$PGF_{2\alpha}$ ELISA

For the quantitative determination of Prostaglandin $F_{2\alpha}$ in Plasma, Saliva, Serum, Cell Culture and Urine.

For Research Use Only. Not For Use In Diagnostic Procedures**.**

Catalog Number: 74-PGFHU-E05
Size: 5 x 96 wells
Version: 121917 - ALPCO 1.0

Intended Use

The $PGF_{2α}$ ELISA kit is a competitive immunoassay for the quantitative determination of Prostaglandin $F_{2α}$ in cell culture, plasma, saliva, serum, and urine. For Research Use Only. Not for use in diagnostic procedures.

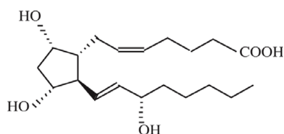
Description

The kit uses a polyclonal antibody to $PGF_{2α}$ to bind, in a competitive manner, the $PGF_{2α}$ in the sample or an alkaline phosphatase molecule which has $PGF_{2α}$ covalently attached to it. After a simultaneous incubation at room temperature the excess reagents are washed away, and substrate is added. After a short incubation time the enzyme reaction is stopped, and the yellow color generated read on a microplate reader at 405 nm. The intensity of the bound yellow color is inversely proportional to the concentration of $PGF_{2α}$ in either standards or samples. The measured optical density is used to calculate the concentration of $PGF_{2α}$. For further explanation of the principles and practice of immunoassays please see the excellent books by Chard[1] or Tijssen[2].

Introduction

Prostaglandin $F_{2α}$ ($PGF_{2α}$) is formed in a variety of cells from PGH_2, which itself is synthesized from arachidonic acid by the enzyme prostaglandin synthetase[3]. $PGF_{2α}$ is often viewed as an antagonist to PGE_2 due to their opposing effects on various tissues[4]. $PGF_{2α}$ is a potent bronchoconstrictor.[5,6]. $PGF_{2α}$ is also involved in reproductive functions[5,7-11].

Prostaglandin F2



Precautions

1. Some kit components contain azide, which may react with lead or copper plumbing. When disposing of reagents always flush with large volumes of water to prevent azide build-up.
2. Stop Solution is a solution of trisodium phosphate. This solution is caustic; care should be taken in use.
3. The activity of the alkaline phosphatase conjugate is dependent on the presence of Mg^{2+} and Zn^{2+} ions. The activity of the conjugate is affected by concentrations of chelators (>10 mM) such as EDTA and EGTA.
4. The kit's performance was tested with a variety of samples; however, it is possible that high levels of interfering substances may cause variation in assay results.
5. The Prostaglandin $F_{2α}$ Standard provided is supplied in ethanolic buffer at a pH optimized to maintain $PGF_{2α}$ integrity. Care should be taken handling this material because of the known and unknown effects of prostaglandins.

Materials Supplied

1. **Donkey anti-Sheep IgG Microtiter Plate, Five Plates of 96 Wells**
   A plate using break-apart strips coated with donkey antibody specific to sheep IgG.
2. **$PGF_{2α}$ ELISA Conjugate, 25 mL**
   A blue solution of alkaline phosphatase conjugated with $PGF_{2α}$.
3. **$PGF_{2α}$ ELISA Antibody, 25 mL**
   A yellow solution of a monoclonal antibody to $PGF_{2α}$.
4. **Assay Buffer, 27 mL**
   Tris buffered saline containing proteins and sodium azide as preservative.

