Prostaglandin $F_{2\alpha}$ Immunoassay

Catalog Number DE1150

For the quantitative determination of Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) concentrations in cell culture supernates, saliva, urine, plasma and serum.

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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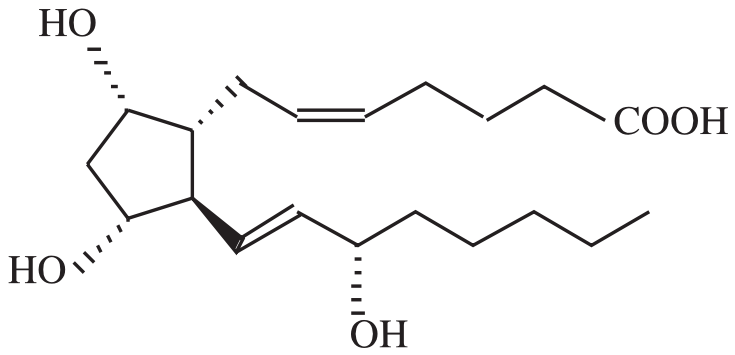
INTRODUCTION

The cyclooxygenase and lipoxygenase pathways are two major synthetic pathways relevant to human disease. 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 $F_{2\alpha}$ ($PGF_{2\alpha}$) is formed in a variety of cells from Prostaglandin H_2, which is synthesized from arachidonic acid by the enzyme prostaglandin synthetase (3). $PGF_{2\alpha}$ is often viewed as an antagonist to PGE_2 because of their opposing effects on various tissues (4). $PGF_{2\alpha}$ has been implicated in asthma attacks (5, 6). It is also involved in reproductive functions including corpus luteum regulation (7), uterine contractions (8), and sperm motility (9). This has led to its use in terminating pregnancies and inducing labor at term (5, 7, 8, 10). High levels of $PGF_{2\alpha}$ have also been associated with pre-eclampsia (11).

$PGF_{2\alpha}$



R&D Systems' $PGF_{2\alpha}$ Immunoassay is a 3.0 hour competitive enzyme immunoassay designed to measure $PGF_{2\alpha}$ in cell culture supernates, saliva, urine, plasma and serum.

PRINCIPLE OF THE ASSAY

This assay is based on the competitive binding technique in which $PGF_{2\alpha}$ present in a sample competes with a fixed amount of alkaline phosphatase-labeled $PGF_{2\alpha}$ for sites on a sheep polyclonal antibody. During the incubation, the polyclonal antibody becomes bound to the donkey anti-sheep 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. The color development is stopped and the absorbance is read at 405 nm. The intensity of the color is inversely proportional to the concentration of $PGF_{2\alpha}$ 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 tip 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 ED1 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 R80-0045) - 96 well microplate coated with a donkey anti-sheep polyclonal antibody.

$PGF_{2\alpha}$ Conjugate (Part R80-0027) - 5 mL of $PGF_{2\alpha}$ conjugated to alkaline phosphatase, with blue dye and preservatives.

$PGF_{2\alpha}$ Standard (Part R80-0029) - 0.5 mL of $PGF_{2\alpha}$ (500,000 pg/mL) in buffer, with preservatives.

$PGF_{2\alpha}$ Antibody Solution (Part R80-0639) - 5 mL of sheep polyclonal antibody to $PGF_{2\alpha}$, with yellow dye and preservatives.

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

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

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 - 1 adhesive strip.

STORAGE

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at 2 - 8° C. 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="6">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>Conjugate</td></tr>
<tr><td>Antibody Solution</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.
- 500 mL graduated cylinder for preparation of Wash Buffer.
- Deionized or distilled water.
- Squirt bottle, or manifold dispenser.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 $\pm$ 50 rpm.
- Prostaglandin synthetase inhibitor, *i.e.*, indomethacin or equivalent (Sigma).

Reagents required for sample extraction:

- 2 N HCl.
- Ethanol (ACS Grade).
- Hexane (ACS Grade).
- Ethyl Acetate (ACS Grade).
- C_{18} Reverse phase column.
- $PGF_{2\alpha}$ 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 $PGF_{2\alpha}$ Standard provided with this kit is supplied in ethanolic buffer at a pH optimized to maintain $PGF_{2\alpha}$ integrity. Care should be taken when handling this material because of the known and unknown effects of prostaglandins.

The activity of the $PGF_{2\alpha}$ 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 sheep IgG may interfere with this assay.

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

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

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

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

If samples are not assayed immediately, a prostaglandin synthetase inhibitor, such as indomethacin, should be added to all samples at approximately 10 μg/mL before storage.

SAMPLE PREPARATION

All serum, plasma, saliva and urine samples require at least a 20-fold dilution. A suggested 20-fold dilution is 20 μL sample + 380 μL Assay Buffer ED1.

