*Oxis*Research™

A Division of OXIS Health Products, Inc.

BIOXYTECH® Urinary 8-Epi-Prostaglandin $F_{2\alpha}$

Enzyme Immunoassay for Urinary Isoprostane

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

Catalog Number 21048 This product is patent protected.

INTRODUCTION

The Analyte

Isoprostanes are prostanglandin-like compounds that are produced by peroxidation of lipoproteins (1,2). 8-epi-prostaglandin-$F_{2\alpha}$ has been shown to be a potent vasoconstrictor in rat kidneys (3) and rabbit lungs (4). Isoprostanes may also play a role in atherosclerosis (5,6). Measurement of isoprostanes concentration may be helpful in assessment of oxidative stress, hepatorenal syndrome, rheumatoid arthritis, atherosclerosis and carcinogenesis (7). This kit can be used for the quantitation of free 8-epi-prostaglandin-$F_{2\alpha}$ in urine samples without the need for prior purification or extraction.

Principles of the Procedure

The BIOXYTECH® Urinary 8-Epi-Prostaglandin $F_{2\alpha}$ Assay is a competitive enzyme-linked immunoassay (ELISA) for determining levels of 8-epi-prostaglandin-$F_{2\alpha}$ in urine samples. Briefly, the samples are mixed with an enhancing reagent that essentially eliminates interferences due to non-specific binding. The 8-epi-prostaglandin-$F_{2\alpha}$ in the sample or standard then competes with 8-epi-prostaglandin-$F_{2\alpha}$ conjugated to horseradish peroxidase (HRP Conjugate) for binding to a polyclonal antibody specific for 8-epi-prostaglandin-$F_{2\alpha}$ coated on the microplate. Following substrate addition, the intensity of the color is inversely proportional to the amount of unconjugated 8-epi-prostaglandin- $F_{2\alpha}$ in the sample or standard.

REAGENTS

Materials Provided

• 96-well micortiter plate, pre-coated with 8-epi-prostaglandin-$F_{2\alpha}$ antibody	1
• 8-epi-prostaglandin-$F_{2\alpha}$ Standards (1μg/mL)	2 x 100 μL
• Pretreatment Reagent	10 mL
• Dilution Buffer	(5x) 20 mL
• Wash Buffer	(5x) 40 mL
• TMB Substrate (Tetramethylbenzidine)	25 μL
• HRP Conjugate	8 μL
• Disposable reagent troughs for a multichannel pipettor	2
• ELISA Template	1
• Stop Solution	5 mL

Materials Required But Not Provided

- Precision pipettes with disposable tips. A multichannel pipette is helpful, but not required.
- 96-well microplate reader for measurement of absorbance at 450 nm.
- Reagents for the quantification of creatinine for normalization.
- Deionized water.

Warnings and Precautions

- Do not smoke, eat or drink in areas where samples and reagents are handled.
- Wear disposable gloves when handling samples and reagents.
- Do not pipette reagents or samples by mouth.
- In case of accidental exposure of skin, mucous membranes or eyes to the components of this kit, thoroughly wash the exposed area with water.
- Reagents may contain sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azides. On disposal of reagents, flush with large volumes of water to prevent azide accumulation.
- For *in vitro* use only. For research purposes only. Not for use in diagnostic procedures.

Reagent Storage and Handling

Store all components at 4°C until immediately before use. Do not freeze.

PROCEDURE

Note: This product is intended for use with urine samples and has not been validated for use with serum, tissue culture supernatants or tissue extracts.

The following instructions are based on using the entire kit (all of the wells at one time). If portions of the kit are to be used at a later time, one may desire to prepare smaller quantities and save the remaining stock for later use.

Reagent Preparation

1. Substrate**:** Ready to use.
2. Add the 5x Wash Buffer (40 mL) to 160 ml of deionized water, mix well.
3. Add the Dilution Buffer (20 mL) to 80 ml of deionized water, mix well
4. HRP Conjugate:
   a. Centrifuge vial before removing the cap.
   b. Prepare a 1/250 dilution (Pipet 4 µL of the HRP Conjugate to 1 mL Dilution Buffer (1x) and mix well.
   c. Further dilute HRP Conjugate 1/50 (Pipet 300 µL of the "1:250 mixture" to 14.7 mL of Dilution Buffer (1x). This is your working solution.

Preparation of Standards

Prepare a series of standards by diluting the (1µg/ml) to the following concentrations: 100, 50, 10, 5, 1, 0.1 and 0.05 ng/ml

- S7: Add 100 µL of Standard to 900µL of Dilution buffer (1x) = 100 ng/ml
- S6: Add 400 µL of S7 to 400 µL of Dilution Buffer (1x) = 50 ng/ml
- S5: Add 200 µL of S6 to 800 µL of Dilution Buffer (1x) = 10 ng/ml
- S4: Add 400 µL of S5 to 400 µL of Dilution Buffer (1x) = 5 ng/ml
- S3: Add 200 µL of S4 to 800 µL of Dilution Buffer (1x) = 1 ng/ml
- S2: Add 100 µL of S3 to 900 µL of Dilution Buffer (1x) = 0.1 ng/ml
- S1: Add 400 µL of S2 to 400 µL of Dilution Buffer (1x) = 0.05 ng/ml
- S0: Dilution Buffer (1x) only.

