

PGF2 α high sensitivity ELISA kit

Catalog #: *ADI-931-069*

480 Well Enzyme Immunoassay Kit

For use with culture supernates, saliva, urine, serum, and plasma)

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Product Manual

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Carefully note the handling and storage conditions of each kit component.

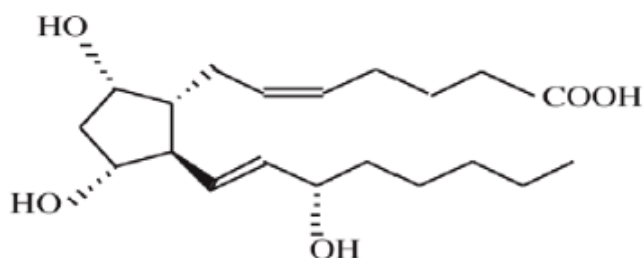
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INTRODUCTION

The PGF_{2α} high sensitivity ELISA kit is a competitive immunoassay for the quantitative determination of Prostaglandin F_{2α} in milk, culture supernates, saliva, urine, serum and plasma. Please read the complete kit insert before performing this assay.

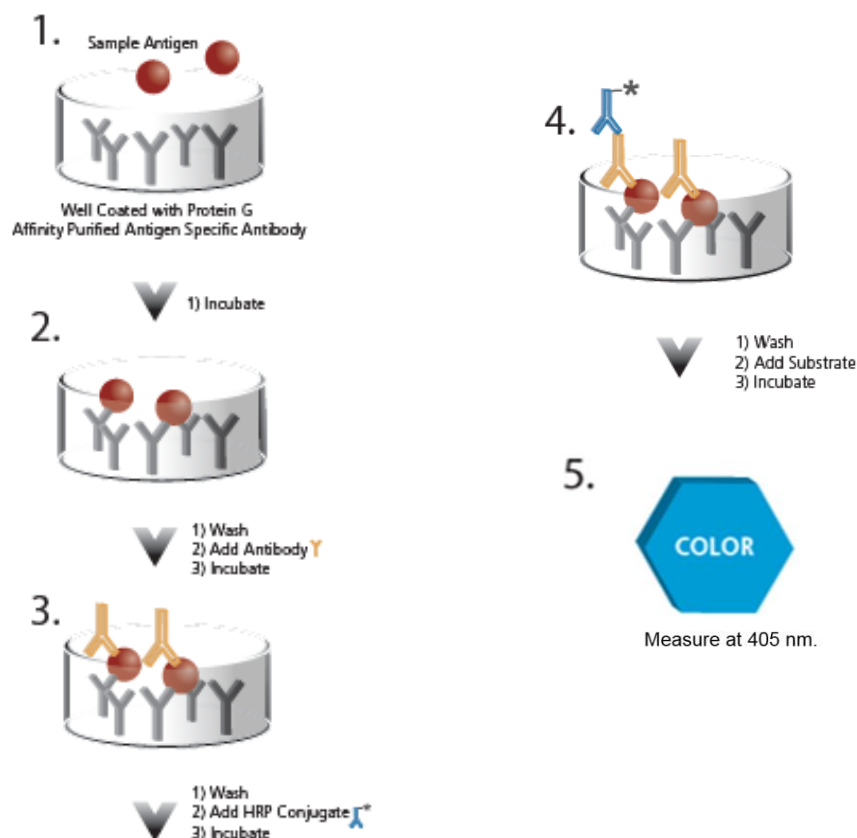
Prostaglandin F_{2α} (PGF_{2α}) is formed in a variety of cells from PGH₂, which itself is synthesized from arachidonic acid by the enzyme prostaglandin synthetase¹. PGF_{2α} is often viewed as an antagonist to PGE₂ due to their opposing effects on various tissues². PGF_{2α} is a potent bronchoconstrictor and has been implicated in asthma attacks^{3,4}. PGF_{2α} is also involved in reproductive functions including corpus luteum regulation⁵, uterine contractions⁶, and sperm motility⁷. This has led to its use in terminating pregnancies and inducing labor at term^{3,5,6,8}. High levels of PGF_{2α} have also been associated with pre-eclampsia⁹.

