Human PGE2 Chemiluminescent Immunoassay Kit

CATALOG NO: IRAAKT2529 **LOT NO**: SAMPLE

INTENDED USE

The Prostaglandin E2 (PGE2) Chemiluminescent 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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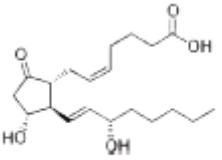
BACKGROUND

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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ASSAY PRINCIPLE

The Prostaglandin E2 (PGE2) Chemiluminescent Immunoassay (CLIA) kit is designed to quantitatively measure very low concentrations of 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 white 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 a special chemiluminescent substrate is added. The substrate reacts with the bound PGE2-peroxidase conjugate to produce light. The generated light is detected in a microtiter plate reader capable of reading luminescence. 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 White 96 Well Plates:** White 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 CLIA Antibody:** A mouse monoclonal antibody specific for Prostaglandin E2.
- **Prostaglandin E2 CLIA Conjugate Concentrate Must be stored at -20°C.**
- **A Prostaglandin E2-peroxidase conjugate concentrate in a special stabilizing solution.**
- **Conjugate Diluent:** Contains special stabilizers and additives.
- **Assay Buffer (or Concentrate):** One plate kit uses a ready-to-use Assay Buffer. Five plate kit uses a 5X concentrate that should be diluted with deionized or distilled water.
- **Wash Buffer Concentrate:** A 20X concentrate that should be diluted with deionized or distilled water.
- **Substrate Solution A**
- **Substrate Solution B**
- **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 PGE2 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 with disposable tips capable of dispensing 25 µL and 100 µL.
- A microplate shaker.
- A 4°C refrigerator.
- 96 well microplate reader capable of reading glow chemiluminescence. All luminometers read Relative Light Units (RLU). These RLU readings will vary with make or model of plate reader. **The number of RLUs obtained is dependant on the sensitivity and gain of the reader used. If you are unsure of how to properly configure your reader contact your plate reader manufacturer or carry out the following protocol:**
- Dilute 5 µL of the Prostaglandin E2 CLIA Conjugate Concentrate into 95 µL of Conjugate Diluent. Dilute 5 µL of this diluted Prostaglandin E2 CLIA Conjugate into 45 µL of deionized water. Pipet 5 µL of this diluted conjugate into a white well and add 100 µL of prepared CLIA substrate (see page 8 for details). This well will give you an intensity slightly above the maximum binding for the assay. Adjust the gain or sensitivity so that your reader is giving close to the maximum signal.
- **To properly analyze the data software will be required for converting raw RLU 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**.

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/spe_alm/index.htm#ext .
- Prostaglandin E2 is identical across all species and we expect this kit may measure Prostaglandin E2 from sources other than human. The end user should evaluate recoveries of Prostaglandin E2 in other samples being tested.

SAMPLE PREPARATION

Serum and Plasma Samples

Serum and plasma samples should be diluted ≥ 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.

Assay Buffer

Once diluted this is stable at 4°C for 3 months. Do not dilute the Assay Buffer in the One Plate Kit.

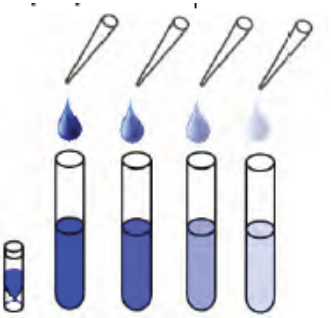
Wash Buffer

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 one test tube as Stock 2 and seven test tubes as #1 through #7. Pipet 135 µL of Assay Buffer into the Stock 2 tube and 525 µL of Assay Buffer into tube #1. Pipet 300 µL of Assay Buffer 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 15 µL of the PGE2 stock solution to the Stock 2 tube and vortex completely. Take 100 µL of the PGE2 solution in the Stock 2 tube and add it to tube #1 and vortex completely. Take 300 µL of the PGE2 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 320, 160, 80, 40, 20, 10 and 5 pg/mL.

Use all Standards within 2 hours of preparation



	Stock 2	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7
Assay Buffer (µL)	**135**	**525**	300	300	300	300	300	300
Addition	PGE_2 Std.	Stock 2	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Vol of Addition (µL)	**15**	**100**	300	300	300	300	300	300
Final Conc (pg/mL)	2,000	320	160	80	40	20	10	5

PGE_2 Conjugate

The supplied PGE_2 Conjugate Concentrate should be diluted 1:20 with the Conjugate Diluent as indicated in the table below. Once diluted the PGE_2 conjugate is to be used the same day.

