ab133041 – PGF_2 alpha ELISA Kit

Instructions for Use

For quantitative detection of PGF_2 alpha in Porcine Plasma, Porcine and Human Serum, Saliva, Urine and Tissue Culture Media

This product is for research use only and is not intended for diagnostic use.

Version 4 Last Updated 6 August 2019

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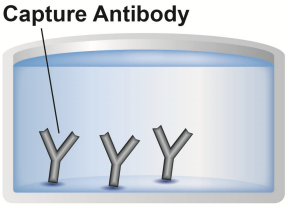
1. BACKGROUND

Abcam's PGF_2 alpha *in vitro* competitive ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of PGF_2 alpha in Porcine Plasma, Porcine and Human Serum, Saliva, Urine and Tissue Culture Media.

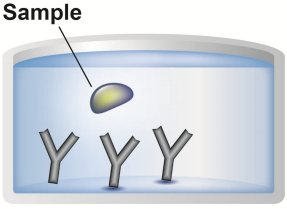
A donkey anti-sheep IgG antibody has been precoated onto 96-well plates. Standards or test samples are added to the wells, along with an alkaline phosphatase (AP) conjugated-PGF_2 alpha antigen and a polyclonal sheep antibody specific to PGF_2 alpha. After incubation the excess reagents are washed away. pNpp substrate is added and after a short incubation the enzyme reaction is stopped and the yellow color generated is read at 405 nm. The intensity of the yellow coloration is inversely proportional to the amount of PGF_2 alpha captured in the plate.

Prostaglandin F_2 alpha (PGF_2 alpha) is formed in a variety of cells from PGH_2, which itself is synthesized from arachidonic acid by the enzyme prostaglandin synthetase. PGF_2 alpha is often viewed as an antagonist to PGF_2 alpha due to their opposing effects on various tissues. PGF_2 alpha is a potent bronchoconstrictor and has been implicated in asthma attacks. PGF_2 alpha is also involved in reproductive functions including corpus luteum regulation, uterine contractions, and sperm motility. This has led to its use in terminating pregnancies and inducing labor at term. High levels of PGF_2 alpha have also been associated with pre-eclampsia.

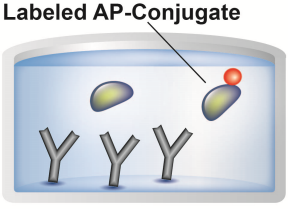
2. ASSAY SUMMARY



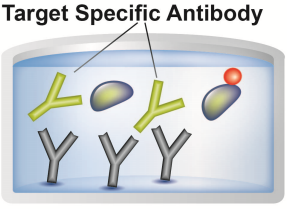
Prepare all reagents and samples as instructed.



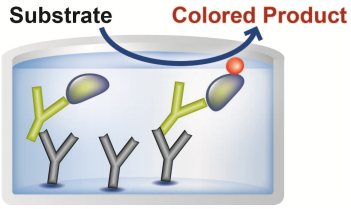
Add standards and samples to appropriate wells.



Add prepared labeled AP-conjugate to appropriate wells.



Add PGF_2 alpha antibody to appropriate wells. Incubate at room temperature.



Add pNpp substrate to each well. Incubate at room temperature. Add Stop Solution to each well. Read immediately.

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

- 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
- Stop Solution is a solution of trisodium phosphate. This solution is caustic; care should be taken in use
- 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
- We test this kit's performance with a variety of samples, however it is possible that high levels of interfering substances may cause variation in assay results
- The $PGF_{2\alpha}$ alpha Standard provided is supplied in ethanolic buffer at a pH optimized to maintain $PGF_{2\alpha}$ alpha integrity. Care should be taken handling this material because of the known and unknown effects of prostaglandin

4. STORAGE AND STABILITY

Store kit at +4°C immediately upon receipt. Avoid multiple freeze-thaw cycles.

