ab133056 – $PGF_{2\alpha}$ High Sensitivity ELISA Kit

Instructions for Use

For quantitative detection of $PGF_{2\alpha}$ High Sensitivity in milk, culture supernatants, saliva, urine, serum and plasma.

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

Version 6b Last Updated 15 January 2019

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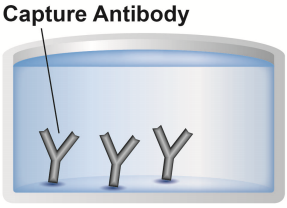
1. BACKGROUND

Abcam's $PGF_{2\alpha}$ High Sensitivity *in vitro* competitive ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of $PGF_{2\alpha}$ in milk, culture supernatants, saliva, urine, serum and plasma.

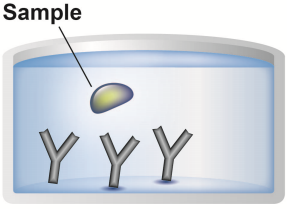
A donkey anti-sheep IgG antibody has been precoated onto 96-well plates. Standards or test samples are added to the wells, along with an alkaline phosphatase (AP) conjugated-$PGF_{2\alpha}$ antigen and a polyclonal sheep antibody specific to $PGF_{2\alpha}$. After incubation the excess reagents are washed 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.

$PGF_{2\alpha}$ 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 PGE_2 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.

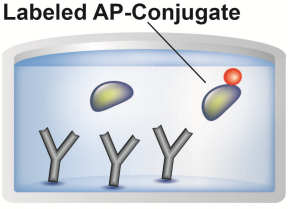
2. ASSAY SUMMARY



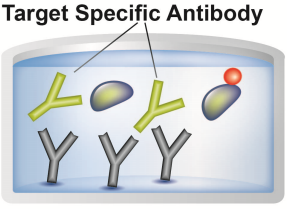
Prepare all reagents and samples as instructed.



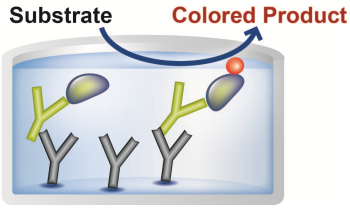
Add standards and samples to appropriate wells.



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.

- 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

4. STORAGE AND STABILITY

Store all components at 4°C immediately upon receipt.

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

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
Donkey anti-sheep IgG Microplate (12 x 8 wells)	96 Wells	+4ºC
$PGF_{2\alpha}$ Alkaline Phosphatase Conjugate	2.5 mL	+4ºC
$PGF_{2\alpha}$ Antibody	2.5 mL	+4ºC
$PGF_{2\alpha}$ Standard	500 µL	+4ºC
20X Wash Buffer Concentrate	27 mL	+4ºC
pNpp Substrate	20 mL	+4ºC
Stop Solution	5 mL	+4ºC
Assay Buffer	27 mL	+4ºC
Plate Sealer	1 Unit	+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
- Deionized water
- Absorbent paper for blotting
- Washing buffer, (see Section 9 for recipes)
- 2M hydrochloric acid (only required for extraction of samples containing low levels of $PGF_{2\alpha}$)
- Ethanol
- Hexane
- Ethyl Acetate
- 200 mg C18 Reverse Phase Extraction Columns (only required for extraction of samples containing low levels of $PGF_{2\alpha}$)

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 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. This can be stored at room temperature until the kit expiration date, or for 3 months, whichever is earlier.

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 reconstituted 500,000 pg/mL $PGF_{2\alpha}$ **Stock Standard** solution to equilibrate to room temperature.

10.2 Label two tubes A and B, six tubes #1 through #6.

10.3 Add 900 µL of standard diluent (Assay Buffer or culture media) into tubes A and B.

10.4 Add 600 µL of standard diluent (Assay Buffer or culture media) into tube #1

10.5 Add 750 µL of standard diluent (Assay Buffer or culture media) into tubes #2 - #6.

10.6 Transfer 100 µL of the 500,000 pg/mL standard to tube A. Vortex thoroughly.

10.7 Transfer 100 µL of tube A to tube B and Vortex thoroughly.

10.8 Prepare **Standard 1** by transferring 400 µL of tube B to tube #1 and Vortex thoroughly.

10.9 Prepare **Standard 2** by transferring 250 µL of tube #1 to tube #2 and Vortex.

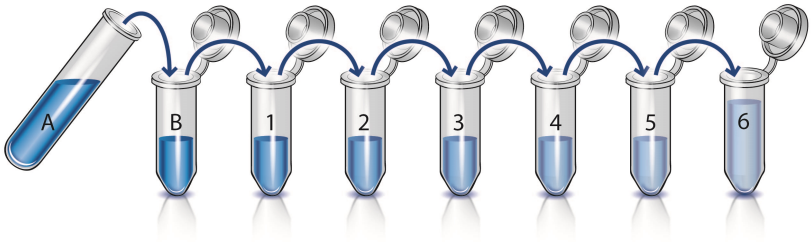
10.10 Using the table below as a guide repeat for tubes #3 through #6.

