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Product Manual

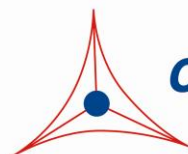
# OxiSelect™ 8-iso-Prostaglandin F2 $\alpha$ ELISA Kit

## Catalog Numbers

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

**FOR RESEARCH USE ONLY**  
**Not for use in diagnostic procedures**

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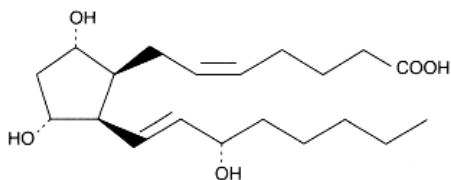


**CELL BIOLABS, INC.**  
*Creating Solutions for Life Science Research*

## **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 F<sub>2</sub>α (also known as 8-epi-PGF<sub>2</sub>α, 8-isoprostane, or 15-isoprostane F<sub>2</sub>t), 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 F<sub>2</sub>α (8-iso-PGF<sub>2</sub>α) 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-PGF<sub>2</sub>α circulates in the plasma and is excreted in the urine. 8-iso PGF<sub>2</sub>α circulates as an esterified LDL Phospholipid and as a free acid. Normal human plasma and urine 8-iso PGF<sub>2</sub>α is about 40-100 pg/mL and about 190 pg/mg of creatinine respectively. Methods for determining total 8-iso PGF<sub>2</sub>α usually require alkaline hydrolysis of 8-iso PGF<sub>2</sub>α esters from tissues followed by extractions, phase separations and thin layer chromatography.



**8-iso-Prostaglandin F<sub>2</sub>α (8-iso-PGF<sub>2</sub>α)**

The OxiSelect™ 8-iso-Prostaglandin F<sub>2</sub>α ELISA Kit is an enzyme immunoassay developed for rapid detection and quantification of 8-iso-PGF<sub>2</sub>α. The quantity of 8-iso-PGF<sub>2</sub>α in samples is determined by comparing its absorbance with that of a known 8-iso-PGF<sub>2</sub>α 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-PGF<sub>2</sub>α kit is a competitive enzyme-linked immunoassay (ELISA) for determining levels of 8-iso-PGF<sub>2</sub>α in a variety of biological samples such as plasma, urine, serum, or tissue extracts. An antibody to 8-iso-PGF<sub>2</sub>α is incubated in pre-coated microtiter plate wells. Upon washing, 8-iso-PGF<sub>2</sub>α standards or treated samples are mixed with an 8-iso-PGF<sub>2</sub>α-HRP conjugate and added simultaneously to the wells. The unconjugated, or free 8-iso-PGF<sub>2</sub>α and 8-iso-PGF<sub>2</sub>α-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-PGF<sub>2</sub>α conjugate bound to the plate and inversely proportional to the amount of free 8-iso-PGF<sub>2</sub>α in the samples or standards. The 8-iso-PGF<sub>2</sub>α 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)
6. STA-816: OxiSelect™ N-epsilon-(Carboxymethyl) Lysine (CML) Competitive ELISA Kit
7. STA-817: OxiSelect™ Advanced Glycation End Products (AGE) Competitive ELISA Kit
8. STA-832: OxiSelect™ MDA Competitive ELISA Kit
9. STA-838: OxiSelect™ HNE Adduct Competitive ELISA Kit

## **Kit Components**

1. Goat Anti-Rabbit Antibody Coated Plate (Part No. 250001): One 96-well strip plate.
2. Anti-8-iso-PGF2 $\alpha$  Antibody (Part No. 233701): One 20  $\mu$ L tube of anti-8-iso-PGF2 $\alpha$  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-PGF2 $\alpha$  Standard (Part No. 233703): One 25  $\mu$ L tube of 200  $\mu$ g/mL 8-iso-PGF2 $\alpha$  in DMSO.
9. 8-iso-PGF2 $\alpha$ -HRP Conjugate (Part No. 233704): One 70  $\mu$ L tube of 8-iso-PGF2 $\alpha$ -HRP conjugate.

