ELISA PRODUCT INFORMATION & MANUAL

6-Keto-Prostaglandin F1A

NBP2-61286

Enzyme-linked Immunosorbent Assay for quantitative detection of 6-Keto-Prostaglandin F1A. For research use only. Not for diagnostic or therapeutic procedures.

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Product Manual

Please read entire booklet before proceeding with the assay.

Carefully note the handling and storage conditions of each kit component.

Please contact Novus Biologicals Technical Support if necessary.

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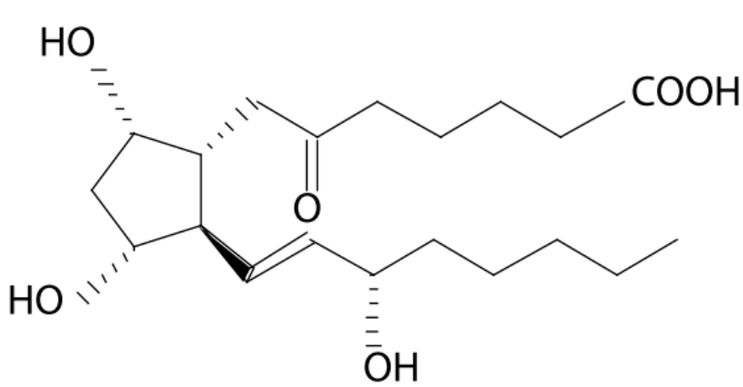
DESCRIPTION

The 6-keto-$PGF_{1\alpha}$ ELISA kit is a competitive immunoassay for the quantitative determination of 6-keto$PGF_{1\alpha}$ in biological fluids. Please read the complete kit insert before performing this assay. The kit uses a polyclonal antibody to 6-keto-$PGF_{1\alpha}$ to bind, in a competitive manner, the 6-keto-$PGF_{1\alpha}$ in the standards or sample or an alkaline phosphatase molecule which has 6-keto-$PGF_{1\alpha}$ 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 6-keto-$PGF_{1\alpha}$ in either standards or samples. The measured optical density is used to calculate the concentration of 6-keto-$PGF_{1\alpha}$. For further explanation of the principles and practice of immunoassays please see the excellent books by Chard[1] or Tijssen[2].

INTRODUCTION

Prostacyclin (PGI_2) is involved in platelet aggregation, vasoconstriction, and reproductive functions[2-5]. However, PGI_2 has a half life of 60 minutes in plasma but only 2 to 3 minutes in buffer[5]. 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[5]. A number of pharmaceuticals alter and/or inhibit the synthesis of PGI_2[6]. Methods to measure PGI_2 in blood and urine typically involve HPLC[7], gas chromatography/mass spectrometry[8], radioimmunoassay[9,10], or enzyme immunoassay[11].

6-keto-Prostaglandin F1α



SAFETY WARNINGS & PRECAUTIONS

Handle with care

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

- 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.

Avoid freeze / thaw cycles

- The 6-keto-prostaglandin $F_{1\alpha}$ Standard provided, Catalog No. 80-0044, 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.

MATERIALS SUPPLIED

1. **Donkey anti-Sheep IgG Microtiter Plate, One Plate of 96 Wells.**

   A plate using break-apart strips coated with donkey antibody specific to sheep IgG.

2. **6-keto-PGF1α ELISA Conjugate, 5 mL.**

   A blue solution of alkaline phosphatase conjugated with 6-keto-$PGF_{1\alpha}$.

3. **6-keto-$PGF_{1\alpha}$ ELISA Antibody, 5 mL.**

   A yellow solution of a sheep polyclonal antibody to 6-keto-$PGF_{1\alpha}$.

4. **Assay Buffer, 27 mL.**

   Tris buffered saline containing proteins and sodium azide as preservative.

5. **Wash Buffer Concentrate, 27 mL.**

   Tris buffered saline containing detergents.

6. **6-keto-Prostaglandin $F_{1\alpha}$ Standard, 0.5 mL.**

   A solution of 500,000 pg/mL 6-keto-$PGF_{1\alpha}$.

7. **pNpp Substrate, 20 mL.**

   A solution of p-nitrophenyl phosphate in buffer. Ready to use.

8. **Stop Solution, 5 mL.**

   A solution of trisodium phosphate in water. Keep tightly capped. Caution: Caustic.

9. **6-keto-$PGF_{1\alpha}$ Assay Layout Sheet, 1 each.**

10. **Plate Sealer, 1 each.**

Reagants require separate storage conditions.

STORAGE

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

OTHER MATERIALS NEEDED

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 beakers for diluting buffer concentrates.
5. Graduated cylinders.
6. A microplate shaker.
7. Adsorbent paper for blotting.
8. Microplate reader capable of reading at 405 nm, preferably with correction between 570 and 590 nm.

