# ab133023 – 6-keto-$PGF_1$ alpha ELISA Kit

Instructions for Use

For quantitative detection of 6-keto-$PGF_1$ alpha in serum, urine, tissue culture media and other biological fluids.

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

Version 5a Last Updated 11 January 2019

# Table of Contents

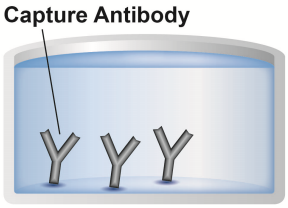
## 1. BACKGROUND

Abcam's 6-keto-$PGF_1$ alpha *in vitro* competitive ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of 6-keto-$PGF_1$ alpha in serum, urine, tissue culture media and other biological fluids.

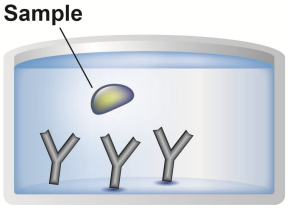
A donkey anti-sheep IgG antibody has been precoated onto 96-well plates. Standards or test samples are added to the wells, along with an alkaline phosphatase (AP) conjugated-6-keto-$PGF_1$ alpha antigen and a polyclonal sheep antibody specific to 6-keto-$PGF_1$ alpha. After incubation the excess reagents are washed away. pNpp substrate is added. After a short incubation the enzyme reaction is stopped and the yellow color generated by alkaline phosphatase is read at 405 nm. The intensity of the yellow coloration is inversely proportional to the amount of 6-keto-$PGF_{1\alpha}$ captured in the plate.

Prostacyclin ($PGI_2$) is involved in platelet aggregation, vasoconstriction, and reproductive functions. However, $PGI_2$ has a half life of 60 minutes in plasma but only 2 to 3 minutes in buffer. The production of $PGI_2$ is typically monitored by measurement of 6-keto-$PGF_1$ alpha. 6-keto-$PGF_1$ alpha is produced by the non-enzymatic hydration of $PGI_2$, and has been shown to be stable. A number of pharmaceuticals alter and/or inhibit the synthesis of $PGI_2$. Methods to measure $PGI_2$ in blood and urine typically involve HPLC, gas chromatography/mass spectrometry, radioimmunoassay, or enzyme immunoassay.

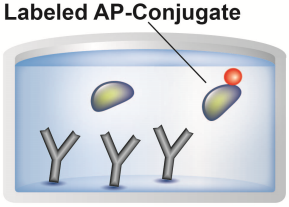
## 2. ASSAY SUMMARY



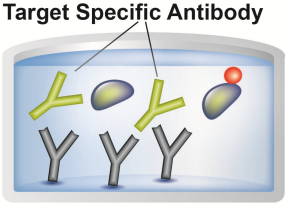

Prepare all reagents and samples as instructed.



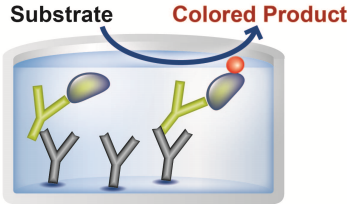

Add standards and samples to appropriate wells.




Add prepared labeled AP-conjugate to appropriate wells.




Add 6-keto-$PGF_1$ 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 6-keto-$PGF_1$ alpha Standard provided is supplied in ethanolic buffer at a pH optimized to maintain 6-keto-$PGF_1$ alpha integrity. Care should be taken handling this material because of the known and unknown effects of prostaglandins.

## 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 (Before Preparation) |
|---|---|---|
| Donkey anti-sheep IgG coated Microplate (12 x 8 wells) | 96 Wells | +4ºC |
| 6-keto-$PGF_1$ alpha Alkaline Phosphatase Conjugate | 5 mL | +4ºC |
| 6-keto-$PGF_1$ alpha EIA Antibody | 5 mL | +4ºC |
| 6-keto-$PGF_1$ alpha Standard | 500 µL | +4ºC |
| Assay Buffer | 27 mL | +4ºC |
| 20X Wash Buffer Concentrate | 27 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 6-keto-$PGF_1$ alpha)
- 2M hydrochloric acid (only required for extraction of samples containing low levels of 6-keto-$PGF_1$ 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 Conjugate 1:10 Dilution for Total Activity Measurement

Prepare the Conjugate 1:10 Dilution by diluting 50 µL of the supplied Conjugate with 450 µL of Assay Buffer. The dilution should be used within 3 hours of preparation.

*This 1:10 dilution is intended for use in the Total Activity wells only.*

### 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. Preparation of the 6-keto-$PGF_{1\alpha}$ standard should be done no more than 1 hour prior to the experiment. Diluted standards should be used within 60 minutes of preparation.

