Product Manual

OxiSelect™ 8-iso-Prostaglandin F2α ELISA Kit

Catalog Numbers

STA-337	96 assays
STA-337-5	5 x 96 assays

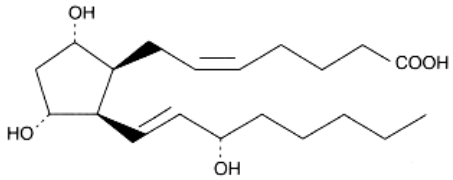
FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Lipid peroxidation is a well-defined mechanism of cellular damage in animals and plants. Lipid peroxides are unstable indicators of oxidative stress in cells that decompose to form more complex and reactive compounds such as isoprostanes. The isoprostanes are a type of eicosanoids produced non-enzymatically through the oxygen radical induced peroxidation of tissue phospholipids and lipoproteins. Isoprostanes are prostaglandin-like compounds that appear in normal plasma and urine samples, but are elevated by oxidative stress in tissue, plasma, and urine.

8-iso-Prostaglandin F2α (also known as 8-epi-PGF2α, 8-isoprostane, or 15-isoprostane F2t), is an isoprostane that has been shown to be useful for the assessment of oxidative stress *in vivo*. It is produced in membrane phospholipids from non-cyclooxygenase and cyclooxygenase peroxidation pathways derived from arachidonic acid. 8-iso-Prostaglandin F2α (8-iso-PGF2α) is a potent vasoconstrictor, a mutagen in 3T3 cells as well as vascular smooth muscle cells, and also a possible pathophysiological mediator that can alter membrane integrity. It has been implicated in atherogenesis and elevated levels are associated with hepatorenal syndrome, rheumatoid arthritis, carcinogenesis, as well as atherosclerosis. 8-iso-PGF2α circulates in the plasma and is excreted in the urine. 8-iso PGF2α circulates as an esterified LDL Phospholipid and as a free acid. Normal human plasma and urine 8-iso PGF2α is about 40-100 pg/mL and about 190 pg/mg of creatinine respectively. Methods for determining total 8-iso PGF2α usually require alkaline hydrolysis of 8-iso PGF2α esters from tissues followed by extractions, phase separations and thin layer chromatography.



8-iso-Prostaglandin F2α (8-iso-PGF2α)

The OxiSelect™ 8-iso-Prostaglandin F2α ELISA Kit is an enzyme immunoassay developed for rapid detection and quantification of 8-iso-PGF2α. The quantity of 8-iso-PGF2α in samples is determined by comparing its absorbance with that of a known 8-iso-PGF2α standard curve. Each kit provides sufficient reagents to perform up to 96 assays, including the standard curve and unknown samples.

Assay Principle

Cell Biolabs' 8-iso-PGF2α kit is a competitive enzyme-linked immunoassay (ELISA) for determining levels of 8-iso-PGF2α in a variety of biological samples such as plasma, urine, serum, or tissue extracts. An antibody to 8-iso-PGF2α is incubated in pre-coated microtiter plate wells. Upon washing, 8-iso-PGF2α standards or treated samples are mixed with an 8-iso-PGF2α-HRP conjugate and added simultaneously to the wells. The unconjugated, or free 8-iso-PGF2α and 8-iso-PGF2α-HRP conjugate compete for binding to the antibody bound to the plate. After this brief incubation and wash, a substrate to the HRP is added. The HRP activity results in color development that is directly proportional to the amount of 8-iso-PGF2α conjugate bound to the plate and inversely proportional to the amount of free 8-iso-PGF2α in the samples or standards. The 8-iso-PGF2α content in an unknown sample is determined by comparing with the known predetermined standard curve. Please read the complete kit insert prior to performing the assay.

Related Products

1. STA-320: OxiSelect™ Oxidative DNA Damage ELISA Kit (8-OHdG Quantitation)
2. STA-325: OxiSelect™ Oxidative RNA Damage ELISA Kit (8-OHG Quantitation)
3. STA-330: OxiSelect™ TBARS Assay Kit (MDA Quantitation)
4. STA-344: OxiSelect™ Hydrogen Peroxide/Peroxidase Assay Kit
5. STA-347: OxiSelect™ In Vitro ROS/RNS Assay Kit (Green Fluorescence)

Kit Components (shipped at room temperature)

1. Goat Anti-Rabbit Antibody Coated Plate (Part No. 250001): One 96-well strip plate.
2. Anti-8-iso-PGF2α Antibody (Part No. 233701): One 20 µL tube of anti-8-iso-PGF2α rabbit IgG.
3. Sample Diluent (Part No. 233702): One 50 mL bottle.
4. Neutralization Solution (Part No. 233705): One 20 mL bottle.
5. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
6. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
7. Stop Solution (Part. No. 310808): One 12 mL bottle.
8. 8-iso-PGF2α Standard (Part No. 233703): One 25 µL tube of 200 µg/mL 8-iso-PGF2α in DMSO.
9. 8-iso-PGF2α-HRP Conjugate (Part No. 233704): One 70 µL tube of 8-iso-PGF2α-HRP conjugate.