SAMPLE HANDLING

The 6-keto-$PGF_{1\alpha}$ ELISA kit is compatible with 6-keto-$PGF_{1\alpha}$ samples in a wide range of matrices after dilution in Assay Buffer. Please refer to the Sample Recovery recommendations on page 12 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 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. Users should only use standard curves generated in media or buffer to calculate concentrations of 6-keto-$PGF_{1\alpha}$ in the appropriate matrix. 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. 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:

Materials Needed

1. 6-keto-$PGF_{1\alpha}$ 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.

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 perml 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 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 0.5 mL/ minute. Wash the column with 10 mL of water, followed by 10ml 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 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. 6-keto-PGF$_{1\alpha}$ Standard

Allow the 500,000 pg/mL 6-keto-PGF$_{1\alpha}$ standard solution to warm to room temperature. Label seven 12 x 75 mm glass tubes #1 through #7. Pipet 900 µL of standard diluent (Assay Buffer or Tissue Culture Media) into tube #1 and 800 µL standard diluent to #2 through #7. Add 100 µL of the 500,000 pg/mL standard to tube #1. Vortex thoroughly. Add 200 µL of tube #1 to tube #2 and vortex thoroughly. Add 200 µL of tube #2 to tube #3 and vortex. Continue this for tubes #4 through #7. The concentration of 6-keto-PGF$_{1\alpha}$ in tubes #1 through #7 will be 50,000, 10,000, 2000, 400, 80, 16, and 3.2 pg/mL respectively. See the 6-keto-PGF$_{1\alpha}$ Assay Layout Sheet for dilution details. Diluted standards should be used within 60 minutes of preparation.

2. Conjugate 1:10 Dilution for Total Activity Measurement

Prepare the Conjugate 1:10 Dilution by diluting 50µl 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.

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 ziploc. 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 #7 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 light blue Conjugate 1:10 Dilution (see step 2, Reagent Preparation, on page 7) 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 every well. This stops the reaction and 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 6-keto-$PGF_{1\alpha}$ in the samples. We recommend that the data be handled by an immunoassay software package utilizing a 4 parameter curve fitting program. If this sort of data reduction software is not readily available, the concentration of 6-keto-PGF1α can be calculated as follows:

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:

   $$\textbf{Percent Bound} = \frac{\textbf{Net OD}}{\textbf{Net Bo OD}} \times 100$$

3. Plot Percent Bound versus Concentration of 6-keto-$PGF_{1\alpha}$ for the standards. Approximate a straight line through the points. The concentration of 6-keto-$PGF_{1\alpha}$ in the unknowns can be determined by interpolation.

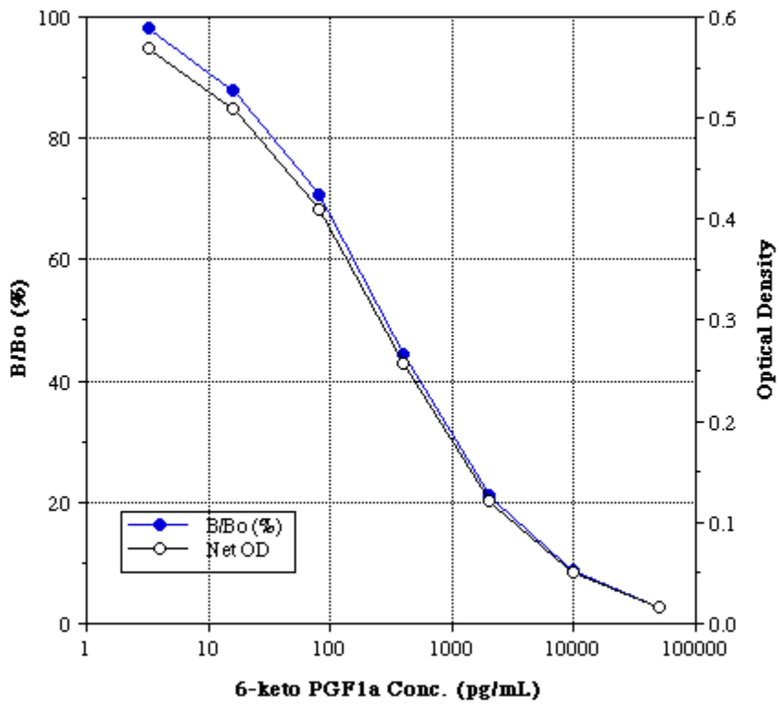
TYPICAL RESULTS

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

Sample	Mean OD (Blank)	Average Net OD	Percent Bound	6-keto-$PGF_{1\alpha}$ (pg/mL)
Blank	(0.090)			
TA	0.414			
NSB	0.092	(0.002)	0.0%	
Bo	0.583	0.581	100%	0
S1	0.019	0.017	2.9%	50,000
S2	0.054	0.052	9.0%	10,000
S3	0.125	0.123	21.2%	2,000
S4	0.261	0.259	44.6%	400
S5	0.413	0.411	70.7%	80
S6	0.512	0.510	87.8%	16
S7	0.573	0.571	98.3%	3.2
Unknown 1	0.148	0.146	25.0%	1,492
Unknown 2	0.414	0.412	70.9%	76

TYPICAL STANDARD CURVES

A typical standard curve is shown below. This curve must not be used to calculate 6-keto-PGF$_{1\alpha}$ concentrations; each user must run a standard curve for each assay used.



