Prostaglandin E_2 High Sensitivity Immunoassay

Catalog Number DE2100

For the quantitative determination of Prostaglandin E_2 (PGE_2) concentrations in cell culture supernates, saliva, urine, serum, plasma, and other biological fluids.

This package insert must be read in its entirety before using this product.

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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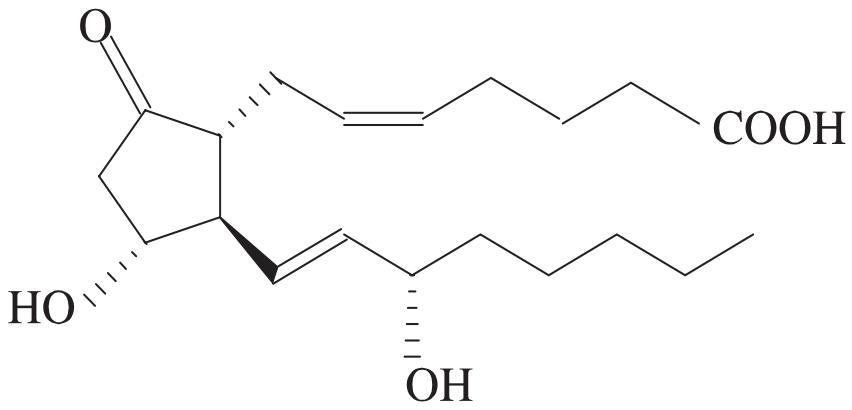
INTRODUCTION

The cyclooxygenase and lipoxygenase pathways are two major synthetic pathways relevant to human physiology. The initial synthetic step for both pathways involves the cleavage of arachidonic acid. Arachidonic acid is stored esterified in phospholipids (1) of cell membranes. It is released from the cell membrane upon demand via phospholipase A_2 (2). The free arachidonic acid is then oxygenated by either the cyclooxygenase or lipoxygenase pathway. The end products of these pathways are called eicosanoids. Prostaglandins and thromboxane are products of the cyclooxygenase pathway and leukotrienes are products of the lipoxygenase pathway. Eicosanoids are synthesized in response to immediate need and are not stored in significant amounts for later release.

Prostaglandin E_2 (PGE_2) is formed in a variety of cells from prostaglandin H_2, which is synthesized from arachidonic acid by the enzyme prostaglandin synthetase (1 - 4). PGE_2 has been shown to have a number of biological actions, including vasodilation (5), both anti- and proinflammatory action (6, 7), modulation of sleep/wake cycles (8), and facilitation of the replication of human immunodeficiency virus (9). It elevates cAMP levels (10), stimulates bone resorption (11), and has thermoregulatory effects. It has been shown to be a regulator of sodium excretion and renal hemodynamics (12).

PGE_2



R&D Systems' PGE_2 High Sensitivity Immunoassay is a 19 - 25 hour competitive enzyme immunoassay designed to measure PGE_2 in cell culture supernates, saliva, urine, serum, plasma, and other biological fluids.

PRINCIPLE OF THE ASSAY

This assay is based on the competitive binding technique in which PGE_2 present in a sample competes with a fixed amount of alkaline phosphatase-labeled PGE_2 for sites on a mouse monoclonal antibody. During the incubation, the mouse monoclonal antibody becomes bound to the goat anti-mouse antibody coated onto the microplate. Following a wash to remove excess conjugate and unbound sample, a substrate solution is added to the wells to determine the bound enzyme activity. Immediately following color development, the absorbance is read at 405 nm. The intensity of the color is inversely proportional to the concentration of PGE_2 in the sample.