Materials Not Supplied

1. Protein samples such as purified protein, plasma, serum, cell lysate
2. Deionized water
3. Reagents and materials necessary for sample extraction and purification

Storage

Upon receipt, store the Anti-8-iso-PGF2α Antibody, 8-iso-PGF2α-HRP Conjugate, and 8-iso-PGF2α Standard at -20ºC. Make aliquots as necessary to avoid freeze/thaw cycles. Store all other kit components at 4ºC. Any partial or unused components should return to their proper storage temperatures.

Safety Considerations

- Some kit components contain azide, which can react with copper or lead piping. Flush with large volumes of water when disposing of reagents.
- Some kit reagents are caustic or hazardous and should be handled accordingly.

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer Concentrate to 1X with deionized water. Stir to homogeneity.
- Anti-8-iso-PGF2α Antibody: Immediately before use, dilute the Anti-8-iso-PGF2α Antibody 1:1000 with Sample Diluent.

- 8-iso-PGF2α-HRP Conjugate: Immediately before use, dilute the conjugate 1:80 with Sample Diluent. Only prepare enough of the diluted conjugate for the number of wells immediately used.
- Substrate Solution: Prior to use, warm the Substrate Solution to room temperature.
  Note: Do not store diluted Anti-8-iso-PGF2α Antibody, 8-iso-PGF2α-HRP Conjugate, or 8-iso-PGF2α Standard solutions.

Preparation of Samples

Hydrolysis of lipoprotein or phospholipid coupled 8-iso-Prostaglandin F2α (8-iso-PGF2α) is required to measure both free and esterified isoprostane. To hydrolyze this ester bond, the sample is usually treated with 2N NaOH at 45°C for 2 hours.

- Serum, plasma, tissue lysate samples: Use 1 part of 10N NaOH for every 4 parts of liquid sample. After incubation at 45°C for 2 hours, add 100 µL of concentrated (10N) HCl per 500 µL of hydrolyzed sample. The sample could turn milky after this addition. Centrifuge the samples for 5 minutes at 12,000 rpm in a microcentrifuge. The clear supernatant can be used in the assay or stored at -20°C or below for future use. Before assaying, check to be sure each neutralized sample is in the pH range of 6-8. If it is not, adjust the pH to this range by adding 100 µL of the sample to 100 µL of the provided Neutralization Solution.
- Urine samples: Acid hydrolysis of urine samples is necessary to break the bonds which hold lipid and non-lipid components together prior to ELISA. Urine sample is acidified to pH 3.0 by adding 1/10 volume of 1N HCl (Example: Add 100 µL of 1N HCl to 1 mL of urine sample). Acidified urine sample should be further diluted in PBS or Sample Diluent 1:4 to 1:8 before ELISA.

Preparation of 8-iso-PGF2α Standards

1. Prepare fresh standards by diluting the 8-iso-PGF2α Standard from 200µg/mL to 0.2 µg/mL in Sample Diluent for a 1:1000 final dilution. (Example: Add 5 µL of 8-iso-PGF2α Standard stock tube to 4.995 mL of Sample Diluent)
2. Prepare a series of the remaining 8-iso-PGF2α standards according to Table 1.

Standard Tubes	8-iso-PGF2α Standard (µL)	Sample Diluent (µL)	8-iso-PGF2α Standard (pg/mL)
1	5 µL of Standard Stock	4995 µL	200,000
2	250 µL of Tube #1	750 µL	50,000
3	250 µL of Tube #2	750 µL	12,500
4	250 µL of Tube #3	750 µL	3,125
5	250 µL of Tube #4	750 µL	781
6	250 µL of Tube #5	750 µL	195
7	250 µL of Tube #6	750 µL	49
8	0 µL	200 µL	0

Table 1. Preparation of 8-iso-PGF2α Standard Curve.
Note: Do not store diluted 8-iso-PGF2α Standard solutions.

Assay Protocol

Note: Each 8-iso-PGF2α Standard and unknown samples should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed.

1. Add 100 µL of the diluted Anti-8-iso-PGF2α Antibody to the Goat Anti-Rabbit Antibody Coated Plate. Incubate 1 hour at 25ºC on an orbital shaker.
2. Remove the antibody solution from the wells. Wash wells 5 times with 300 µL 1X Wash Buffer per well. After the last wash, empty the wells and tap microwell plate on absorbent pad or paper towel to remove excess wash solution.
   Note: Thorough washing is necessary to remove all of the azide present in the antibody solution.