## **Materials Not Supplied**

1. Protein samples such as purified protein, plasma, serum, cell lysate
2. Deionized water
3. 5  $\mu$ L to 1000  $\mu$ L adjustable single channel precision micropipettes with disposable tips
4. 50  $\mu$ L to 300  $\mu$ L adjustable multichannel micropipette with disposable tips
5. Bottles, flasks, and conical or microtubes necessary for reagent preparation
6. Reagents and materials necessary for sample extraction and purification
7. Multichannel micropipette reservoir
8. Plate orbital shaker or rotator
9. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

## **Storage**

Upon receipt, store the Anti-8-iso-PGF2 $\alpha$  Antibody, 8-iso-PGF2 $\alpha$ -HRP Conjugate, and 8-iso-PGF2 $\alpha$  Standard at -20°C. Make aliquots as necessary to avoid freeze/thaw cycles. Store all other kit components at 4°C. 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-PGF2 $\alpha$  Antibody: Immediately before use, dilute the Anti-8-iso-PGF2 $\alpha$  Antibody 1:1000 with Sample Diluent.
- 8-iso-PGF2 $\alpha$ -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-PGF2 $\alpha$  Antibody, 8-iso-PGF2 $\alpha$ -HRP Conjugate, or 8-iso-PGF2 $\alpha$  Standard solutions.*

## **Preparation of Samples**

Hydrolysis of lipoprotein or phospholipid coupled 8-iso-Prostaglandin F2 $\alpha$  (8-iso-PGF2 $\alpha$ ) 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  $\mu$ L of concentrated (10N) HCl per 500  $\mu$ L 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  $\mu$ L of the sample to 100  $\mu$ L 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  $\mu$ L 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-PGF2 $\alpha$ Standards**

1. Prepare fresh standards by diluting the 8-iso-PGF2 $\alpha$  Standard from 200 $\mu$ g/mL to 0.2  $\mu$ g/mL in Sample Diluent for a 1:1000 final dilution. (Example: Add 5  $\mu$ L of 8-iso-PGF2 $\alpha$  Standard stock tube to 4.995 mL of Sample Diluent)
2. Prepare a series of the remaining 8-iso-PGF2 $\alpha$  standards according to Table 1.

<b>Standard Tubes</b>	<b>8-iso-PGF2<math>\alpha</math> Standard (<math>\mu</math>L)</b>	<b>Sample Diluent (<math>\mu</math>L)</b>	<b>8-iso-PGF2<math>\alpha</math> Standard (pg/mL)</b>
1	5 $\mu$ L of Standard Stock	4995 $\mu$ L	200,000
2	250 $\mu$ L of Tube #1	750 $\mu$ L	50,000
3	250 $\mu$ L of Tube #2	750 $\mu$ L	12,500
4	250 $\mu$ L of Tube #3	750 $\mu$ L	3,125
5	250 $\mu$ L of Tube #4	750 $\mu$ L	781
6	250 $\mu$ L of Tube #5	750 $\mu$ L	195
7	250 $\mu$ L of Tube #6	750 $\mu$ L	49
8	0 $\mu$ L	200 $\mu$ L	0

**Table 1. Preparation of 8-iso-PGF2 $\alpha$  Standard Curve.**

*Note: Do not store diluted 8-iso-PGF2 $\alpha$  Standard solutions.*

## **Assay Protocol**

*Note: Each 8-iso-PGF2 $\alpha$  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  $\mu$ L of the diluted Anti-8-iso-PGF2 $\alpha$  Antibody to the Goat Anti-Rabbit Antibody Coated Plate. Incubate 1 hour at 25°C on an orbital shaker.
2. Remove the antibody solution from the wells. Wash wells 5 times with 300  $\mu$ L 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.

