-12].

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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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   1. 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.
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.

ASSAY PRINCIPLE

The 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.

SUPPLIED COMPONENTS

- **Coated Clear 96 Well Plates:** Clear plastic microtiter plate(s) coated with goat anti-mouse IgG.
- **Prostaglandin E2 Standard Must be stored at -20°C.**
- **Prostaglandin E2 at 20,000 pg/mL in a special stabilizing solution.**
- **Prostaglandin E2 High Sensitivity Antibody:** A mouse monoclonal antibody specific for Prostaglandin E2.
- **Prostaglandin E2 Conjugate Must be stored at -20°C.**
- **A Prostaglandin E2-peroxidase conjugate in a special stabilizing solution.**
- **Assay Buffer (or Concentrate):** One plate kit uses a ready-to-use Assay Buffer.
- **Wash Buffer Concentrate:** A 20X concentrate that should be diluted with deionized or distilled water.
- **TMB Substrate**
- **Stop Solution:** A 1M solution of hydrochloric acid. CAUSTIC.
- **Plate Sealer**

STORAGE INSTRUCTIONS

- **The unopened kit should be stored at -20°C.**
- Once opened the kit can be stored at 4°C up to the expiration date on the kit label, **except for the PGE_2 Standard and PGE2 Conjugate. These must be stored at -20°C. 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.
- 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.
- The antibody coated plate needs to be stored desiccated. The silica gel pack included in the foil ziploc bag will keep the plate dry. The silica gel pack will turn from blue to pink if the ziploc has not been closed properly.
- This kit utilizes a peroxidase-based readout system. Buffers, including other manufacturers Wash Buffers, containing sodium azide will inhibit color production from the enzyme. Make sure **all** buffers used for samples are **azide free**. Ensure that any plate washing system is rinsed well with deionized water prior to using the supplied Wash Buffer as prepared on Page 8.
- The Stop Solution is acid. The solution should not come in contact with skin or eyes. Take appropriate precautions when handling this reagent.

SAMPLE TYPES

- This assay has been validated for saliva, urine, serum, EDTA and heparin plasma samples and for tissue culture samples. A general cyclooxygenase inhibitor, such as meclofenamic acid or indomethacin at 15 µM should be added immediately after collection of any biological samples, such as serum and plasma. All samples should be frozen rapidly in dry ice/ethanol and **stored at -80°C**.
- Samples containing visible particulate should be centrifuged prior to using. Severely hemolyzed samples should not be used in this kit. All samples containing lipids may interfere with the measurement of 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/spealm/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 ≥ 1:10 with the supplied Assay Buffer prior running in the assay. Mouse serum and plasma samples need to be diluted ≥ 1:20 with the supplied Assay Buffer prior running in the assay to minimize any interference of mouse IgG on the assay. Typical normal mouse PGE2 serum levels are 45-150 ng/mL.

Urine Samples

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

Saliva Samples

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

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

The ethanol concentration in the final Assay Buffer dilution added to the well should be <5%.

Use all samples within 2 hours of preparation.

REAGENT PREPARATION

Allow the kit reagents to thaw and come to room temperature for 30-60 minutes. We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine prostaglandin E2 concentrations. Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit.

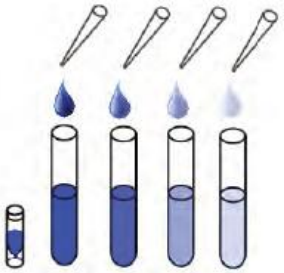
Wash Buffer

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

ASSAY PROTOCOL

1. Use the plate layout sheet on the back page to aid in proper sample and standard identification. Determine the number of wells to be used and return unused wells to the foil pouch with desiccant. Seal the ziploc plate bag and store at 4˚C.
2. Pipet 100 µL of samples or standards into wells in the plate.
3. Pipet 125 µL of Assay Buffer into the non-specific binding (NSB) wells.
4. Pipet 100 µL of Assay Buffer into wells to act as maximum binding wells (B0 or 0 pg/mL).
5. Add 25 µL of the Prostaglandin E2 Conjugate to each well using a repeater pipet.
6. Add 25 µL of the 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.

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.

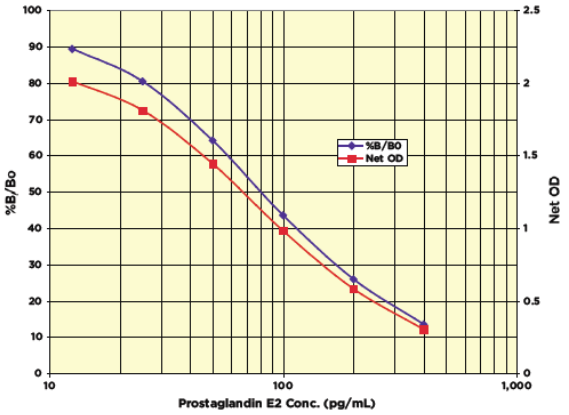
TYPICAL DATA

Sample	Mean OD	Net OD	% B/B0	PGE_2 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
B0	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 E_2 is equivalent to 283.7 pM.