TECHNICAL HINTS

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- Allow reagents to warm to room temperature before use.
- The unopened bottle of pNPP Substrate should be colorless to pale yellow.
- Stop Solution should be added to the plate in the same order as the pNPP Substrate. The color developed in the wells will remain yellow upon the addition of Stop Solution.
- When mixing or reconstituting protein solutions, always avoid foaming.
- Pre-rinse the pipette tips when pipetting standards.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- Pipette standards and samples to the bottom of the wells.
- Add all other reagents to the side of the wells to avoid contamination.
- If samples generate values higher than the highest standard, further dilute the samples with Assay Buffer and repeat the assay.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Alkaline Phosphatase is a temperature sensitive enzyme. Optical Density (OD) units may vary with temperature changes.

REAGENTS

Microplate (Part R80-0050) - 96 well microplate coated with a goat anti-mouse polyclonal antibody.

PGE_2 HS Conjugate (Part R80-0287) - 6 mL of PGE_2 conjugated to alkaline phosphatase, with blue dye and preservative.

PGE_2 HS Standard (Part R80-0004) - 0.5 mL of PGE_2 (50,000 pg/mL) in buffer, with preservative.

PGE_2 HS Antibody Solution (Part R80-0286) - 6 mL of mouse monoclonal antibody to PGE_2, with yellow dye and preservative.

Assay Buffer ED1 (Part R80-0010) - 30 mL of a buffered protein base with preservative.

Wash Buffer Concentrate (Part R80-1286) - 30 mL of a 20-fold concentrated solution of a buffered surfactant with preservative.

pNPP Substrate (Part R80-0075) - 20 mL of p-nitrophenyl phosphate in a buffered solution.

Stop Solution (Part R80-0247) - 5 mL of a trisodium phosphate (TSP) solution. **Keep tightly capped. Caution: caustic.**

Plate Cover - 2 adhesive strips.

STORAGE

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at ≤ -20° C in a manual defrost freezer. Do not use past kit expiration date.</td></tr>
<tr><td rowspan="8">Opened Reagents</td><td>Diluted Wash Buffer</td><td>May be stored at room temperature for up to 3 months.*</td></tr>
<tr><td>Stop Solution</td><td rowspan="4">May be stored at 2 - 8° C until the expiration date of the kit.</td></tr>
<tr><td>Assay Buffer ED1</td></tr>
<tr><td>pNPP Substrate</td></tr>
<tr><td>Antibody Solution</td></tr>
<tr><td>Conjugate</td><td rowspan="2">The conjugate and standard must be stored at ≤ -20° C in a manual defrost freezer.* Aliquot and freeze the conjugate after initial use. Avoid multiple freeze-thaw cycles.</td></tr>
<tr><td>Standard</td></tr>
<tr><td>Microplate Wells</td><td>Return unused wells to the pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored at 2 - 8° C until the expiration date of the kit.</td></tr>
</table>

*Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 405 nm with wavelength correction set between 570 nm and 590 nm.
- Pipettes and pipette tips.
- 1000 mL graduated cylinder for preparation of Wash Buffer.
- Deionized or distilled water.
- Squirt bottle or manifold dispenser.
- 37° C incubator or water bath.
- Prostaglandin synthetase inhibitor, *i.e.,* indomethacin or equivalent (Sigma).

If extracting PGE_2 from the sample matrix, the following supplies are also required:

- 2 N HCl, ethanol, hexane, and ethyl acetate.
- 200 mg C_{18} Reverse Phase Columns.
- PGE_2 Standard (for determination of extraction efficiency).

PRECAUTIONS

Some components of this kit contain sodium azide which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

The Stop Solution provided with the kit is a caustic material. Wear eye, hand, face, and clothing protection when using this material.

The PGE_2 Standard provided with this kit is in ethanolic buffer at a pH optimized to maintain PGE_2 integrity. Care should be taken when handling this material because of the known and unknown effects of prostaglandins.

The activity of the PGE_2 Conjugate is dependent on the presence of Mg^{2+} and Zn^{2+} ions and is affected by high concentrations of chelators, such as EDTA and EGTA. Samples that contain < 10 mM EDTA or EGTA can be assayed without interference. Samples that contain higher concentrations of chelators must be diluted prior to assay.