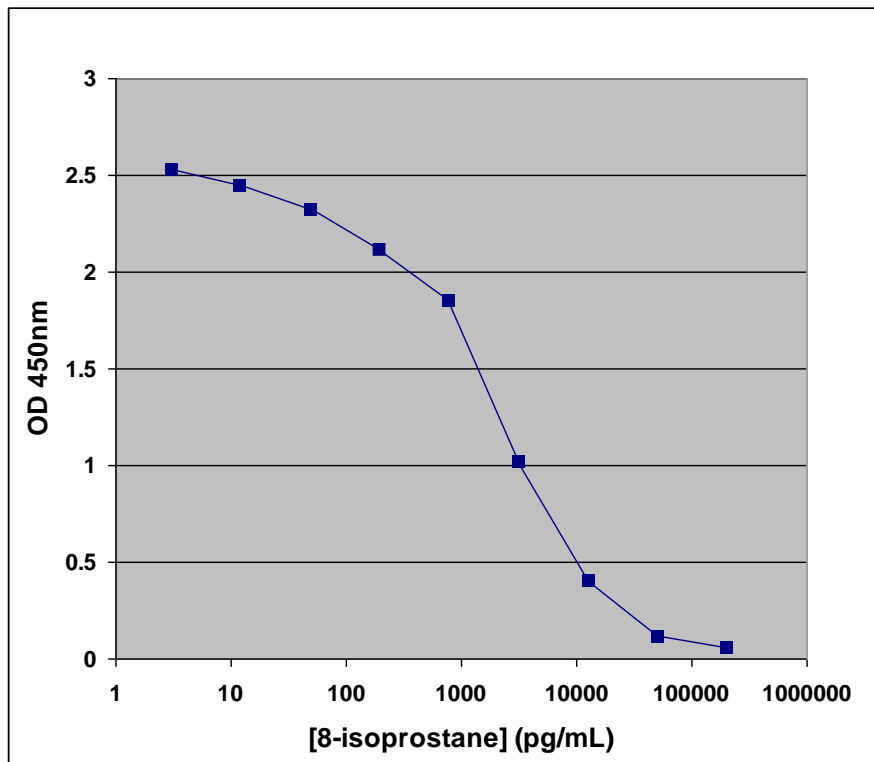
*Note: Thorough washing is necessary to remove all of the azide present in the antibody solution.*

3. Combine 55  $\mu$ L of the 8-iso-PGF2 $\alpha$  standard or sample and 55  $\mu$ L of 8-iso-PGF2 $\alpha$ -HRP conjugate in a microtube and mix thoroughly. Transfer 100  $\mu$ L of the combined solution per well. A well containing Sample Diluent can be used as a control. Incubate 1 hour at 25°C on an orbital shaker.
4. Remove the combined solution from the wells. Wash 5 times with 300  $\mu$ L 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.