SAMPLE EXTRACTION

If diluted samples measure below the lowest standard, then it is recommended that the samples be extracted for accurate measurement of $PGF_{2\alpha}$. The following extraction protocol is a general extraction method and is supplied as a guideline. For more information on extraction protocols, refer to references 12 - 15.

1. Acidify the sample by addition of 2 N HCl to a pH of 3.5 (approximately 50 µL of 2 N HCl per 1 mL of sample).
2. Incubate for 15 minutes at 2 - 8° C. Centrifuge in a microcentrifuge for 2 minutes to remove any precipitate.
3. Prepare the C_{18} reverse phase column by washing with 10 mL of ethanol followed by 10 mL of deionized water.
4. Apply the sample under a slight positive pressure to obtain a flow rate of 0.5 mL/minute. Wash the column with 10 mL deionized water, followed by 10 mL 15% ethanol, and finally 10 mL hexane. Elute the sample from the column by addition of 10 mL ethyl acetate.
5. Immediately prior to assay, evaporate samples under a stream of nitrogen. Add 250 µL of Assay Buffer ED1. Vortex well then allow to sit for 5 minutes at room temperature. Repeat twice more. If samples cannot be assayed the same day, store samples as the eluted ethyl acetate solutions at ≤ -70° C. Avoid repeated freeze-thaw cycles. Evaporate the organic solvent under a stream of nitrogen prior to running the assay and reconstitute as described above.

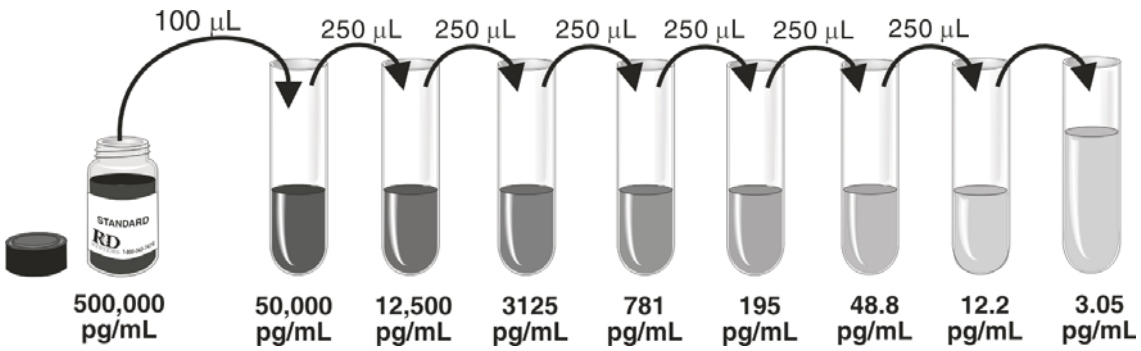
REAGENT PREPARATION

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).

$PGF_{2\alpha}$ Standard - Pipette 900 µL of Assay Buffer ED1 into the 50,000 pg/mL tube. Pipette 750 µL of Assay Buffer ED1 into the remaining tubes. *When pipetting standards, it is important to pre-rinse the pipette tip*. Use the 500,000 pg/mL standard stock to produce a dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 50,000 pg/mL standard serves as the high standard and the Assay Buffer ED1 serves as the zero standard (B_0) (0 pg/mL). **Use 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. There will be a small change in binding associated when 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 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 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 $PGF_{2\alpha}$ Conjugate to each well (excluding the TA and Substrate Blank wells).
8. Add 50 µL of $PGF_{2\alpha}$ Antibody Solution to each well (excluding the NSB, TA, and Substrate Blank wells). Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.

   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 (200 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 towels.
10. Add 5 µL of $PGF_{2\alpha}$ Conjugate to the TA wells.
11. Add 200 µL of pNPP Substrate to **all wells**. Incubate for 45 minutes at room temperature **on the benchtop**.
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. See Sample Preparation Section.

CALCULATION OF RESULTS

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

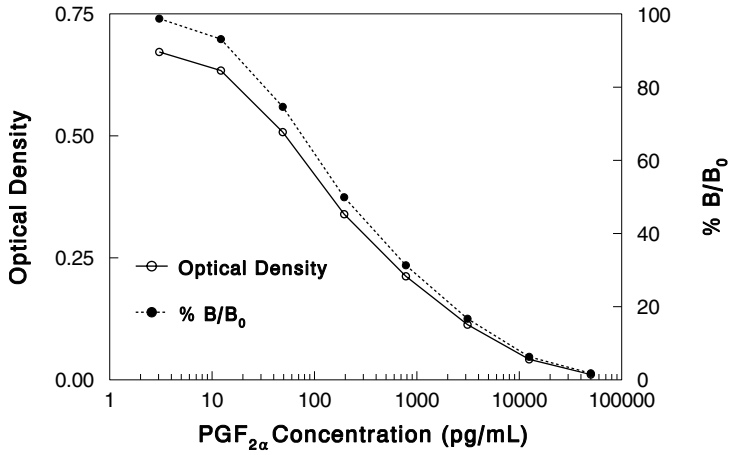
Create a standard curve by reducing the data using computer software capable of generating a four paramater logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on a linear y-axis against the concentration on a logarithmic x-axis and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