5. **Wash Buffer Concentrate, 100 mL**
   Tris buffered saline containing detergents.
6. **Prostaglandin $F_{2α}$ Standard, 3 X 0.5 mL**
   A solution of 500,000 pg/mL $PGF_{2α}$.
7. **pNpp Substrate, 100 mL**
   A solution of p-nitrophenyl phosphate in buffer. Ready to use.
8. **Stop Solution, 30 mL**
   A solution of trisodium phosphate in water. Keep tightly capped. Caution: **Caustic.**
9. **$PGF_{2α}$ Assay Layout Sheet, 1 each**
10. **Plate Sealers, 5 each**

Storage

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

Materials Needed but Not Supplied

1. Deionized or distilled water
2. Precision pipets for volumes between 5 µL and 1,000 µL
3. Repeater pipets for dispensing 50 µL and 200 µL
4. Disposable beaker for diluting buffer concentrates
5. Graduated cylinders
6. A microplate shaker
7. Absorbent paper for blotting
8. Microplate reader able to read at 405 nm, preferably with correction between 570 and 590 nm

Sample Handling

The $PGF_{2α}$ ELISA kit is compatible with $PGF_{2α}$ samples in a wide range of matrices. Sample diluted sufficiently into Assay Buffer can be read directly from the standard curve. Please refer to the Sample Recovery recommendations on page 8 for details of suggested dilutions. However, the end user **must verify** that the recommended dilutions are appropriate for their samples. **Samples containing sheep IgG may interfere with the assay.**

Samples in most tissue culture media, including those containing fetal bovine serum, can also be read in the assay, provided the standards have been diluted into the tissue culture media instead of Assay Buffer. There will be a small change in binding associated with running the standards and samples in media. Users should only use standard curves generated in media or buffer to calculate concentrations of $PGF_{2α}$ in the appropriate matrix. For tissue, urine and plasma samples, prostaglandin synthetase inhibitors such as indomethacin or meclofenamic acid at concentrations up to 10 µg/mL should be added to either the tissue homogenate or urine and plasma samples.

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 C_{18} Reverse Phase Extraction Columns

Procedure

1. Acidify the plasma, urine, or tissue homogenate 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 C_{18} reverse phase column by washing with 10mL of ethanol followed by 10mL 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 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 sample. Vortex well, then allow to sit for five minutes at room temperature. Repeat 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 12-15 for details of extraction protocols.

Procedural Notes

1. Do not mix components from different kit lots or use reagents beyond the kit expiration date.
2. Allow all reagents to warm to room temperature for at least 30 minutes before opening.
3. Standards can be made up in either glass or plastic tubes.
4. Pre-rinse the pipet tip with the reagent, use fresh pipet tips for each sample, standard and reagent.
5. Pipet standards and samples to the bottom of the wells.
6. Add the reagents to the side of the well to avoid contamination.
7. This kit uses break-apart microtiter strips, which allow the user to measure as many samples as desired. Unused wells must be kept desiccated at 4°C in the sealed bag provided. The wells should be used in the frame provided.
8. **Care must be taken to minimize contamination by endogenous alkaline phosphatase.** Contaminating alkaline phosphatase activity, especially in the substrate solution, may lead to high blanks. Care should be taken not to touch pipet tips and other items that are used in the assay with bare hands.
9. **Prior to addition of substrate, ensure that there is no residual wash buffer in the wells. Any remaining wash buffer may cause variation in assay results.**

Reagent Preparation

1. **Assay Buffer**

   Just before use, prepare the Assay Buffer by diluting 10 mL of the supplied concentrate with 90 mL of deionized water. Discard unused buffer or add up to 0.09% sodium azide (w/v) for storage.

2. **$PGF_{2\alpha}$ Standard**

   Allow the 500,000 pg/mL $PGF_{2\alpha}$ standard solution to warm to room temperature. Label eight 12 x 75 mm glass tubes #1 through #8. Pipet 900 µL of standard diluent (Assay Buffer or Tissue Culture Media) into tube #1. Pipet 750 µL of standard diluent into tubes #2 through #8. Add 100 µL of the 500,000 pg/mL standard to tube #1. Vortex thoroughly. Add 250 µL of tube #1 to tube #2 and vortex thoroughly. Add 250 µL of tube #2 to tube #3 and vortex thoroughly. Continue this for tubes #4 through #8.