Refer to list of materials supplied for storage conditions of individual components.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition
Donkey anti-sheep IgG Microplate (12 x 8 wells)	96 Wells	+4ºC
PGF_2 alpha Alkaline Phosphatase Conjugate	5 mL	+4ºC
PGF_2 alpha Antibody	5 mL	+4ºC
PGF_2 alpha Standard	500 µL	+4ºC
Assay Buffer	30 mL	+4ºC
20X Wash Buffer Concentrate	30 mL	+4ºC
pNpp Substrate	20 mL	+4ºC
Stop Solution	5 mL	+4ºC

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Standard microplate reader - capable of reading at 405 nm, preferably with correction between 570 and 590 nm
- Automated plate washer (optional)
- Adjustable pipettes and pipette tips. Multichannel pipettes are recommended when large sample sets are being analyzed
- Eppendorf tubes
- Microplate Shaker
- Absorbent paper for blotting
- 200 mg C18 Reverse Phase Extraction Columns (only required for extraction of samples containing low levels of PGF_2 alpha)
- 2M hydrochloric acid (only required for extraction of samples containing low levels of PGF_2 alpha)
- Deionized water
- Ethanol
- Hexane
- Ethyl acetate

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted

8. TECHNICAL HINTS

- Standards can be made up in either glass or plastic tubes
- Pre-rinse the pipette tip with the reagent, use fresh pipette tips for each sample, standard and reagent
- Pipette standards and samples to the bottom of the wells
- Add the reagents to the side of the well to avoid contamination
- 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
- 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
- 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
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions**

9. REAGENT PREPARATION

Equilibrate all reagents and samples to room temperature (18 - 25°C) prior to use.

9.1 PGF_2 alpha Alkaline Phosphatase Conjugate

Allow the PGF_2 alpha Alkaline Phosphatase Conjugate to equilibrate to room temperature. Any unused conjugate should be aliquoted and re-frozen at or below -20°C.

9.2 1X Wash Buffer

Prepare the 1X Wash Buffer by diluting 5 mL of the 20X Wash Buffer Concentrate in 95 mL of deionized water. Mix thoroughly and gently.

10. STANDARD PREPARATIONS

Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use. Diluted standards should be used within 60 minutes of preparation.

10.1 Allow the 500,000 pg/mL PGF_2 alpha **Stock Standard** solution to equilibrate to room temperature. The standard solution should be stored at -20°C. Avoid repeated freeze-thaw cycles.

10.2 Label 8 tubes #1 – #8.

10.3 Add 900 µL of appropriate diluent (either Assay Buffer or Tissue Culture Media) to tube #1.

10.4 Add 750 µL of appropriate diluent to tube #2 - #8.

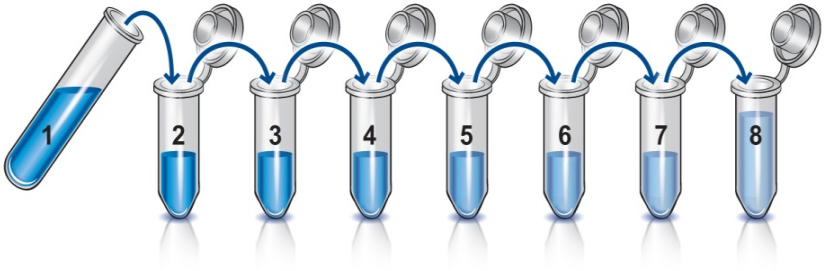
10.5 Prepare a 50,000 pg/mL **Standard 1** by adding 100 µL of the 500,000 pg/mL Stock Standard to tube #1. Mix thoroughly and gently.

10.6 Prepare **Standard 2** by transferring 250 µL from tube #1 to tube #2. Mix thoroughly and gently.

10.7 Prepare **Standard 3** by transferring 250 µL from tube #2 to tube #3. Mix thoroughly and gently.

10.8 Using the table below as a guide, repeat for tubes #4 through #8.

Standard	Sample to Dilute	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	Standard	100	900	500,000	50,000
2	Standard 1	250	750	50,000	12,500
3	Standard 2	250	750	12,500	3,125
4	Standard 3	250	750	3,125	781.25
5	Standard 4	250	750	781.25	195.31
6	Standard 5	250	750	195.31	48.83
7	Standard 6	250	750	48.83	12.2
8	Standard 7	250	750	12.2	3.05