Typical Quality Control Parameters

Total Activity Added = 0.414 x 10 x 10 = 41.4

%NSB =	0.07%
%Bo/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

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[16].

Sensitivity

Sensitivity was calculated in Assay Buffer by determining the average optical density bound for sixteen (16) wells run as Bo, and comparing to the average optical density for sixteen (16) wells run with Standard #7. The detection limit was determined as the concentration of 6-keto-PGF1α measured at two (2) standard deviations from the zero along the standard curve.

Average Optical Density for the Bo = 1.029 ± 0.009 (0.89%)

Average Optical Density for Standard #7 = 0.988 ± 0.010 (1.06%)

Delta Optical Density (0-3.2 pg/mL) = 1.029 - 0.988 = 0.041

2 SD's of the Zero Standard = 2 x 0.009 = 0.018

$$\text{Sensitivity} = \frac{0.018}{0.041} \times 3.2 \text{ pg/mL} = 1.4 \text{ pg/mL}$$

Linearity

A sample containing 50,000 pg/mL 6-keto-$PGF_{1\alpha}$ was diluted serially 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 with a correlation coefficient of 0.9999.

Precision

Intra-assay precision was determined by taking samples containing low, medium and high concentrations of 6-keto-$PGF_{1\alpha}$ and running these samples multiple times (n=10) in the same assay. Inter-assay precision was determined by measuring two samples with low, medium, and high concentrations of 6-keto-$PGF_{1\alpha}$ in multiple assays (n=8). The precision numbers listed below represent the percent coefficient of variation for the concentrations of 6-keto-$PGF_{1\alpha}$ determined in these assays as calculated by a 4 parameter logistic curve fitting program.

	6-keto-$PGF_{1\alpha}$ (pg/mL)	Intra-assay %CV	Inter-assay %CV
Low	41	13.1	
Medium	240	4.8	
High	4,886	2.9	
Low	239		7.9
Medium	1,220		5.2
High	4,828		6.0

Cross Reactivities

The cross reactivities for a number of 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 6-keto-$PGF_{1\alpha}$ assay, and the measured 6-keto-$PGF_{1\alpha}$ 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
6-keto-PGF1α	100%
2,3-dinor-6-keto-PGF1α	3.17%
PGF2α	1.67%
PGD2	0.60%
PGF1α	0.60%
PGE1	0.20%
6,15-diketo-13,14-dihydro-PGF1α	0.12%
13,14-dihydro-15-keto-PGF1α	<0.01%
15-keto-PGF2α	<0.01%
PGA2	<0.01%
PGB1	<0.01%
PGE2	<0.01%
Thromboxane B2	<0.01%
15-HpETE*	<0.01%
2-Arachidonoylglycerol	<0.01%
Anandamide	<0.01%

*Data from S. Rawlinson, Royal Veterinary College, London,U.K.

SAMPLE RECOVERIES

Please refer to pages 5-7 for Sample Handling recommendations and Standard preparation. 6-keto-$PGF_{1\alpha}$ concentrations were measured in a variety of different samples including tissue culture media, human saliva, serum, and urine. 6-keto-$PGF_{1\alpha}$ was spiked into the undiluted samples of these media, which were diluted with the appropriate diluent and then assayed in the kit. The following results were obtained:

Sample	% 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

*See Sample Handling instructions on page 5 for details.

REFERENCES

1. T. Chard,"An Introduction to Radioimmunoassay & Related Techiques 4th Ed.", (1990) Amsterdam: Elsevier.
2. P. Tijssen,"Practice & Theory of Enz. Immunoassays", (1985) Amsterdam: Elsevier.
3. A.S. Nies, Clin.Pharmacol. Ther., (1986) 39: 481.
4. K.L. Brigham, et al., Prostaglandins and the Cardiovascular System, (1982) Raven Press: New York.
5. O. Ylikorkala & U.M. Makila, Am. J. Obstet. Gynecol., (1985) 152: 318.
6. J.M. Gerrard, Can. J. Physiol. Pharmacol., (1989) 67: 922.
7. W.S. Powell, Anal. Biochem., (1987) 164: 117.
8. C. Chiabrando, et al., J. Chromatography, (1989) 495: 1.
9. A. Riutta, et al., Anal. Biochem., (1994) 220: 351.
10. W. Schramm, et al., Clin. Chem., (1990) 36: 509.
11. T. Tonai, et al., Biochim. Biophys, Acta, (1985) 836: 335.
12. K. Green, et al., Anal. Biochem, (1973) 54: 434.
13. J. Frolich, et al., J. Clin. Invest., (1975) 55: 763.
14. J.E. Shaw & P.W. Ramwell, Meth. Biochem. Anal., (1969) 17: 325.
15. K. Green, et al., Adv. Prostaglandin & Thromboxane Res., (1978) 5: 15.
16. National Committee for Clinical Laboratory Standards Evaluation Protocols, SC1, (1989) Villanova, PA: NCCLS.