3. Combine 55 µL of the 8-iso-PGF2α standard or sample and 55 µL of 8-iso-PGF2α-HRP conjugate in a microtube and mix thoroughly. Transfer 100 µL of the combined solution per well. A well containing Sample Diluent can be used as a control. Incubate 1 hour at 25ºC on an orbital shaker.
4. Remove the combined solution from the wells. Wash 5 times with 300 µL of 1X Wash Buffer per well. After the last wash, empty wells and tap microwell plate on absorbent pad or paper towel to remove excess wash solution.
5. Add 100 µL of Substrate Solution to each well. Incubate at room temperature for 10-30 minutes on an orbital shaker.
6. Stop the enzyme reaction by adding 100 µL of Stop Solution to each well. Results should be read immediately (color will fade over time).
7. Read absorbance of each well on a microplate reader using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical 8-iso-PGF2α results. One should use the data below for reference only. This data should not be used to interpret actual results.

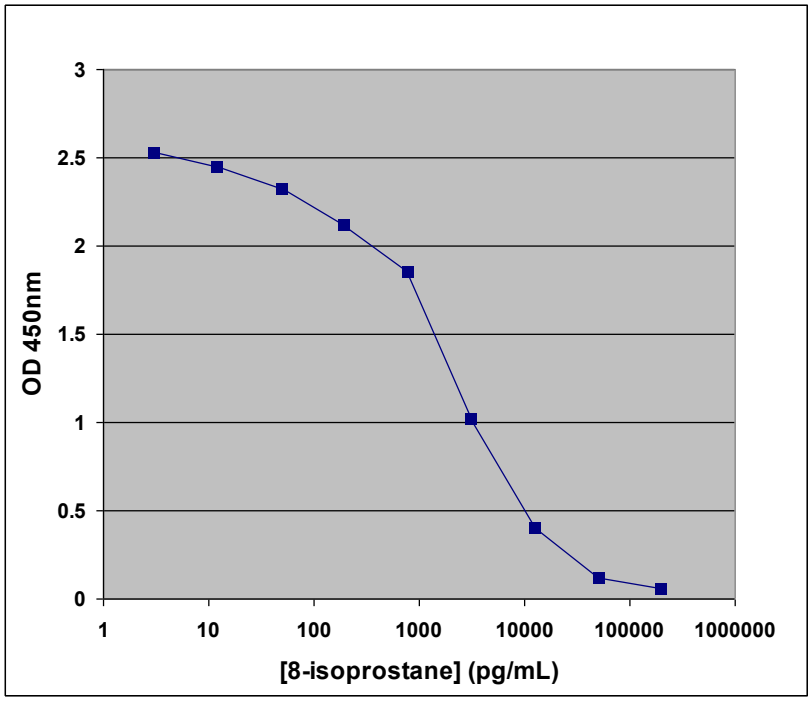


Figure 1: 8-iso-PGF2α ELISA Standard Curve.

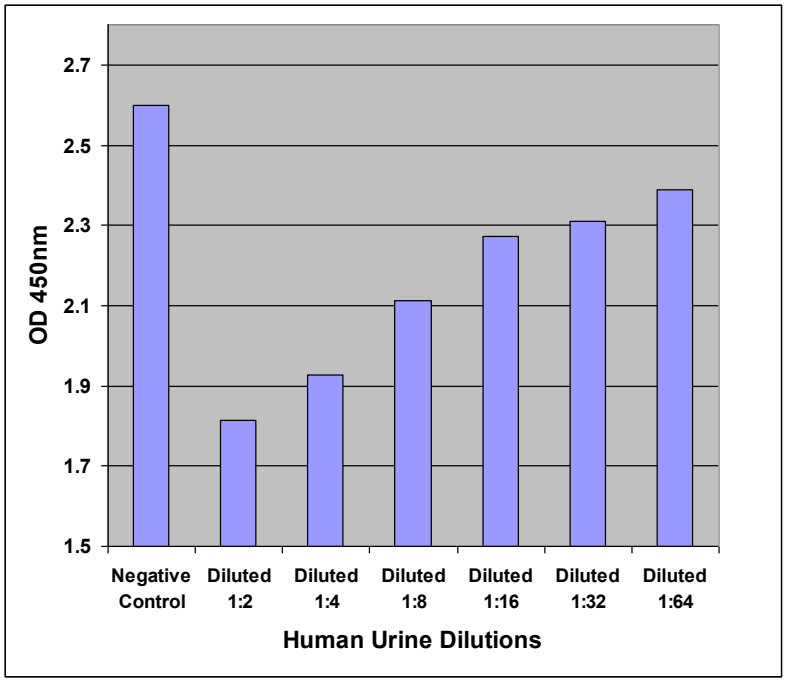


Figure 2: Dilutions of Human Urine tested with 8-iso-PGF2α ELISA.

Cross reactivity of 8-iso-Prostaglandin F2α ELISA Kit

Compounds	Cross Reactivity
8-iso-PGF2α	100%
PGF1α	4.6%
PGF2α	1.85%
PGE1	0.19%
TXB2	0.023%
PGB1	0.02%
PGE3	0.012%
6-keto-PGF1α	0.008%
13,14-dihydro-15-keto-PGF2α	0.008%
6,15-keto-13,14-dihydro-PGF1α	0.005%
8-iso-PGE1	<0.001%
PGA2	<0.001%
PGJ2	<0.001%

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