11. SAMPLE COLLECTION AND STORAGE

- The PGF_2 alpha kit is compatible with PGF_2 alpha samples in a wide range of matrices after dilution in Assay Buffer. However, the end user must verify that the recommended dilutions are appropriate for their samples. Samples containing mouse IgG may interfere with the assay.
- Samples in the majority of 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.
- 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_2 alpha present and extraction may be necessary for accurate measurement. A suitable extraction procedure is outlined below:

11.1. **Materials Needed**

11.1.1. PGF_2 alpha Standard to allow extraction efficiency to be accurately determined.

11.1.2. 2M hydrochloric acid, deionized water, ethanol, hexane and ethyl acetate.

11.1.3. 200 mg C18 Reverse Phase Extraction Columns.

11.2. **Procedure**.

11.2.1. 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.

11.2.2. Prepare the C18 reverse phase column by washing with 10 mL of ethanol followed by 10 mL of deionized water.

11.2.3. 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.

11.2.4. 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 allow to sit 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

12. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused well strips should be returned to the plate packet and stored at 4°C.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Well effects have not been observed with this assay.

Recommended plate layout

	1	**2**	**3**	**4**
A	B_s	Std 1	Std 5	Sample 1
B	B_s	Std 1	Std 5	Sample 1
C	TA	Std 2	Std 6	Sample 2
D	TA	Std 2	Std 6	Sample 2
E	NSB	Std 3	Std 7	etc
F	NSB	Std 3	Std 7	etc
G	B_0	Std 4	Std 8	
H	B_0	Std 4	Std 8	

Plate layout shows controls, blanks and standards required for each assay. Use additional strips of wells to assay all your samples.

Key:

B_s = Blank; contains substrate only.

TA = Total Activity; contains conjugate (5 µL) and substrate.

NSB = Non-specific binding; contains assay buffer, conjugate and substrate.

B_0 = 0 pg/mL standard; contains assay buffer, conjugate, antibody and substrate.

13. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature prior to use**
- **It is recommended to assay all standards, controls and samples in duplicate**
- **Refer to the recommended plate layout in Section 12 before proceeding with the assay.**

13.1 Add 100 µL appropriate diluent* into the NSB (non-specific binding) wells. (*Use the same diluent used to prepare standards in section 10, either Assay Buffer or Tissue Culture Media).

13.2 Add 100 µL appropriate diluent (Assay Buffer or tissue culture media) into the B_0 (0 pg/mL standard) wells.

13.3 Add 100 µL of prepared standards and 100 µL diluted samples to appropriate wells.

13.4 Add 50 µL of Assay Buffer into the NSB wells.

13.5 Add 50 µL of PGF_2 alpha Alkaline Phosphatase Conjugate (blue) into NSB, B_0, standard and sample wells, i.e. not TA (Total Activity) and B_s (blank) wells.

13.6 Add 50 µL of PGF_2 alpha Antibody (yellow) into B_0, standard and sample wells, i.e. not B_s, TA and NSB wells.

Note: Every well used should be green except the NSB wells which should be blue. The B_s and TA wells are empty at this point and have no color.

13.7 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.

13.8 Empty the contents of the wells and wash by adding 400 µL of 1X Wash Buffer to every well. Repeat the wash 2 more times for a total of 3 washes. 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.

13.9 Add 5 µL of the $PGF_{2\alpha}$ Alkaline Phosphatase Conjugate (blue) to the TA wells.

13.10 Add 200 µL of the pNpp Substrate solution to every well. Incubate at room temperature for 45 minutes without shaking.

13.11 Add 50 µL Stop Solution into each well. The plate should be read immediately.

13.12 After blanking the plate reader against the B_s (blank) wells, read 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 B_s wells, manually subtract the mean optical density of the blank wells from all readings.

14. CALCULATIONS

14.1 Calculate the average net absorbance measurement (Average Net OD) for each standard and sample by subtracting the average NSB absorbance measurement from the average absorbance measurement (Average OD) for each standard and sample.