Note: 150 µL of Pretreatment Reagent and 150 µL of HRP Conjugate can be added to 300 µL of each of the standards (S0 to S8) when the sample preparation is ready. 200 µL of the total volume of 600 µL will be used for the assay.

Sample Preparation

1. Make multiple dilutions of each sample, e.g.1:2; 1:4; 1:8 using Dilution Buffer (1x).
2. Add 150 µL of Pretreatment Reagent and 150 µL of HRP Conjugate to 300 µL of the diluted samples and mix well. Use 200 µL of this mixture for the assay.

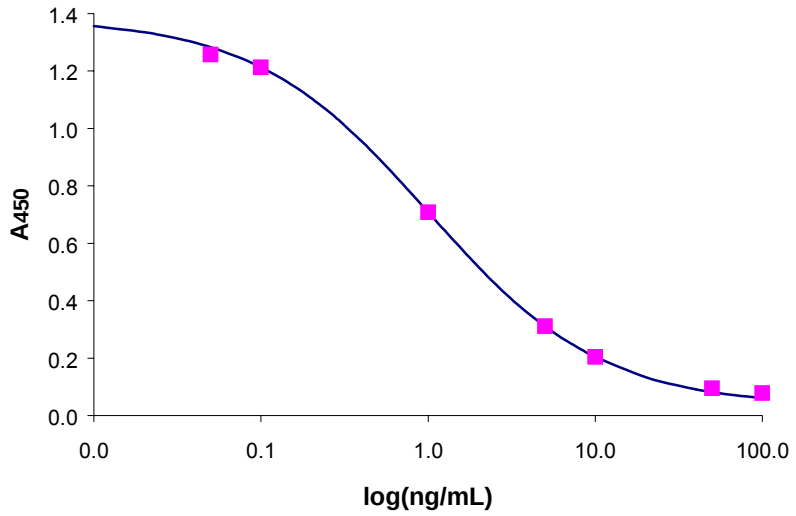
Assay

1. Remove microplate from foil pouch.
2. Pipet 200 µL of the prepared standard or sample mixture into each well.
3. Seal plate or place in humidity chamber.
4. Allow the plate to stand at room temperature for 2 hours.
5. Empty the contents and blot the plate on a lint free towel.
6. Wash the plate 3 times by adding 300 µL of Wash Buffer (1x) to each well.
7. Empty the contents and blot the plate on a lint free towel.
8. Add 200 µL TMB Substrate to each well. Incubate for 30 minutes at room temperature.
9. Add 50 µL of Stop Solution to each well and read absorbance at 450 nm.

Calculations

The Standard Curve is obtained by fitting the Standard absorbances at 450 nm to the concentration of 8-epi-prostaglandin- $F_{2\alpha}$ by the 4-parameter logistic curve fit method.

Figure 1: 8-epi-Prostaglandin-$F_{2\alpha}$ Standard Curve



PERFORMANCE CHARACTERISTICS

Specificity

8-epi-Prostaglandin $F_{2\alpha}$	**100.0%**
9_{α},11β-Prostaglandin $F_{2\alpha}$	4.1%
13,14-Dihydro-15-Keto-$PGF_{2\alpha}$	3.0%
9_{α},11β-Prostaglandin $F_{2\alpha}$	<0.01%
Prostaglandin $F_{2\alpha}$	<0.01%
6-Keto-Prostaglandin $F_{1\alpha}$	<0.01%
Prostaglandin E_2	<0.01%
Prostaglandin D_2	<0.01%
Arachidonic Acid	<0.01%

*Cross reactivity at mid-point of the Standard Curve.

REFERENCES

1. Roberts LJ, Morrow JD (1997). The generation and action of isoprostanes. Biochim Biophys Acta **1345**: 121-135.
2. Morrow JD, Roberts LJ (1997). The isoprostanes: unique bioactive products of lipid peroxidation. Prog. Lipid Res. **36(1)**: 1-21.
3. Kanji VK, Wang C, Salahudeen AK (1999). Vitamin E suppresses cyclosporin A-induced increase in the urinary excretion of arachidonic acid metabolites inducing F2-isoprostanes in the rat model. Transplant Proc. **31(3):** 1724-28.
4. Banerjee M, Kang KH, Morrow JD, Roberts LJ, Newman JH (1992). Effects of a novel prostaglandin 8-epi PGF2 alpha in rabbit lung in situ. Am. J. Physiol. **263**: H660-H663.
5. Gniwotta C, Morrow JD, Roberts LJ II, Kuhn H (1997). Prostaglandin F2-like compounds, F2-isoprostanes, are present in increased amounts in human atherosclerotic lesion. Arteriosceler. Thromb. Vasc. Biol. **17**: 3236-3241.
6. Tangirala RK et al. (2001). Reduction of isoprostanes and regression of advanced atherosclerosis by apolipoprotein E. J. Biol. Chem. **276(1):** 261-6.
7. Roberts LJ and Morrow JD (2000). Measurement of F2-isoprstanes as an index of oxidative stress *in vivo*. Free Rad. Biol. Medicine. **28(4):** 505-513.

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TECHNICAL SUPPORT

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