Inorganic phosphate is a strong competitive inhibitor of alkaline phosphatase; avoid the use of PBS based wash buffers and other sources of inorganic phosphate contamination.

SAMPLE COLLECTION AND STORAGE

Samples containing mouse IgG may interfere with this assay.

Cell Culture Supernates - Remove particulates by centrifugation. Assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, assay immediately or aliquot and store at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at approximately 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Saliva - Collect 2 - 3 mL of saliva in a polypropylene tube. Vortex briefly and centrifuge for 6 minutes at 14,000 rpm in microcentrifuge tubes. Collect the aqueous layer (no pellet) and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Note: *Cell culture supernate, urine, serum, plasma, and saliva samples containing low levels of PGE_2 may require extraction for measurement within the dynamic range of the assay. See the Sample Extraction section for a procedural outline.*

Other Biological Fluids - The recovery and linearity characteristics of this assay using other biological fluids has not been evaluated. Therefore, we recommend sample extraction before running the assay.

A prostaglandin synthetase inhibitor, such as indomethacin, should be added to all samples at approximately 10 μg/mL.

SAMPLE PREPARATION

All saliva, urine, serum, and plasma samples require at least a 10-fold dilution. A suggested 10-fold dilution is 50 μL sample + 450 μL Assay Buffer ED1.

Note: *To avoid dilution of samples containing low levels of PGE_2, we recommend extraction of PGE_2 from the sample matrix as outlined in the Sample Extraction section.*

SAMPLE EXTRACTION

1. Acidify the sample by the addition of 2 N HCl to a pH of 3.5. Approximately 50 µL of HCl will be needed per mL of sample. Allow to sit at 2 - 8° C for 15 minutes. Centrifuge samples in a microcentrifuge for 2 minutes to remove any precipitate.
2. Prepare the C_{18} reverse phase column by washing with 10 mL of ethanol followed by 10 mL of deionized water.
3. Apply the sample under a slight positive pressure to obtain a flow rate of about 0.5 mL/minute. Wash the column with 10 mL of deionized ice water, followed by 10 mL of 15% ethanol, and 10 mL of hexane. Elute the sample from the column by the addition of 10 mL of ethyl acetate.
4. If analysis is to be carried out immediately, evaporate samples under a stream of nitrogen. Add at least 250 µL of Assay Buffer. Vortex well then allow to sit for 5 minutes at room temperature. Repeat twice more. If analysis is to be delayed, store samples as the eluted ethyl acetate solutions at ≤ -70° C until the immunoassay is to be run. Evaporate the organic solvent under a stream of nitrogen prior to running the assay and reconstitute as described above.

Note: *Please refer to references 13 - 16 for details of extraction protocols.*

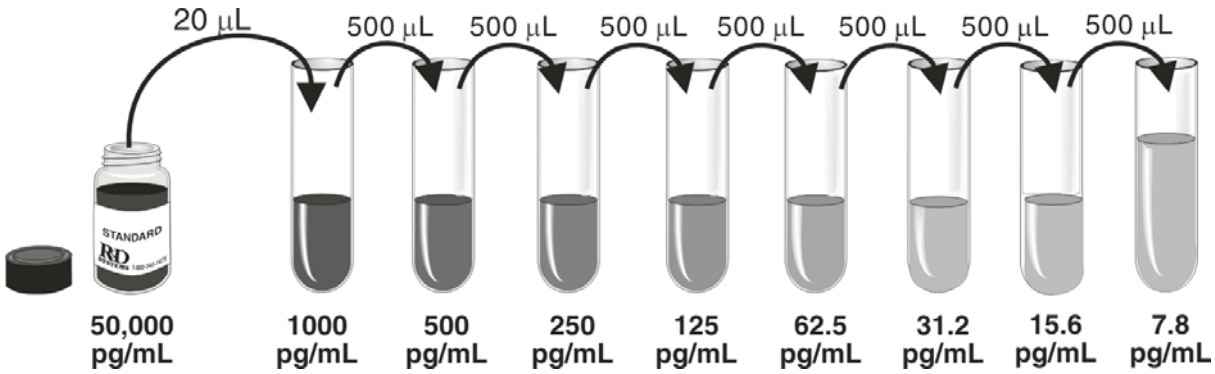
REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer - Dilute 30 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 600 mL of Wash Buffer (1X).