10.1 **Standard preparation for serum/urine samples:**

dilute the 6-keto-$PGF_1$ alpha standard with Assay Buffer.

10.2 **Standard preparation for culture supernatants samples**: dilute the 6-keto-$PGF_1$ alpha standard with tissue culture media.

10.3 Allow the 500,000 pg/mL 6-keto-$PGF_1$ alpha stock standard solution to equilibrate to room temperature. The standard solution should be stored at -20°C. Avoid repeated freeze-thaw cycles.

10.4 Label 7 tubes #1 – #7.

10.5 Add 900 µL of appropriate sample diluent (refer to step 10.1 and 10.2) to tube #1.

10.6 Add 800 µL of the appropriate sample diluent (refer to step step 10.1 and 10.2) to tubes #2 – #7.

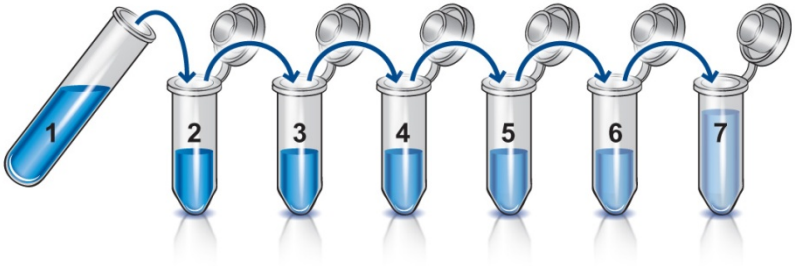
10.7 Prepare a 50,000 pg/mL Standard 1 by adding 100 µL of the 500,000 pg/mL Stock Standard to tube #1. Vortex thoroughly.

10.8 Prepare **Standard 2** by transferring 200 µL from tube #1 to tube 2. Mix thoroughly and gently.

10.9 Prepare **Standard 3** by transferring 200 µL from tube #2 to tube #3. Mix thoroughly and gently.

10.10 Using the table below as a guide, repeat for tubes #4 through #7.

| 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 | 200 | 800 | 50,000 | 10,000 |
| 3 | Standard 2 | 200 | 800 | 10,000 | 2,000 |
| 4 | Standard 3 | 200 | 800 | 2,000 | 400 |
| 5 | Standard 4 | 200 | 800 | 400 | 80 |
| 6 | Standard 5 | 200 | 800 | 80 | 16 |
| 7 | Standard 6 | 200 | 800 | 16 | 3.2 |



## 11. SAMPLE COLLECTION AND STORAGE

- The 6-keto-$PGF_1$ alpha kit is compatible with 6-keto-$PGF_1$ 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 serum samples, prostaglandin synthetase inhibitors such as indomethacin or meclofenamic acid at concentrations up to 10 µg/mL should be added to the samples. Some samples normally have very low levels of 6-keto-$PGF_1$ 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 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

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

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 diluted samples to appropriate wells.

13.1 Add 50 µL of Assay Buffer into the NSB wells only.

13.2 Add 50 µL of 6-keto-$PGF_1$ alpha Alkaline Phosphatase Conjugate (blue) into NSB, $B_0$, standard and sample wells, i.e. not the Total Activity (TA) and $B_s$ wells.

13.3 Add 50 µL of 6-keto-$PGF_1$ 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 Blank and TA wells are empty at this point and have no color.

13.4 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.

13.5 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.

13.6 Add 5 µL of the Conjugate 1:10 Dilution (Section 9.1) to the TA wells.

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

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

13.9 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

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

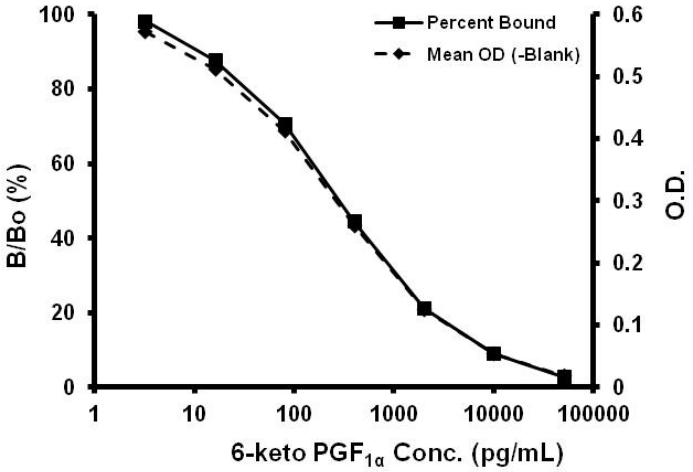
14.3 Plot the Percent Bound ($B/B_0$) and the net OD versus concentration of 6-keto-$PGF_1$ alpha for the standards. The concentration of 6-keto-$PGF_1$ alpha in the unknowns can be determined by interpolation of net OD values.