	1 Plate	2 Plates	3 Plates	4 Plates	5 Plates
Conjugate Concentrate	125 µL	250 µL	375 µL	500 µL	625 µL
Conjugate Diluent	2.375 mL	4.75 mL	7.125 mL	9.5 mL	11.375 mL
Final Mixture	2.5 mL	5 mL	7.5 mL	10 mL	12.5 mL

Chemiluminescent Substrate

Mix one part of the Substrate Solution A with one part of Substrate Solution B in a brown bottle. Once mixed the substrate is stable for one month when stored at 4°C.

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 diluted Prostaglandin E2 CLIA Conjugate to each well using a repeater pipet.
6. Add 25 µL of the Prostaglandin E2 CLIA 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 the mixed Chemiluminescent Substrate to each well, using a repeater pipet.
11. Incubate the plate at room temperature for 5 minutes without shaking.
12. Read the luminescence generated from each well in a mutimode or chemiluminescent plate reader using a 0.1 second read time per well. The chemiluminescent signal will decrease about 40% over 60 minutes.
13. Use the plate reader's built-in 4PLC software capabilities to calculate cAMP concentration for each sample.

CALCULATION OF RESULTS

All luminometers read Relative Light Units (RLU). These RLU readings will vary with make or model of plate reader. Average the duplicate RLU 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 RLU'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.

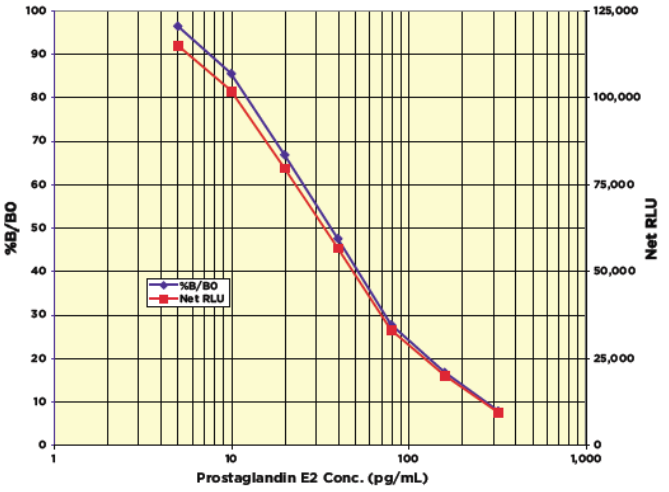
TYPICAL DATA

Sample	Mean RLU	Net RLU	% B/B0	PGE_2 Conc. (pg/mL)
NSB	3,960	-	-	-
Standard 1	13,265	9,305	7.8%	320
Standard 2	23,870	19,910	16.7%	160
Standard 3	36,935	32,975	27.7%	80
Standard 4	60,620	56,660	47.5%	40
Standard 5	83,560	79,600	66.8%	20
Standard 6	105,845	101,885	85.5%	10
Standard 7	118,880	114,920	96.4%	5
B0	123,145	119,185	100%	0
Sample 1	36,785	32,825	27.5%	83.7
Sample 2	66,515	62,555	52.5%	33.2

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



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

VALIDATION DATA

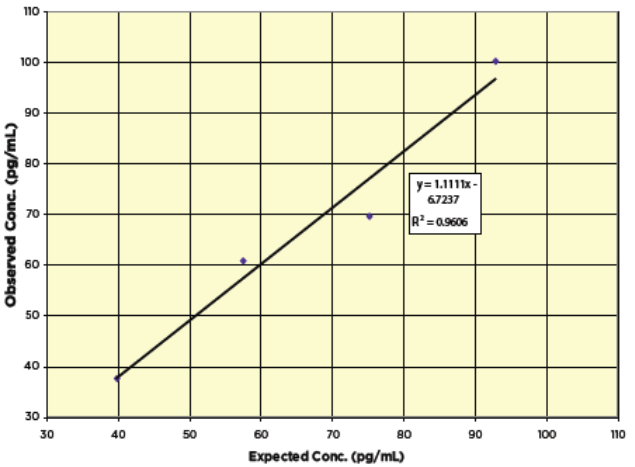
Sensitivity

- Sensitivity was calculated by comparing the RLU's for twenty wells run for each of the B0 and standard #7. The detection limit was determined at two (2) standard deviations from the B0 along the standard curve.
- **Sensitivity was determined as 4.81 pg/mL. This is equivalent to 481 fg PGE2 per sample or 1.365 fmol PGE2 per sample.**