PGE_2 Standard* - Pipette 980 µL of Assay Buffer ED1 into the 1000 pg/mL tube. Pipette 500 µL of Assay Buffer ED1 into the remaining tubes. *When pipetting standards, it is important to pre-rinse the pipette tips.* Use the 50,000 pg/mL standard stock to produce a dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 1000 pg/mL standard serves as the high standard and the Assay Buffer ED1 serves as the zero standard (B_0) (0 pg/mL). **Use the diluted standards within 60 minutes of preparation.**

***When running cell culture supernate samples, use cell culture media (CCM) instead of Assay Buffer ED1 to produce the standard dilution series and as the zero standard. Changes in binding have been associated with running the standards and samples in media.**



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples and standards be assayed in duplicate.

Total Activity (TA), Non-Specific Binding (NSB), Maximum Binding (B_0), and Substrate Blank wells should be run in the assay as a means of quality control for each assay.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal.
3. Reserve wells for TA and Substrate Blank.
4. Add 150 µL of Assay Buffer (or CCM) to the NSB wells.
5. Add 100 µL of Assay Buffer (or CCM) to the zero standard (B_0) wells.
6. Add 100 µL of Standard or sample* to the remaining wells.
7. Add 50 µL of PGE_2 HS Conjugate to each well (excluding the TA and Substrate Blank wells).
8. Add 50 µL of PGE_2 HS Antibody Solution to each well (excluding the NSB, TA, and Substrate Blank wells). Cover with the adhesive strip provided. Incubate for 18 - 24 hours at 2 - 8° C.

   Note: *The TA and Substrate Blank wells are empty at this point. The NSB wells should be blue and all other wells should be green.*
9. Aspirate or decant each well and wash, repeating the process twice for a total of three washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle or manifold dispenser. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper toweling.
10. Add 5 µL of PGE_2 HS Conjugate to the TA wells.
11. Add 200 µL of pNPP Substrate to **all wells**. Cover with the adhesive strip provided. Incubate for 1 hour at 37° C.
12. Add 50 µL of Stop Solution to each well.
13. Determine the optical density of each well **immediately** using a microplate reader set to 405 nm with wavelength correction set between 570 nm and 590 nm.

*Samples may require dilution or extraction as directed in the Sample Preparation section.

CALCULATION OF RESULTS

Average the duplicate readings for each standard and sample and subtract the average NSB optical density.

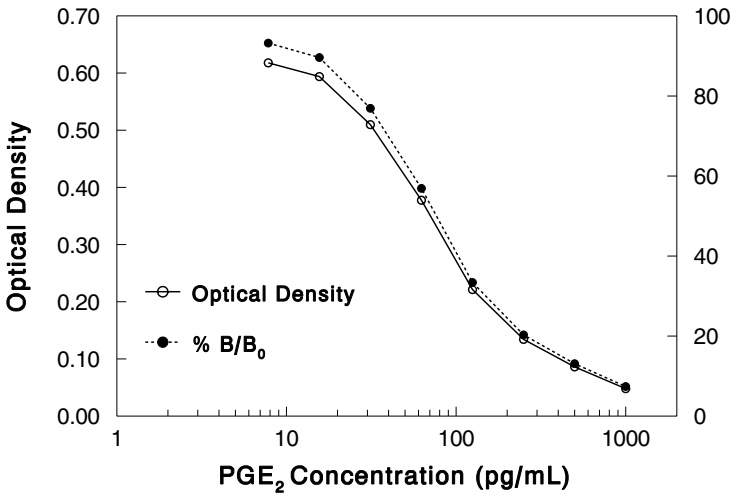
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on a linear y-axis against the concentration on a logarithmic x-axis.