Typical Standard Curves



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VALIDATION DATA

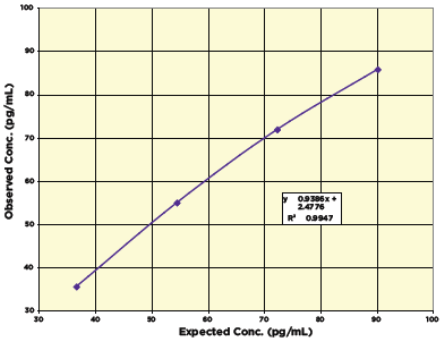
Sensitivity

- 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 E_2 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 E_2 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

SAMPLE VALUES

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

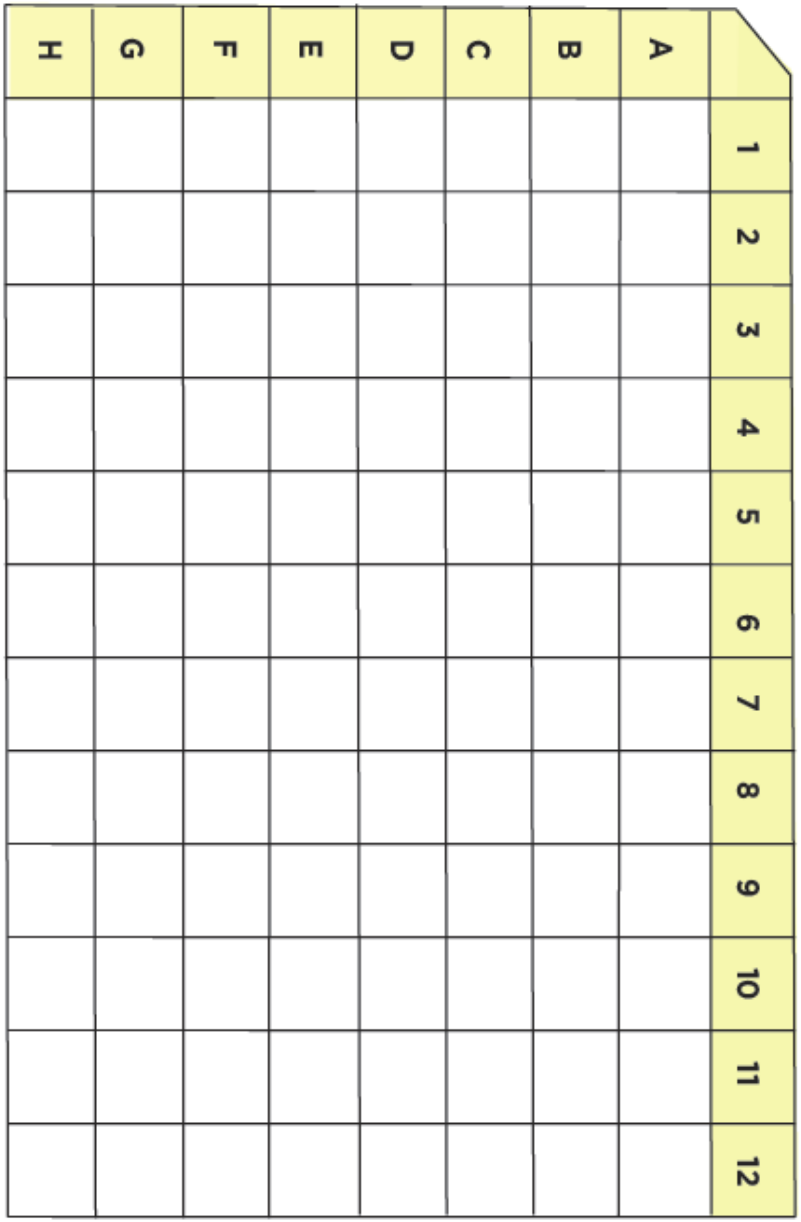
The following cross reactants were tested in the assay and calculated at the 50% binding point.

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

INTERFERENTS

- A variety of solvents were tested as possible interfering substances in the assay. Organic solvents such as DMSO, Dimethylformamide (DMF), methanol and ethanol were tested in the assay at 0.1%. DMSO and DMF caused a 1.2% and 0.8% decrease in measured 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.

ASSAY TEMPLATE



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