Prostaglandin F2 alpha ELISA Kit

Catalog Number KA0327

96 assays

Version: 04

Intended for research use only

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Table of Contents

Introduction

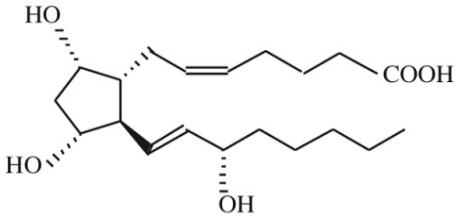
Intended Use

The High Sensitivity Prostaglandin F2α Enzyme Immunometric Assay (EIA) kit is a competitive immunoassay for the quantitative determination of Prostaglandin F2α in milk, culture supernates, saliva, urine, serum and plasma. Please read the complete kit insert before performing this assay.

Background

Prostaglandin F2α (PGF2α) is formed in a variety of cells from PGH2, which itself is synthesized from arachidonic acid by the enzyme prostaglandin synthetase[1]. PGF2α is often viewed as an antagonist to PGE2 due to their opposing effects on various tissues[2]. PGF2α is a potent bronchoconstrictor and has been implicated in asthma attacks[3,4]. PGF2α is also involved in reproductive functions including corpus luteum regulation[5], uterine contractions[6], and sperm motility[7]. This has led to its use in terminating pregnancies and inducing labor at term[3,5,6,8]. High levels of PGF2α have also been associated with pre-eclampsia[9].

Prostaglandin F2α



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 PGF2α is then added, followed by a blue solution of PGF2α conjugated to alkaline phosphatase.
2. During a simultaneous overnight incubation at 4℃, the antibody to PGF2α binds, in a competitive manner, the PGF2α in the sample or conjugate. The plate is washed, leaving only bound PGF2α.
3. pNpp substrate solution is added. The substrate generates a yellow color when catalyzed by the alkaline phosphatase on the PGF2α conjugate.
4. Stop solution is added. The yellow color is read at 405nm. The amount of signal is indirectly proportional to the amount of PGF2α in the sample.

General Information

Materials Supplied

List of component

Component	Description	Amount
Donkey anti-Sheep IgG Microtiter Plate	A clear plate of break-apart strips coated with donkey antibody specifc to sheep IgG	One Plate of 96 Wells
PGF2α HS EIA Conjugate	A blue solution of alkaline phosphatase conjugated to PGF2α	2.5 mL
PGF2α HS EIA Antibody	A yellow solution of sheep polyclonal antibody to PGF2α	2.5 mL
Assay Buffer	Tris buffered saline containing proteins and sodium azide	27 mL
Wash Buffer Concentrate	Tris buffered saline containing detergents	27 mL
Prostaglandin F2α Standard	A solution of 500,000 pg/mL PGF2α	0.5 mL
pNpp Substrate	A solution of p-nitrophenyl phosphate	20 mL
Stop Solution	A solution of trisodium phosphate in water.	5 mL
Plate Sealer	-	1 each

Storage Instruction

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

Materials Required but Not Supplied

- ✓ 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.

Precautions for Use

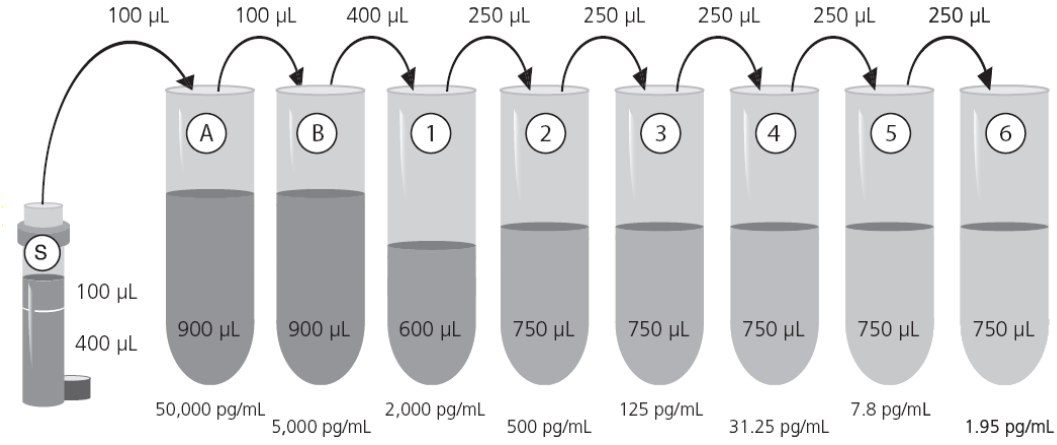
- ✓ Do not mix components from different kit lots or use reagents beyond the expiration date of the kit.
- ✓ The activity of conjugate is affected by concentrations of chelators > 10 mM (such as EDTA and EGTA).
- ✓ The standard should be handled with care due to the known and unknown effects of the antigen.
- ✓ Avoid contamination by endogenous alkaline phosphatase. Do not expose reagents or supplies to bare skin.
- ✓ Stop solution is caustic. Keep tightly capped.