   The concentration of $PGF_{2\alpha}$ in tubes #1 through #8 will be 50,000, 12,500, 3,215, 781.25, 195.31, 48.83, 12.2, and 3.05 pg/mL, respectively. See $PGF_{2\alpha}$ Assay Layout Sheet for dilution details. STORE STANDARD AT -20°C, avoid repeated freeze-thaws.

   Diluted standards should be used within 60 minutes of preparation.

3. **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.
All standards and samples should be run in duplicate.

1. Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells with the desiccant back into the pouch and seal the ziplock. 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 #8 into the appropriate wells.
4. Pipet 100 µL of the Samples into the appropriate wells.
5. Pipet 50 µL of Assay Buffer into the NSB wells.
6. Pipet 50 µL of blue Conjugate into each well, except the Total Activity (TA) and Blank wells.
7. Pipet 50 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 2 hours at ~500 rpm. The plate may be covered with the plate sealer provided, if so desired.
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 5 µL of the blue Conjugate to the TA wells.
12. Add 200 µL of the pNpp Substrate solution to every well. Incubate at room temperature for 45 minutes without shaking.
13. Add 50 µL of Stop Solution to all wells to stop the reaction. The plate should be read immediately.
14. Blank the plate reader against the Blank wells, read the optical density at 405 nm, preferably with correction between 570 and 590 nm. If the plate reader is not able to be blanked against the Blank wells, manually subtract the mean optical density of the Blank wells from all readings.

Calculation of Results

Several options are available for the calculation of the concentration of $PGF_{2\alpha}$ in the samples. It is recommended that the data be handled by an immunoassay software package utilizing a 4parameter logistic curve fitting program.

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

   Average Net OD = Average Bound 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 both the Percent Bound and the Net OD versus Concentration of $PGF_{2\alpha}$ for the standards. Sample concentrations may be calculated off Net OD values using the desired curve fitting.

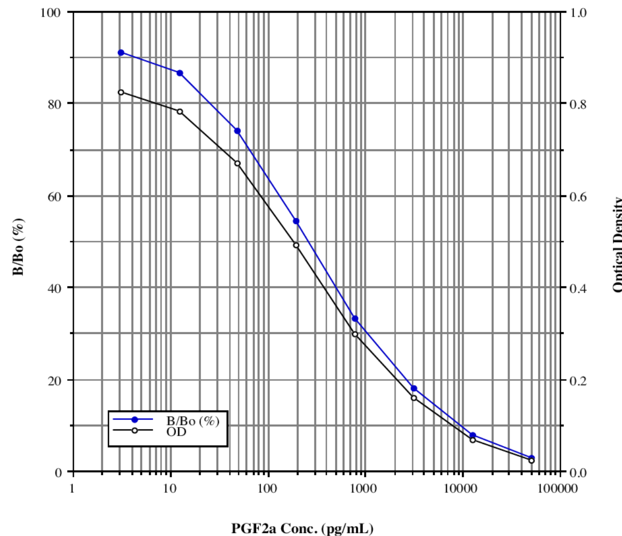
Typical Results

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

Sample	Mean OD (-Blank)	Average Net OD	Percent Bound	Percent (pg/mL)
Blank OD	(0.071)			
TA	1.021	1.021		
NSB	0.008	0.000	0.00%	
Bo	0.689	0.681	100%	**0**
S1	0.020	0.012	1.8%	**50,000**
S2	0.050	0.042	6.2%	**12,500**
S3	0.121	0.113	16.6%	**3,125**
S4	0.220	0.212	31.1%	**781**
S5	0.347	0.339	49.8%	**195**
S6	0.516	0.508	74.6%	**48.8**
S7	0.642	0.634	93.1%	**12.2**
S8	0.680	0.672	98.7%	**3.05**
Unknown 1	0.507	0.499	73.3%	**58**
Unknown 2	0.110	0.102	15.0%	**2,717**

Typical Standard Curve

A typical standard curve is shown below. This curve **must not** be used to calculate $PGF_{2\alpha}$ concentrations; each user must run a standard curve for each assay.