Prostaglandin F_{2α}



PRINCIPLE OF THE ASSAY

1. Standards and samples are added to wells coated with a DxS IgG antibody. A yellow solution of sheep polyclonal antibody to PGF_{2α} is then added, followed by a blue solution of PGF_{2α} conjugated to alkaline phosphatase.
2. During a simultaneous overnight incubation at 4°C, the antibody to PGF_{2α} binds, in a competitive manner, the PGF_{2α} in the sample or conjugate. The plate is washed, leaving only bound PGF_{2α}.
3. pNpp substrate solution is added. The substrate generates a yellow color when catalyzed by the alkaline phosphatase on the PGF_{2α} conjugate.
4. Stop solution is added. The yellow color is read at 405nm. The amount of signal is indirectly proportional to the amount of PGF_{2α} in the sample.



MATERIALS SUPPLIED

Do not mix components from different kit lots or use reagents beyond the expiration date of the kit.



The activity of conjugate is affected by concentrations of chelators > 10 mM (such as EDTA and EGTA).



The standard should be handled with care due to the known and unknown effects of the antigen.



Avoid contamination by endogenous alkaline phosphatase. Do not expose reagents or supplies to bare skin.



Stop solution is caustic. Keep tightly capped.

Donkey anti-Sheep IgG Microtiter Plate

5 Plate of 96 Wells each, Catalog No. 80-0045

A clear plate of break-apart strips coated with donkey antibody specific to sheep IgG

PGF_{2α} HS ELISA Conjugate

17 mL, Catalog No. 80-1708

A blue solution of alkaline phosphatase conjugated to PGF_{2α}

PGF_{2α} HS ELISA Antibody

17 mL, Catalog No. 80-1709

A yellow solution of sheep polyclonal antibody to PGF_{2α}

Assay Buffer Concentrate

27 mL, Catalog No. 80-0011

Tris buffered saline containing proteins and sodium azide

Wash Buffer Concentrate

100 mL, Catalog No. 80-1287

Tris buffered saline containing detergents

Prostaglandin F_{2α} Standard

3 x 0.5 mL, Catalog No. 80-0029

A solution of 500,000 pg/mL PGF_{2α}

pNpp Substrate

100 mL, Catalog No. 80-0076

A solution of p-nitrophenyl phosphate

Stop Solution

27 mL, Catalog No. 80-0248

A solution of trisodium phosphate in water.

High Sensitivity PGF_{2α} Assay Layout Sheet

1 each, Catalog No. 30-0246

Plate Sealer

5 each, Catalog No. 30-0012



Storage temp

STORAGE & STABILITY

All components of this kit are stable at 4°C until the kit's expiration date.

ADDITIONAL MATERIALS NEEDED

- *Deionized or distilled water*
- *Precision pipets for volumes between 2.5 μL and 1,000 μL*
- *Repeater pipets for dispensing 25 μL and 200 μL*
- *Disposable beakers for diluting buffer concentrates*
- *Graduated cylinders*
- *Microplate shaker*
- *Lint-free paper toweling for blotting.*
- *Microplate reader capable of reading at 405 nm.*
- *For optional extraction procedure, the following additional materials are required: 2M HCl, ethanol, hexane, ethyl acetate, and 200 mg C18 Reverse Phase Extraction columns.*



Important/ Warning

SAFETY WARNINGS & PRECAUTIONS

1. Wear appropriate personnel protective apparel. Avoid contact with clothes and exposed skin. In case of accidental skin exposure, flush with water immediately. Consult a physician if required.
2. Use a safety pipetting device for all pipetting. Never pipet by mouth.
3. Interpretation of the results is the sole responsibility of the user.

SAMPLE HANDLING

The High Sensitivity PGF_{2α} ELISA Kit is compatible with PGF_{2α} in a wide range of matrices. Samples containing sheep IgG will interfere with the assay.

Samples diluted sufficiently into Assay Buffer can be read directly from the standard curve.

Samples in the majority of culture media, including those containing fetal bovine serum, can also be read in the assay, provided the standards have been diluted into the same non-conditioned culture media instead of the assay buffer. There will be a small change in binding associated with running the standards and samples in media. For tissue, urine, and blood samples, prostaglandin synthetase inhibitors such as indomethacin or meclofenamic acid at concentrations up to 10 µg/mL should be added.



If buffers other than those provided are used in the assay, the end-user must determine the appropriate dilution and assay validation.

SAMPLE RECOVERIES

PGF_{2α} concentrations were measured in a variety of different samples including milk, tissue culture media, saliva, urine, serum, and porcine plasma. For samples in tissue culture media, ensure that the standards have been diluted into the same media. PGF_{2α} was spiked into the undiluted samples of these media, which were diluted with the kit Assay Buffer and assayed in the kit. The following results were obtained:

| Sample | % Recovery | Recommended Dilution |
|-----------------------|------------|----------------------|
| Tissue Culture Media | -- | Neat |
| Human Saliva | 103.5 | ≥ 1:20 |
| Human Urine | 107.0 | ≥ 1:20 |
| Porcine & Human Serum | 100.3 | ≥ 1:10 |
| Porcine Plasma | 96.9 | ≥ 1:10 |



Check our website for additional protocols, technical notes and FAQs.

