*Oxis*Research™

A Division of OXIS Health Products, Inc.

BIOXYTECH® F2-Isoprostane Metabolite

Enzyme Immunoassay for F_2-Isoprostane Metabolite
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Catalog Number 21049. This product is patent protected.

INTRODUCTION

The Analyte

F_2-isoprostanes formed as a result of free radical-mediated non-enzymatic peroxidation of membrane-bound arachidonic acid are largely metabolized before excretion. They can be found in esterfied tissues, plasma lipids and other body fluids (1). Thus, they can be used to evaluate local or systemic lipid peroxidation in vivo. Four classes of isoprostanes have been described, each potentially present in 16 isomers. 8-epi-prostaglandin-$F_{2á}$ (8-epi-$PGF_{2á}$ or sometimes called $iPF_{2á}$ -III), a major component of the F_2-isoprostane family with mitogenic and vasoconstrictor capability, can be noninvasively measured in urine to assess in vivo lipid peroxidation (2). In humans, increased F_2-isoprostane levels have been found in different physiopathological conditions such as atherothrombotic disease, diabetes, hypercholesterolemia, Alzheimer's disease and cigarette smoking (2, 3, 4).

However, not all of the amount of F_2-isoprostanes detected in urine may be of systemic lipid peroxidation origin. So far, three major urinary and plasma metabolites of 8-epi-$PGF_{2á}$ have been reported in humans and rats: 2,3-dinor-5,6-dihydro-8-epi-$PGF_{2á}$, 2,3-dinor-8-epi-$PGF_{2á}$ and 2,3,4,5-tetranor-15-keto-13,14-dihydro-8-epi-$PGF_{2á}$ (5, 6). In addition, autoxidation of ã-linolenic acid found in plants will also produce 2,3-dinor-5,6-dihydro-8-epi-$PGF_{2á}$, a major urinary metabolite in humans (5). Thus, the measurement of major metabolites of endogenous 8-epi-$PGF_{2á}$ in addition to the parent compound may be useful for both (a) allowing the researcher to obtain a more accurate evaluation of the overall production of the biomarker in vivo while adding significance to individual measurements; and (b) provide a compound that can be measured without the risk of artificial production ex vivo. This assay may be used for the quantification of metabolites of 8-epi-$PGF_{2á}$ in samples without the need for prior purification or extraction.

Principles of the Procedure

The BIOXYTECH® F2-Isoprostane Metabolite Assay is a competitive enzyme-linked immunoassay (ELISA) for determining levels of F_2-Isoprostane Metabolite. Briefly, the samples are mixed with an pretreatment reagent that essentially eliminates interferences due to non-specific binding. The F_2-Isoprostane Metabolite in the sample or standard then competes with F_2-Isoprostane Metabolite conjugated to horseradish peroxidase (HRP Conjugate) for binding to a polyclonal antibody specific for F_2-Isoprostane Metabolite coated on the microplate. Following substrate addition, the intensity of the color is proportional to the amount of F_2-Isoprostane Metabolite HRP Conjugate bound and inversely proportional to the amount of unconjugated F_2-Isoprostane Metabolite in the sample or standard.

REAGENTS

Materials Provided

Item	Quantity
96 well microtiter plate precoated with anti-F_2-Isoprostane Metabolite	1
F2-Isoprostane Metabolite Standard	2 x 100 µL
Pretreatment Reagent	10 mL
Dilution Buffer	20 mL
Wash Buffer	40 mL
TMB Substrate	25 mL
HRP Conjugate	70 µL

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.
- 3N sulfuric acid.

Warnings and Precautions

- In case of accidental exposure of skin, mucous membranes or eyes, thoroughly wash the exposed area with water.
- For *in vitro* use only. For research purposes only. Not for use in diagnostic procedures.

Reagent Storage and Handling

Store all components at 2-8°C until immediately before use. Do not freeze. Only use the 96-well precoated plate supplied with the kit.

PROCEDURE

The following instructions are based on using the entire kit (all of the wells at one time). To use less than the complete plate, scale preparations appropriately.