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

Calculate the concentration of $PGF_{2\alpha}$ corresponding to the mean absorbance or % 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	OD	Average	Corrected	% B/B_0
Blank	0.071 0.071	0.071	—	—
NSB	0.081 0.077	0.079	0.008	—
0 (B_0)	0.742 0.777	0.760	0.681	100.0
3.05	0.744 0.758	0.751	0.672	98.7
12.2	0.737 0.689	0.713	0.634	93.2
48.8	0.599 0.575	0.587	0.508	74.9
195	0.403 0.432	0.419	0.340	50.5
781	0.287 0.296	0.292	0.213	32.1
3,125	0.197 0.188	0.193	0.114	17.7
12,500	0.122 0.121	0.122	0.043	7.4
50,000	0.093 0.089	0.091	0.012	2.9

QUALITY CONTROL

Each laboratory should establish a quality control program to monitor the performance of the $PGF_{2\alpha}$ Immunoassay. As a part of this program, TA, NSB, B_0, and Substrate Blank wells should be run in each assay.

Typical Quality Control Parameters

Substrate Blank (O.D.)	=	0.071
TA (TA x 10)	=	10.21
%NSB (NSB/TA) x 100	=	0.078%
$\%B_0$ (B_0/TA) x 100	=	6.7%
Quality of Fit	=	0.999

PRECISION

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested ten 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	4	5	6
n	10	10	10	8	8	8
Mean (pg/mL)	82.7	405	916	47.4	290	731
Standard deviation	10.8	27.6	45.4	4.6	15.9	22.3
CV (%)	13.1	6.8	4.9	9.7	5.5	3.1

RECOVERY

The recovery of $PGF_{2\alpha}$ spiked into samples in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Human Saliva*	104	93 - 114%
Human Urine*	107	72 - 126%
Human Serum*	100	66 - 123%
Porcine Serum*	100	66 - 123%
Porcine Plasma*	97	83 - 110%

*Samples were diluted prior to assay as directed in Sample Preparation.

LINEARITY

To assess the linearity of the assay, Assay Buffer ED1 containing $PGF_{2\alpha}$ was assayed using serial 2-fold dilutions.

Dilution	Observed (pg/mL)	Expected (pg/mL)	% Observed / Expected
Neat	4339	—	—
1:2	2320	2170	107%
1:4	1260	1085	116%
1:8	652	542.5	120%
1:16	344	271.2	127%

SENSITIVITY

The sensitivity of the $PGF_{2\alpha}$ assay is typically less than 6.71 pg/mL.

Sensitivity was determined by subtracting two standard deviations from the mean optical density 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 5 pg/mL to 500,000 pg/mL. The cross-reactivity was calculated at 50% B/B_0.

Compound	Cross-reactivity
$PGF_{1\alpha}$	11.82%
PGD_2	3.62%
6-keto-$PGF_{1\alpha}$	1.38%
PGI_2	1.25%
PGE_2	0.77%
TXB_2	0.77%
8-iso-$PGF_{2\alpha}$	0.73%
PGE_1	0.39%
PGA_2	< 0.10%
6,15-keto-13,14-dihydro-$PGF_{1\alpha}$	< 0.01%
2-Arachidonoylglycerol	< 0.01%
Anandamide	< 0.01%

REFERENCES

1. Ramwell, P.W. (1977) Biol. Reprod. **16**:70.
2. Flower, R.J. and G.J. Blackwell (1976) Biochem. Pharmacol. **25**:285.
3. Samuelsson, B. *et al.* (1978) Annu. Rev. Biochem. **47**:997.
4. Anderson, N.H. *et al.* (1981) Prostaglandins **22**:841.
5. Fishburne, J.I. *et al.* (1972) Obstet. Gynecol. **39**:892.
6. Matthe, A.A. *et al.* (1977) New Engl. J. Med. **296**:850.
7. Stormshak, F. *et al.* (1987) Adv. Exp. Med. Biol. **219**:327.
8. Lein, D.H. *et al.* (1989) J. Reprod. Fert. Suppl. **39**:231.
9. Didolkar, A.K. and D. Roychowdhury (1980) Andrologia **12**:135.
10. Jenkin, G. (1992) J. Reprod. Fert. Suppl. **45**:97.
11. Freidman, S.A. (1988) Obstet. Gynecol. **71**:122.
12. Green, K. *et al.* (1973) Anal. Biochem. **54**:434.
13. Frolich, J. *et al.* (1975) J. Clin. Invest. **55**:763.
14. Shaw, J.E. and PW. Ramwell (1969) Meth. Biochem. Anal. **17**:325.
15. Green, K. *et al.* (1978) Adv. Prostaglandin Thromboxane Res. **5**:15.

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