Some samples normally have very low levels of PGF_{2α} present and extraction may be necessary for accurate measurement. A suitable extraction procedure is outlined below:

Materials Needed

1. PGF_{2α} Standard to allow extraction efficiency to be accurately determined.
2. 2M hydrochloric acid, deionized water, ethanol, hexane and ethyl acetate.
3. 200 mg C18 Reverse Phase Extraction Columns.

Extraction Procedure

1. Acidify the sample by addition of 2M HCl to pH of 3.5. Approximately 50 µL of HCl will be needed per mL of plasma. Allow to sit at 4°C for 15 minutes. Centrifuge samples in a microcentrifuge for 2 minutes to remove any precipitate.
2. Prepare the C18 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 0.5mL/ minute. Wash the column with 10 mL of water, followed by 10 mL of 15% ethanol, and finally 10 mL hexane. Elute the

sample from the column by addition of 10 mL ethyl acetate.

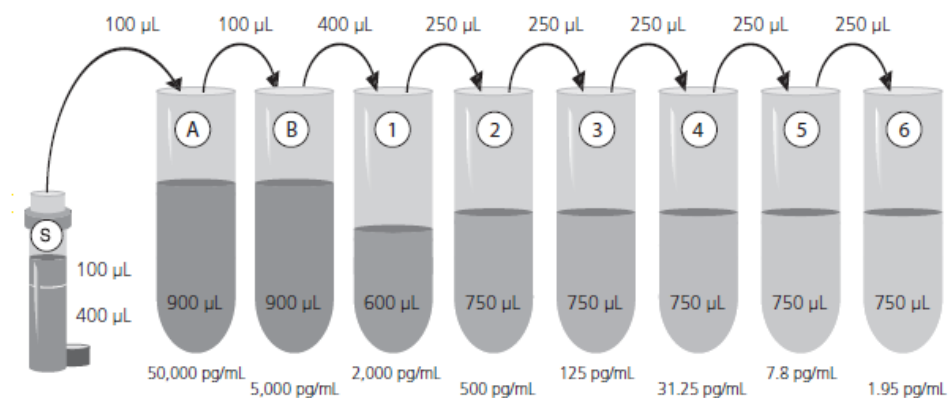
4. If analysis is to be carried out immediately, evaporate samples under a stream of nitrogen. Add at least 250 μ L of Assay Buffer to the dried samples. Vortex well then allow to sit five minutes at room temperature. Repeat vortexing twice more. If analysis is to be delayed, store samples as the eluted ethyl acetate solutions at -80 °C until the immunoassay is to be run. Evaporate the organic solvent under a stream of nitrogen prior to running assay and reconstitute as above.

Please refer to references 10-13 (see References section) for details of extraction protocols.

REAGENT PREPARATION

PGF_{2α} Standard

1. Allow the 500,000 pg/mL PGF_{2α} standard solution to warm to room temperature. Label two 12 x 75 mm glass tubes A and B, and six tubes #1 through #6. Pipet 900 μL of standard diluent (Assay Buffer or culture media) into tubes A and B. Pipet 600 μL of standard diluent into tube #1 and 750 μL of standard diluent into tube #2 through #6. Add 100 μL of the 500,000 pg/mL standard to tube A and vortex thoroughly. Add 100 μL of tube A to tube B and vortex thoroughly. Add 400 μL of tube B to tube #1 and vortex thoroughly. Add 250 μL of tube #2 to tube #3 and vortex. Continue this for tubes #4 through #6.



Diluted standards should be used within 60 minutes of preparation.

The concentrations of PGF_{2α} in the tubes are labeled above.

2. Wash Buffer

Prepare the Wash Buffer by diluting 5 mL of the supplied concentrate with 95 mL of deionized water. This can be stored at room temperature until the kit expiration date, or for 3 months, whichever is earlier.

ASSAY PROCEDURE



Bring all reagents to room temperature for at least 30 minutes prior to opening.



Standards and samples should be run in triplicate.

Pre-rinse each pipet tip with reagent. Use fresh pipet tips for each sample, standard, and reagent.



Pipet the reagents to the sides of the wells to avoid possible contamination.



Prior to the addition of substrate, ensure there is no residual wash buffer in the wells. Remaining wash buffer may cause variation in assay results.