% B/B_0 can be calculated by dividing the corrected OD for each standard or sample by the corrected B_0 OD and multiplying by 100.

Calculate the concentration of PGE_2 corresponding to the mean absorbance or the % B/B_0 from the standard curve. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



pg/mL	O.D.	Average	Corrected	% B/B_0
NSB	0.080 0.081	0.081	—	—
0(B_0)	0.739 0.748	0.744	0.663	100.0
7.8	0.648 0.749	0.699	0.618	93.2
15.6	0.690 0.659	0.675	0.594	89.6
31.2	0.590 0.591	0.591	0.510	76.9
62.5	0.456 0.460	0.459	0.378	57.0
125	0.296 0.308	0.303	0.222	33.5
250	0.214 0.216	0.216	0.135	20.4
500	0.163 0.172	0.168	0.087	13.1
1000	0.128 0.131	0.130	0.049	7.4

QUALITY CONTROL

Each laboratory should establish a quality control program to monitor the performance of the PGE_2 Immunoassay. As a part of this program, TA, NSB, B_0, and Substrate Blank wells should be run in the assay.

Typical Quality Control Parameters

Substrate Blank (O.D.)	=	0.081
TA (TA x 10)	=	1.48
% NSB (NSB/TA x 100)	=	0.0%
% B_0 (B_0/TA x 100)	=	44.8%
Quality of Fit	=	0.999

PRECISION

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty-four times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in eight separate assays to assess inter-assay precision.

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	24	24	24	8	8	8
Mean (pg/mL)	19.4	55.8	110	17.3	51.1	98.2
Standard deviation	1.9	3.4	3.4	2.1	6.4	8.0
CV (%)	9.8	6.1	3.1	12.1	12.6	8.1

RECOVERY

The recovery of PGE_2 spiked into samples in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell Culture Media	104	88 - 127%
Human Saliva*	123	110 - 136%
Human Urine*	109	100 - 118%
Serum*	120	110 - 132%

*Samples were diluted 10-fold prior to assay.

LINEARITY

To assess the linearity of the assay, Assay Buffer ED1 spiked with PGE_2 was assayed using serial 2-fold dilutions.

Dilution	Observed (pg/mL)	Expected (pg/mL)	% $\frac{\text{Observed}}{\text{Expected}}$
Neat	196.8	—	—
1:2	101.9	98.4	104
1:4	49.2	49.2	100
1:8	23.0	24.6	94

SENSITIVITY

The sensitivity of the PGE_2 assay is typically less than 8.26 pg/mL.

Sensitivity was determined by subtracting two standard deviations from the mean absorbance value of sixteen zero standard (B_0) replicates and calculating the corresponding concentration.

CROSS-REACTIVITY

Cross-reactivity for the following compounds was determined by adding the cross-reactant to Assay Buffer ED1 at concentrations ranging from 39 pg/mL to 500,000 pg/mL. The cross-reactivity was calculated at 50% B/B_0.

Compound	% Cross-reactivity
PGE_1	70
PGE_3	16.3
$PGF_{1\alpha}$	1.4
$PGF_{2\alpha}$	0.7
6-keto-$PGF_{1\alpha}$	0.6
PGA_2	0.1
PGB_1	0.1
13,14-dihydro-15-keto-$PGF_{2\alpha}$	< 0.1
6,15-keto-13,14-dihydro-$PGF_{1\alpha}$	< 0.1
Thromboxane B_2	< 0.1
2-Arachidonoylglycerol	< 0.1
Anandamide	< 0.1
PGD_2	< 0.1
Arachidonic acid	< 0.1

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