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 | 6-keto-$PGF_1$ alpha (pg/mL) |
|---|---|---|---|
| $B_s$ | (0.09) | | |
| TA | 0.414 | | |
| NSB | 0.092 | 0 | |
| $B_0$ | 0.583 | 100 | 0 |
| S1 | 0.019 | 2.9 | 50,000 |
| S2 | 0.054 | 9 | 10,000 |
| S3 | 0.125 | 21.2 | 2,000 |
| S4 | 0.261 | 44.6 | 400 |
| S5 | 0.413 | 70.7 | 80 |
| S6 | 0.512 | 87.8 | 16 |
| S7 | 0.573 | 98.3 | 3.2 |
| Unknown 1 | 0.148 | 25 | 1,492 |
| Unknown 2 | 0.414 | 70.9 | 76 |

**TYPICAL QUALITY CONTROL PARAMETERS –**

| | |
|---|---|
| Total Activity Added | = 0.414 x 10 x 10 = 41.4 |
| %NSB | = 0.07% |
| $\%B_0/TA$ | = 1.41% |
| Quality of Fit | = 1.000 (Calculated from 4 parameter logistic curve fit) |
| 20% Intercept | = 2,326 pg/mL |
| 50% Intercept | = 284 pg/mL |
| 80% Intercept | = 38 pg/mL |

## 16. TYPICAL SAMPLE VALUES

**SENSITIVITY –**

The sensitivity, minimum detectable dose of 6-keto-$PGF_1$ alpha using this ELISA kit was found to be 1.4 pg/mL. This was determined by the average optical density of $B_0$ (0 pg/mL standard) and comparing to the average optical density for Standard 7. The detection limit was determined as the concentration of 6-keto-$PGF_1$ alpha measured at 2 standard deviations from the zero along the standard curve.

**SAMPLE RECOVERY –**

Recovery was determined by 6-keto-$PGF_1$ alpha in tissue culture media, Human saliva, serum, and urine. Mean recoveries are as follows:

| Sample Type | Average % Recovery | Recommended Dilution |
|---|---|---|
| Tissue culture media | 106.7 | None |
| Human saliva | 110.7 | 1:10 – 1:100 |
| Human urine | 108.7 | 1:100 – 1:1000 |
| Human serum | 108.4 | 1:100 – 1:1000 |

**LINEARITY OF DILUTION –**

A sample containing 50,000 pg/mL 6-keto-$PGF_1$ alpha was diluted 6 times 1:5 in the kit Assay Buffer and measured in the assay. The data was plotted graphically as actual 6-keto-$PGF_1$ alpha concentration versus measured 6-keto-$PGF_1$ alpha concentration.

The line obtained had a slope of 1.017 and a correlation coefficient of 0.9999.

**PRECISION –**

**Intra-assay**

| | **6-keto-$PGF_1$ alpha (pg/mL)** | **%CV** |
|---|---|---|
| Low | 41 | 13.1 |
| Medium | 240 | 4.8 |
| High | 4,886 | 2.9 |

**Inter-assay**

| | **6-keto-$PGF_1$ alpha (pg/mL)** | **%CV** |
|---|---|---|
| Low | 239 | 7.9 |
| Medium | 1,220 | 5.2 |
| High | 4,828 | 6.0 |

## 17. ASSAY SPECIFICITY

**CROSS REACTIVITY –**

The cross reactivities for a number of related eicosanoid compounds was determined by dissolving the cross reactant in Assay buffer at concentrations from 500,000 to 5 pg/mL. These samples were then measured in the 6-keto-PGF1α assay, and the measured 6-keto-PGF1α concentration at 50% B/B0 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 |
|---|---|
| 6-keto-$PGF_1$ alpha | 100 |
| 2,3-dinor-6-keto-$PGF_1$ alpha | 3.17 |
| $PGF_2$ alpha | 1.67 |
| $PGD_2$ | 0.60 |
| $PGF_1$ alpha | 0.60 |
| $PGE_1$ | 0.20 |
| 6,15-diketo-13,14-dihydro-$PGF_1$ alpha | 0.12 |
| 13,14-dihydro-15-keto-$PGF_1$ alpha | <0.01 |
| 15-keto-$PGF_2$ alpha | <0.01 |
| $PGA_2$ | <0.01 |
| $PGE_2$ | <0.01 |
| $PGB_1$ | <0.01 |
| Thromboxane $B_2$ | <0.01 |
| 15-HpETE | <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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