1. Refer to the Assay Layout Sheet to determine the number of wells to be used. Remove the wells not needed for the assay and return them, with desiccant, to the mylar bag and seal. Store unused wells at 4°C.
2. Pipet 100 µL of standard diluent (Assay Buffer or Tissue Culture Media) into the NSB and the Bo (0 pg/mL Standard) wells.
3. Pipet 100 µL of Standards #1 through #6 into the appropriate wells.
4. Pipet 100 µL of the Samples into the appropriate wells.
5. Pipet 25 µL of Assay Buffer into the NSB wells.
6. Pipet 25 µL of blue Conjugate into each well, except the TA and Blank wells.
7. Pipet 25 µL of yellow Antibody into each well, except the Blank, TA and NSB wells.

NOTE: Every well used should be Green in color except the NSB wells which should be Blue. The Blank and TA wells are empty at this point and have no color.

8. Incubate the plate at room temperature on a plate shaker for 5 minutes at ~500 rpm to thoroughly mix each well. The plate should be covered with the plate sealer provided. Incubate the plate at 4°C overnight without further shaking.
9. Empty the contents of the wells and wash by adding 400 µL of wash solution to every well. Repeat the wash 2 more times for a total of 3 Washes.
10. After the final wash, empty or aspirate the wells, and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
11. Add 2.5 µL of the blue Conjugate Solution to TA wells.
12. Add 200 µL of the pNpp Substrate solution to each well. Seal the plate with the plate sealer provided and incubate at 37°C for 3 hours without shaking.
13. Add 50 µL of Stop Solution to each well.
14. After blanking the plate reader against the substrate blank, read optical density at 405 nm. If plate reader is not capable of adjusting for the blank, manually subtract the mean OD of the substrate blank from all readings.

CALCULATION OF RESULTS

Several options are available for the calculation of the concentration of PGF_{2α} in samples. We recommend that the data be handled by an immunoassay software package utilizing a 4 parameter logistic curve fitting program. If data reduction software is not readily available, the concentration of PGF_{2α} can be calculated as follows:

1. Calculate the average net Optical Density (OD) for each standard and sample by subtracting the average NSB OD from the average OD for each standard and sample.

Average Net OD = Average OD - Average NSB OD

2. Calculate the binding of each pair of standard wells as a percentage of the maximum binding wells (Bo), using the following formula:

$$\text{Percent Bound} = \frac{\text{Net OD}}{\text{Net Bo OD}} \times 100$$

3. Plot the Percent Bound versus Concentration of PGF_{2α} for the standards. Approximate a straight line through the points. The concentration of PGF_{2α} in the unknowns can be determined by interpolation.

Samples with concentrations outside of the standard curve range will need to be reanalyzed using a different dilution.



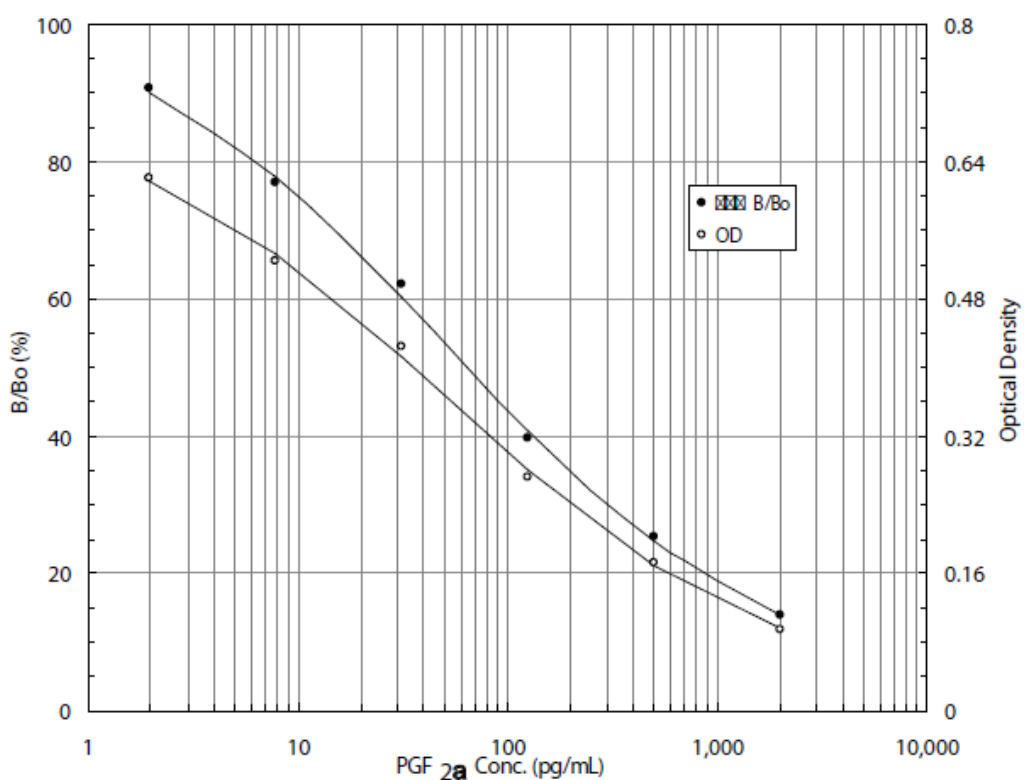
Make sure to multiply sample concentrations by the dilution factor used during sample preparation.