Typical Quality Control Parameters

Total Activity Added	=	1.021 x 10 = 10.21
%NSB	=	0.08%
%Bo/TA	=	6.70%
Quality of Fit (curve fit)	=	0.9999 (Calculated from 4 parameter logistic fit)
20% Intercept	=	2,041 pg/mL
50% Intercept	=	230 pg/mL
80% Intercept	=	33 pg/mL

Performance Characteristics

The following parameters for this kit were determined using the guidelines listed in the National Committee for Clinical Laboratory Standards (NCCLS) Evaluation Protocols[19].

Sensitivity

Sensitivity was calculated by determining the average optical density bound for sixteen (16) wells run as B_o and comparing to the average optical density for sixteen (16) wells run with Standard #8. The detection limit was determined as the concentration of $PGF_{2\alpha}$ measured at two (2) standard deviations from the zero along the standard curve.

Average Optical Density for the Bo =	0.843 ± 0.022 (2.60%)
Average Optical Density for Standard #8 =	0.823 ± 0.018 (2.18%)
Delta Optical Density (0-3.05 pg/mL) =	0.020
2 SD's of the Zero Standard = 2 x 0.022 =	0.044

Sensitivity = $\frac{0.044}{0.020}$ x 3.05 pg/mL = **6.71 pg/mL**

Linearity

A sample containing 10,000 pg/mL $PGF_{2\alpha}$ was diluted serially 4 times 1:10 in the kit Assay Buffer and measured in the assay. The data was plotted graphically as actual $PGF_{2\alpha}$

concentration versus measured $PGF_{2\alpha}$ concentration. The line obtained had a slope of 1.069 and a correlation coefficient of 0.999.

Precision

Intra-assay precision was determined by taking samples containing low, medium, and high concentrations of $PGF_{2\alpha}$ and running these samples multiple times (n=10) in the same assay. Inter-assay precision was determined by measuring three samples with low, medium, and high concentrations of $PGF_{2\alpha}$ in multiple assays (n=8).

The precision numbers listed below represent the percent coefficient of variation for the concentrations of $PGF_{2\alpha}$ determined in these assays as calculated by a 4-parameter logistic curve fitting program

	$PGF_{2\alpha}$ (pg/mL)	Intra-Assay % CV	Inter-Assay % CV
Low	83	13.1	
Medium	405	6.8	
High	916	4.9	
Low	47		9.7
Medium	290		5.5
High	731		3.1

Cross Reactivities

The cross reactivities for several related eicosanoid compounds was determined by dissolving the cross reactant (purity checked by N.M.R. and other analytical methods) in Assay Buffer at concentrations from 500,000 to 5 pg/mL. These samples were then measured in the $PGF_{2\alpha}$ assay, and the measured $PGF_{2\alpha}$ concentration at 50% B/Bo calculated. The % cross reactivity was calculated by comparison with the actual concentration of cross reactant in the sample and expressed as a percentage.

Compound	Cross Reactivity
$PGF_{2\alpha}$	100%
$PGF_{1\alpha}$	11.82%
PGD_2	3.62%
6-keto-$PGF_{1\alpha}$	1.38%
PGI_2	1.25%
PGE_2	0.77%
Thromboxane B_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%

Sample Recoveries
Please refer to pages 3 and 4 for Sample Handling recommendations and Standard preparation.

$PGF_{2\alpha}$ concentrations were measured in a variety of different samples including tissue culture media, human saliva, serum, urine, as well as porcine serum and plasma. $PGF_{2\alpha}$ was spiked into the undiluted samples of these matrices which were then 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
Human and Porcine Serum	100.3	≥1:10
Porcine Plasma	96.9	≥1:10

* See Sample Handling instructions on page 3 and 4 for details.

References

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