
Product Manual

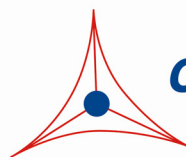
OxiSelect™ 8-iso-Prostaglandin F2 α ELISA Kit

Catalog Numbers

STA-337

96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

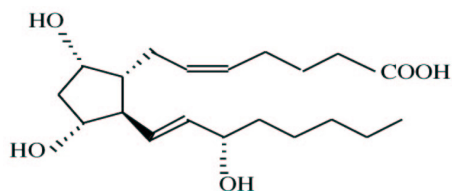


CELL BIOLABS, INC.
Creating Solutions for Life Science Research

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. The total normal plasma 8-iso-PGF₂α is about 40-100 pg/mL and about 190 pg/mg of creatine. 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)

Cell Biolabs' OxiSelect™ 8-iso-Prostaglandin F₂α ELISA Kit is an enzyme immunoassay developed for rapid detection and quantification of 8-iso-Prostaglandin F₂α. 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 phospholipids 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-330: OxiSelect™ TBARS Assay Kit (MDA Quantitation)
2. STA-334: OxiSelect™ HNE-His Adduct ELISA Kit
3. STA-340: OxiSelect™ Superoxide Dismutase Activity Assay
4. STA-341- OxiSelect™ Catalase Activity Assay Kit
5. STA-310: OxiSelect™ Protein Carbonyl ELISA Kit
6. STA-320: OxiSelect™ Oxidative DNA Damage ELISA Kit (8-OHdG Quantitation)
7. STA-325: OxiSelect™ Oxidative RNA Damage ELISA Kit (8-OHG Quantitation)
8. STA-350: OxiSelect™ Comet Assay (3-Well Slides)
9. STA-345: OxiSelect™ ORAC Activity Assay
10. STA-346: OxiSelect™ HORAC Activity Assay

Kit Components

1. Goat Anti-Rabbit Antibody Coated Plate (Part No. 250001): One 96-well strip plate.
2. Anti-8-iso-PGF2 α Antibody (Part No. 233701): One 20 μ L tube of anti-8-iso-PGF2 α rabbit IgG.
3. Sample Diluent (Part No. 233702): One 50 mL bottle.
4. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
5. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
6. Stop Solution (Part. No. 310808): One 12 mL bottle.
7. 8-iso-PGF2 α Standard (Part No. 233703): One 25 μ L tube of 200 μ g/mL 8-iso-PGF2 α in DMSO.
8. 8-iso-PGF2 α -HRP Conjugate (Part No. 233704): One 70 μ L tube of 8-iso-PGF2 α -HRP conjugate.

Materials Not Supplied

1. Protein samples such as purified protein, plasma, serum, cell lysate
2. Deionized water
3. 5 μ L to 1000 μ L adjustable single channel precision micropipettes with disposable tips
4. 50 μ L to 300 μ 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 α Antibody, 8-iso-PGF2 α -HRP Conjugate, and 8-iso-PGF2 α Standard at -20°C. Make aliquots as necessary to avoid freeze/thaw cycles. Store all other kit components at 4°C until their expiration dates. Any partial or unused components should return to their proper storage temperatures.

Safety Considerations

1. Some kit components contain azide, which can react with copper or lead piping. Flush with large volumes of water when disposing of reagents.
2. 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 α Antibody: Immediately before use, dilute the Anti-8-iso-PGF2 α Antibody 1:1000 with Sample Diluent.
- 8-iso-PGF2 α -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 α Antibody, 8-iso-PGF2 α -HRP Conjugate, or 8-iso-PGF2 α Standard solutions.

Preparation of Samples

Hydrolysis of lipoprotein or phospholipid coupled 8-iso-Prostaglandin F2 α (8-iso-PGF2 α) 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 μ L of concentrated (12.1N) HCl per 500 μ 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 \leq -20 °C for future use. If necessary check the pH of the neutralized samples. The pH should be in the range of 6-8. If it is not, adjust the pH to this range.