Reagent Preparation

1. TMB Substrate: Ready to use.
2. Add the 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: Centrifuge vial before removing the cap. Add 64 µL of the HRP Conjugate to 8 mL of the diluted Dilution Buffer.

Preparation of Standards

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

2. Add 150 µL of Pretreatment Reagent and 150 µL of HRP Conjugate to 300 µL of each of the standards (S0 to S8). Use 200 µL per well for the assay.

Sample Preparation

1. Dilute urine samples in Dilution Buffer. The extent of dilution required for accurate quantification may vary depending on the F_2-Isoprostane Metabolite levels in the samples and must be determined by the user. For typical urine specimens, four common dilution factors are 20, 40, 80 and 120.
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 per well for the assay.

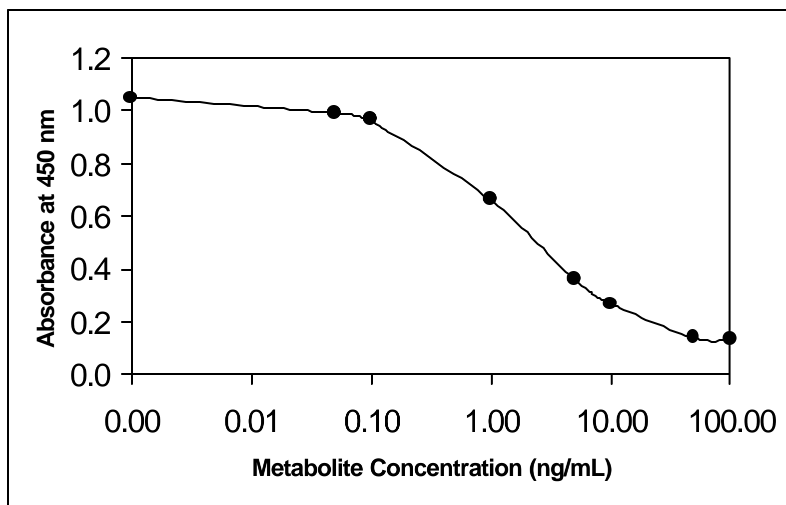
Assay

1. Remove microplate from foil pouch.
2. Pipet 200 µL of the prepared standard or sample dilution mixture containing Pretreatment Reagent and HRP Conjugate into each well.
3. Allow the plate to stand at room temperature for 2 hours.
4. Empty the contents and blot the plate on a lint free paper towel.
5. Wash the plate 3 times with 300 µL of Wash Buffer (1x) in each well.
6. Empty the contents and blot the plate on a lint free paper towel.
7. Add 200 µL TMB Substrate to each well. Allow to incubate at room temperature for at least 45 minutes, but not more than 60 minutes. Blue color will develop.
8. Add 50 µL of 3N sulfuric acid to each well to stop the HRP-catalyzed color development. The color will change from blue to yellow. Read the absorbance at 450 nm.

Calculations

1. The Standard Curve is obtained by fitting each Standard absorbance at 450 nm to the concentration of Metabolite by the 4-parameter logistic curve fit method.

Standard Curve



Typical B/Bo: 20% 5.9 ng/mL; 50% 1.3 ng/mL; 80% 0.4 ng/mL

2. Calculate the diluted sample concentration from its absorbance using the curve.
3. Before multiplying by the dilution factor, identify which diluted sample concentration value is closest to the 50% B/Bo for each sample. This is the "best estimate" dilution.
4. Multiply the selected "best estimate" dilution sample values by their respective dilution factors.

PERFORMANCE CHARACTERISTICS

Specificity

Compound	Percent Cross-Reactivity
F_2-Isoprostane Metabolite	100.00%
F_2-Isoprostane (8-epi Prostaglandin $F_{2\alpha}$)	0.98%
Prostaglandin $F_{1\alpha}$	0.75%
11β-Prostaglandin $F_{2\alpha}$	0.29%
6-keto Prostaglandin $F_{1\alpha}$	< 0.25%
Thromboxane B_2	< 0.25%
Arachidonic Acid	< 0.25%

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