Average Net OD = Average Bound OD - Average NSB OD

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

$$\text{Percent Bound} = \frac{\text{Average Net OD}}{\text{Average Net } B_0 \text{ OD}} \times 100$$

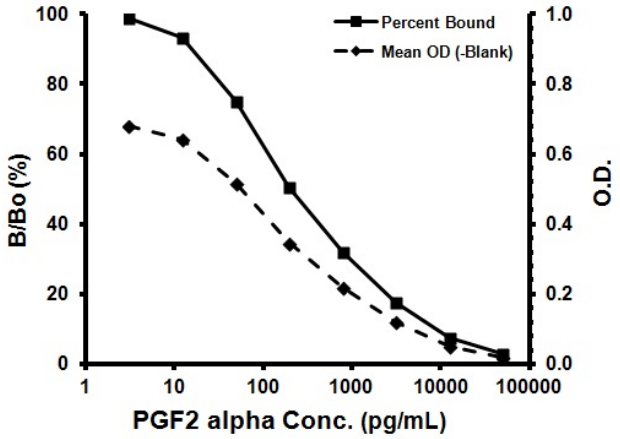
14.3 Plot the Percent Bound (B/B_0) and the net OD versus concentration of $PGF_{2\alpha}$ for the standards. The concentration of $PGF_{2\alpha}$ in the unknowns can be determined by interpolation of net OD values.

A four parameter algorithm (4PL) provides the best fit, though other equations can be examined to see which provides the most accurate (e.g. linear, semi-log, log/log, 4 parameter logistic). Interpolate protein concentrations for unknown samples from the standard curve plotted.

Samples producing signals greater than that of the highest standard should be further diluted and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.

15. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.



Sample	Mean OD ($-B_s$)	% Bound	PGF_2 alpha pg/mL
B_s	(0.071)		
TA	1.021		
NSB	0.008	0	
Standard 1	0.020	2.9	50,000
Standard 2	0.050	7.4	12,500
Standard 3	0.121	17.7	3,125
Standard 4	0.220	32.1	781
Standard 5	0.347	50.5	195
Standard 6	0.516	74.9	48.8
Standard 7	0.642	93.2	12.2
Standard 8	0.680	98.7	3.05
B_O	0.689	100	0
Unknown1	0.507	73.6	182
Unknown 2	0.110	16.0	5,852

Typical Quality Control Parameters

Total Activity Added	= 1.021 x 10 = 10.21
$\%B_0/TA$	= 6.70%
Quality of Fit	= 0.9999 (Calculated from 4 parameter logistic curve fit)
20% Intercept	= 2,041 pg/mL
50% Intercept	= 230 pg/mL
80% Intercept	= 33 pg/mL

16. TYPICAL SAMPLE VALUES

SENSITIVITY –

Sensitivity was calculated by determining the average optical density bound for sixteen (16) wells run as B_0, 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 and was determined to be 6.71 pg/mL.

SAMPLE RECOVERY –

Recovery was determined by $PGF_{2\alpha}$ into tissue culture media, Human saliva, serum, and urine. Mean recoveries are as follows:

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

LINEARITY OF DILUTION –

A sample containing 10,000 pg/mL $PGF_{2\alpha}$ was diluted 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 two samples with low 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
Low	83	13.1
Medium	405	6.8
High	916	4.9

	$PGF_{2\alpha}$ (pg/mL)	Inter-Assay %CV
Low	47	9.7
Medium	290	5.5
High	731	3.1

17. ASSAY SPECIFICITY

The cross reaction of the antibody calculated at 50% is:

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 B2	0.77
8-iso PGF_2 alpha	0.73
PGE_1	0.39
PGA_2	<0.10
6,15-keto-13,14-dihydro- PGF_2 alpha	<0.01
2-Arachidonoylglycerol	<0.01
Anandamide	<0.01

18. TROUBLESHOOTING

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standards dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Samples give higher value than the highest standard	Starting sample concentration is too high.	Dilute the specimens and repeat the assay
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the kit	Store the all components as directed.

19. NOTES

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