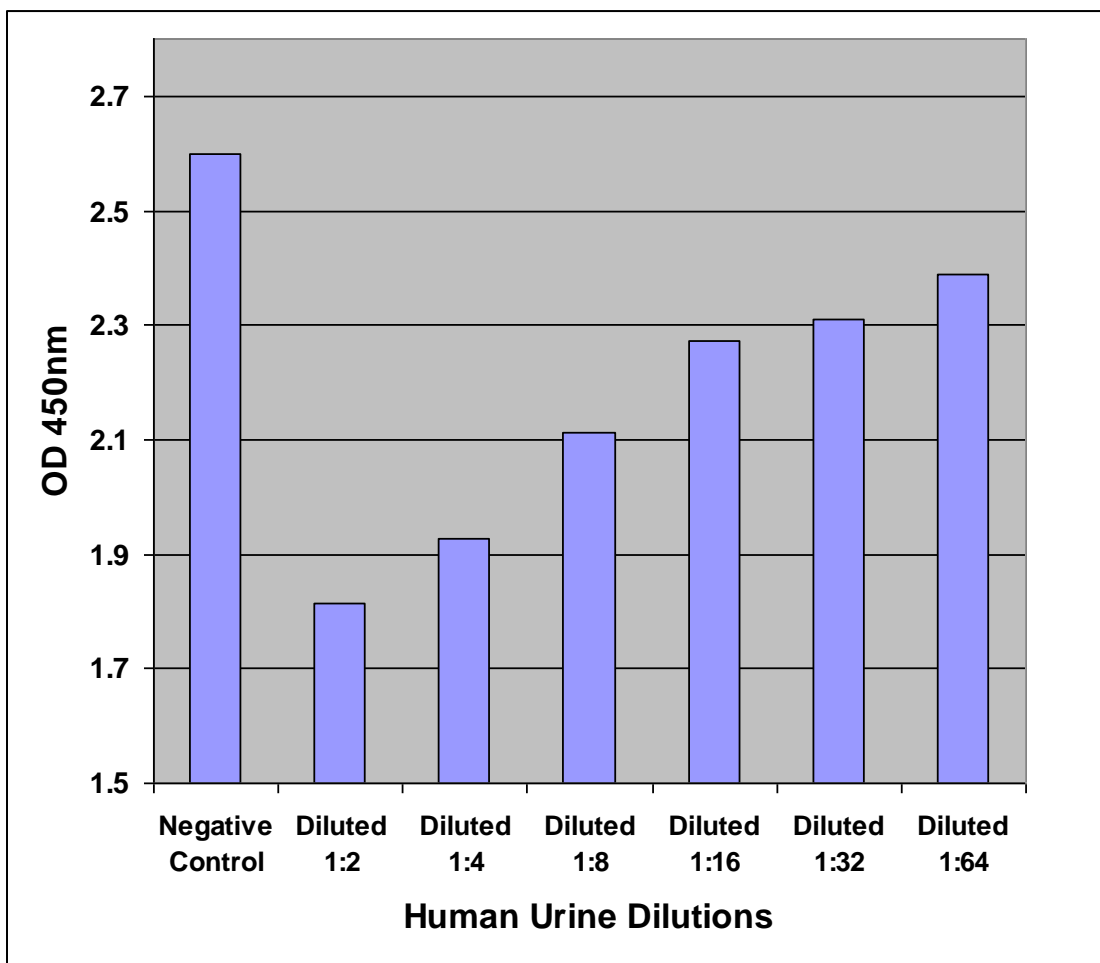
5. Add 100  $\mu\text{L}$  of Substrate Solution to each well. Incubate at room temperature for 10-30 minutes on an orbital shaker.
6. Stop the enzyme reaction by adding 100  $\mu\text{L}$  of Stop Solution to each well. Results should be read immediately (color will fade over time).
7. Read absorbance 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-PGF2 $\alpha$  results. One should use the data below for reference only. This data should not be used to interpret actual results.



**Figure 1: 8-iso-PGF2 $\alpha$  ELISA Standard Curve.**



**Figure 2: Dilutions of Human Urine tested with 8-iso-PGF2 $\alpha$  ELISA.**

**Cross reactivity of 8-iso-Prostaglandin F2 $\alpha$  ELISA Kit**

<u>Compounds</u>	<u>Cross Reactivity</u>
8-iso-PGF2 $\alpha$	100%
PGF1 $\alpha$	4.6%
PGF2 $\alpha$	1.85%
PGE1	0.19%
TXB2	0.023%
PGB1	0.02%
PGE3	0.012%
6-keto-PGF1 $\alpha$	0.008%
13,14-dihydro-15-keto-PGF2 $\alpha$	0.008%
6,15-keto-13,14-dihydro-PGF1 $\alpha$	0.005%
8-iso-PGE1	<0.001%
PGA2	<0.001%
PGJ2	<0.001%

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## Recent Product Citations

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