LINEARITY

Linearity was determined by taking two diluted human serum samples, one with a low Prostaglandin E2 level of 22.2 pg/mL and one with a higher level of 110.6 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%	37.6	39.8	94.3
60%	40%	60.8	57.5	105.7
40%	60%	69.6	75.2	92.5
20%	80%	100.1	92.9	107.8
			Mean Recovery	**100.1%**



Intra Assay Precision

Two 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	104.1	10.0
2	32.9	13.7

Inter Assay Precision

Two human samples were diluted with Assay Buffer and run in duplicates in nineteen 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	93.0	5.8
2	33.2	13.5

SAMPLE VALUES

- Ten 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 from 1:15 to 1:200 in Assay Buffer. Values, not adjusted for dilution, ranged from 3.95 to 260.7 pg/mL. Dilution adjusted values ranged from 395.1 to over 26,000 pg/mL. Four normal human urine samples were diluted from 1:15 to 1:30 in Assay Buffer and values, not adjusted for dilution, ranged from 3.47 to 124.8 pg/mL. Dilution adjusted values ranged from 79.1 to over 1,872 pg/mL. Three normal human saliva samples were diluted from 1:4 in Assay Buffer and values, not adjusted for dilution, ranged from 4.23 to 5.88 pg/mL. Dilution adjusted values ranged from 16.9 to over 23.5 pg/mL.

CROSS REACTIVITY

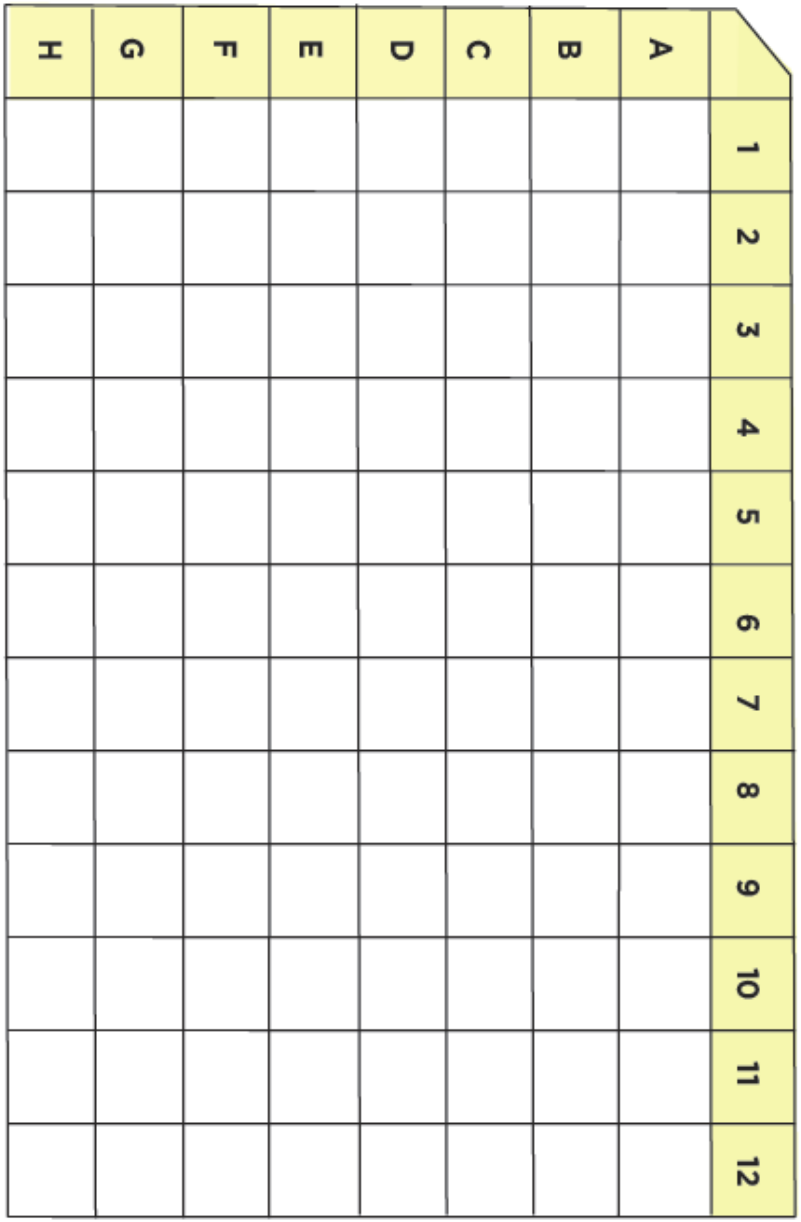
The following cross reactants were tested in the assay and calculated at the 50% binding point.

Eicosanoid	Cross Reactivity (%)
Prostaglandin E_2	100%
Prostaglandin E_1	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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