10.11 Prepare **Standard 2** by transferring 250 µL from Standard 1 to tube 2.

10.12 Using the table below as a guide, repeat for tubes 3 through to 6.

10.13 B_0 contains no protein and is the Blank Activity control

Standard	Sample to Dilute	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
A	Stock	100	900	500,000	50,000
B	Tube A	100	900	50,000	5,000
1	Tube B	400	600	5,000	2,000
2	Standard 1	250	750	2,000	500
3	Standard 2	250	750	500	125
4	Standard 3	250	750	125	31.25
5	Standard 4	250	750	31.25	7.8
6	Standard 5	250	750	7.8	1.95



11. SAMPLE COLLECTION AND STORAGE

- The $PGF_{2\alpha}$ High Sensitivity 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 sheep 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. 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 micro-centrifuge for 2 minutes to remove any precipitate.

  11.2. Prepare the C18 reverse phase column (see Section 6) by washing with 10mL of ethanol followed by 10mL of deionized water.

  11.3. 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.

11.4. 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. Mix well and hold at room temperature for 5 minutes. 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

- Unused well strips should be returned to the plate packet and stored at +4°C
- For each assay performed, a minimum of 2 wells must be used as blanks, omitting primary antibody from well additions
- 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. Contents of each well can be recorded on the template sheet included in the Resources section

	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 standard diluent, assay buffer, conjugate and substrate.

B_0 = 0 pg/mL standard; contains standard diluent, 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 25 µL of the $PGF_{2\alpha}$–AP conjugate (blue) into NSB, B_0, standard and sample wells, i.e. not the Total Activity (TA) and B_s wells.

13.5 Add 25 µL of the $PGF_{2\alpha}$ 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 in color except the NSB wells which should be blue. The B_s and TA wells which are empty at this point and have no color.

13.6 Incubate the plate at room temperature on a plate shaker at ~500 rpm for 5 minutes. The plate should be covered with the plate sealer provided. Incubate the plate at 4°C overnight without further shaking.

13.7 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.

13.8 Add 2.5 µL of the PGF_2-AP Conjugate to the TA wells.

13.9 Add 200 µL of the pNpp Substrate solution to every well. Incubate at 37°C for 3 hours without shaking.

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

13.11 Read the O.D. absorbance at 405 nm, preferably with correction between 570 and 590 nm.

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

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

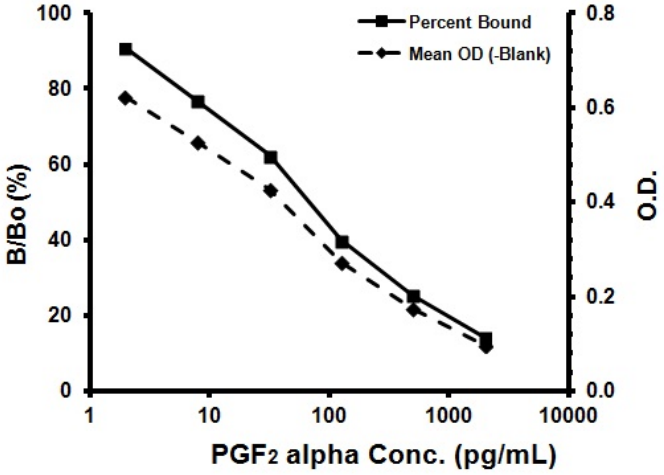
14.3 Plot the Percent Bound (B/B_0) and the net OD versus concentration of PGF_2 for the standards. The concentration of PGF_2 in the unknowns can be determined by interpolation of net OD values.

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	(0.097)	-	-
TA	0.731	-	-
NSB (net)	0.002	0	-
Standard 1	0.097	14.2	2,000
Standard 2	0.175	25.5	500
Standard 3	0.274	40.0	125
Standard 4	0.427	62.3	31.3
Standard 5	0.527	76.9	7.8
Standard 6	0.622	90.8	1.95
B_o	0.685	100	0
Unkonwn1	0.428	62.5	8.8
Unknown 2	0.191	27.9	114.9

16. TYPICAL SAMPLE VALUES

SENSITIVITY –

The sensitivity of the assay, defined 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.

SAMPLE RECOVERY –

Recovery was determined by $PGF_{2\alpha}$ into tissue culture media, Human saliva, serum, urine and Porcine Plasma. 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

PRECISION –

Intra-Assay

	$PGF_{2\alpha}$(pg/mL)	%CV
Low	11.50	7.16
Medium	33.79	4.94
High	107.50	6.53

Inter-Assay

	$PGF_{2\alpha}$ (pg/mL)	%CV
Low	10.10	10.14
Medium	35.81	8.88
High	117.80	10.97

LINEARITY OF DILUTION –

A buffer sample containing $PGF_{2\alpha}$ was serially diluted 1:2 in the kit 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	-
1:2	107.42	107.29	99.9
1:4	53.71	60.21	112.1
1:8	26.85	28.87	107.5
1:16	13.43	14.48	107.8
1:32	6.71	5.81	86.6
1:64	3.36	3.50	104.2
1:128	1.68	1.51	89.9

17. ASSAY SPECIFICITY

CROSS REACTIVITY –

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 (%)
$PGF_{2\alpha}$	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 $PGF2_{\,\alpha}$	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 %

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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