Assay Protocol

Reagent Preparation

- PGF2α Standard

Allow the 500,000 pg/mL PGF2α 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 in the tubes are labeled above.

- 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.

Sample Preparation

- Sample preparation

The High Sensitivity PGF2α EIA Kit is compatible with PGF2α 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.

- Sample Recoveries

PGF2α 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. PGF2α 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

Some samples normally have very low levels of PGF2α present and extraction may be necessary for accurate measurement. A suitable extraction procedure is outlined below:

Materials Needed

1. PGF2α 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℃ 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 µL of Assay Buffer to the dried samples. Vortex well then allows sitting 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 ℃ until the immunoassay is to be run. Evaporate the organic solvent under a stream of nitrogen prior to running assay and reconstitute as above.

Assay Procedure

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

1. Remove the wells not needed for the assay and return them, with desiccant, to the mylar bag and seal. Store unused wells at 4℃.
2. Pipet 100 µL of standard diluents (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℃ 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℃ 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.

Note:

All standards and samples should be run in duplicate.

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.

Data Analysis

Calculation of Results

Several options are available for the calculation of the concentration of PGF2α 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 PGF2α 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:

   Percent Bound = Net OD / Net Bo OD x 100

3. Using Logit-Log paper plot the Percent Bound versus Concentration of PGF2α for the standards. Approximate a straight line through the points. The concentration of PGF2α 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.

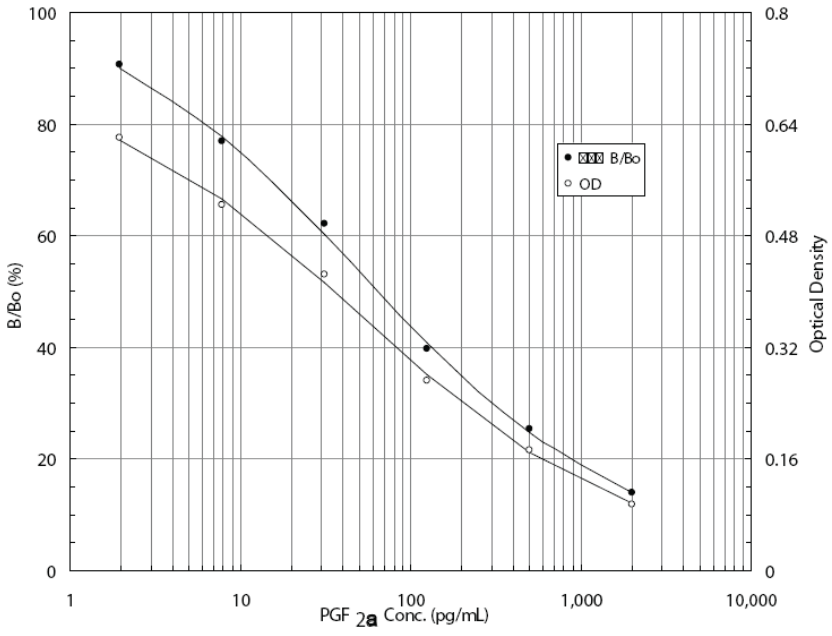
Typical Results

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

A typical standard curve is shown below. This curve must not be used to calculate PGF2α concentrations; each user must run a standard curve for each assay.



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
PGF2α	100%
PGF1α	11.82%
PGD2	3.62%
6-keto-PGF1α	1.38%
PGI2	1.25%
PGE2	0.77%
Thromboxane B2	0.77%
8-iso PGF2α	0.73%
PGE1	0.39%
PGA2	<0.10%
6, 15-keto-13, 14-dihydro-PGF1α	<0.01%
2-Arachidonoylglycerol	<0.01%
Anandamide	<0.01%

- Sensitivity

The sensitivity of the assay, defned as the concentration of PGF2α 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 PGF2α was serially diluted 1:2 in the assay buffer and measured in the assay. The results are shown in the table below.

Dilution	Expected	Observed	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 PGF2α in a single assay.

pg/mL	%CV
107.50	6.53
33.79	4.94
11.50	7.16

Intra-assay precision was determined by measuring buffer controls of various PGF2α concentrations in multiple assays over several days.

pg/mL	%CV
117.80	10.97
35.81	8.88
10.10	10.14

Resources

References

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Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Std 1	Std 5									
B	Blank	Std 1	Std 5									
C	TA	Std 2	Std 6									
D	TA	Std 2	Std 6									
E	NSB	Std 3										
F	NSB	Std 3										
G	Bo	Std 4										
H	Bo	Std 4										