TYPICAL RESULTS

The results shown below are for illustration only and should not be used to calculate results from another assay.

| Sample | Average Net OD | Percent Bound | PGF2α (pg/mL) |
|-----------|----------------|---------------|---------------|
| Blank | (0.097) | --- | --- |
| TA | 0.731 | --- | --- |
| NSB (net) | 0.002 | 0% | --- |
| Bo | 0.685 | 100% | 0 |
| S1 | 0.097 | 14.2% | 2000 |
| S2 | 0.175 | 25.5% | 500 |
| S3 | 0.274 | 40.0% | 125 |
| S4 | 0.427 | 62.3% | 31.3 |
| S5 | 0.527 | 76.9% | 7.8 |
| S6 | 0.622 | 90.8% | 1.95 |
| Unknown 1 | 0.428 | 62.5% | 8.8 |
| Unknown 2 | 0.191 | 27.9% | 114.9 |

TYPICAL STANDARD CURVE



PERFORMANCE CHARACTERISTICS

Specificity

The cross reactivities for a number of related compounds were determined by diluting cross reactants in the assay buffer at a concentration of 600,000 pg/mL. These samples were then measured in the assay.

| Compound | Cross Reactivity |
|---|------------------|
| PGF _{2α} | 100% |
| PGF _{1α} | 11.82% |
| PGD ₂ | 3.62% |
| 6-keto-PGF _{1α} | 1.38% |
| PGI ₂ | 1.25% |
| PGE ₂ | 0.77% |
| Thromboxane B ₂ | 0.77% |
| 8-iso PGF _{2α} | 0.73% |
| PGE ₁ | 0.39% |
| PGA ₂ | <0.10% |
| 6, 15-keto-13, 14-dihydro-PGF _{1α} | <0.01% |
| 2-Arachidonoylglycerol | <0.01% |
| Anandamide | <0.01% |

Sensitivity

The sensitivity of the assay, defined as the concentration of PGF_{2α} measured at 2 standard deviations from the mean of 24 zeros along the standard curve, was determined to be 0.98 pg/mL.

Linearity

A buffer sample containing PGF_{2α} was serially diluted 1:2 in the assay buffer and measured in the assay. The results are shown in the table below.

| Dilution | Expected (pg.mL) | Observed (pg/mL) | Recovery (%) |
|----------|------------------|------------------|--------------|
| Neat | --- | 214.83 pg/mL | --- |
| 1:2 | 107.42 pg/mL | 107.29 pg/mL | 99.9 % |
| 1:4 | 53.71 pg/mL | 60.21 pg/mL | 112.1 % |
| 1:8 | 26.85 pg/mL | 28.87 pg/mL | 107.5 % |
| 1:16 | 13.43 pg/mL | 14.48 pg/mL | 107.8 % |
| 1:32 | 6.71 pg/mL | 5.81 pg/mL | 86.6 % |
| 1:64 | 3.36 pg/mL | 3.50 pg/mL | 104.2 % |
| 1:128 | 1.68 pg/mL | 1.51 pg/mL | 89.9 % |

Precision

Intra-assay precision was determined by assaying 24 replicates of three buffer controls containing PGF_{2α} in a single assay.

| pmol/mL | % CV |
|---------|------|
| 107.50 | 6.53 |
| 33.79 | 4.94 |
| 11.50 | 7.16 |

Inter-assay precision was determined by measuring buffer controls of various PGF_{2α} concentrations in multiple assays over several days.

| pmol/mL | % CV |
|---------|-------|
| 117.80 | 10.97 |
| 35.81 | 8.88 |
| 10.10 | 10.14 |

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