Urine Sample:

Urine samples can be used in the assay directly after diluting in Sample Diluent.

Preparation of 8-iso-PGF2 α Standards

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

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

Table 1. Preparation of 8-iso-PGF2 α Standard Curve.

Note: Do not store diluted 8-iso-PGF2 α Standard solutions.

Assay Protocol

Note: Each 8-iso-PGF2 α 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 μ L of the diluted Anti-8-iso-PGF2 α 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 μ 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 μ L of the 8-iso-PGF2 α standard or sample and 55 μ L of 8-iso-PGF2 α -HRP conjugate in a microtube and mix thoroughly. Transfer 100 μ 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 μ 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 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-PGF 2α results. One should use the data below for reference only. This data should not be used to interpret actual results.

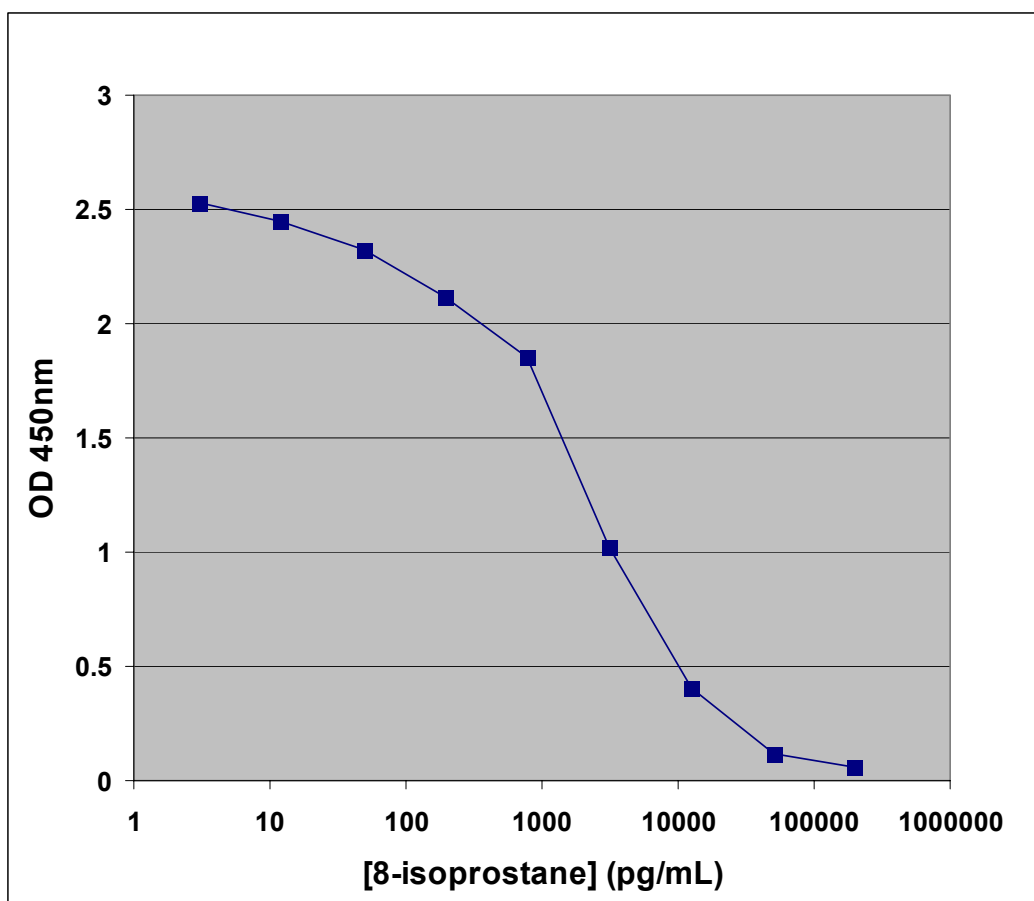


Figure 1: 8-iso-PGF 2α ELISA Standard Curve.

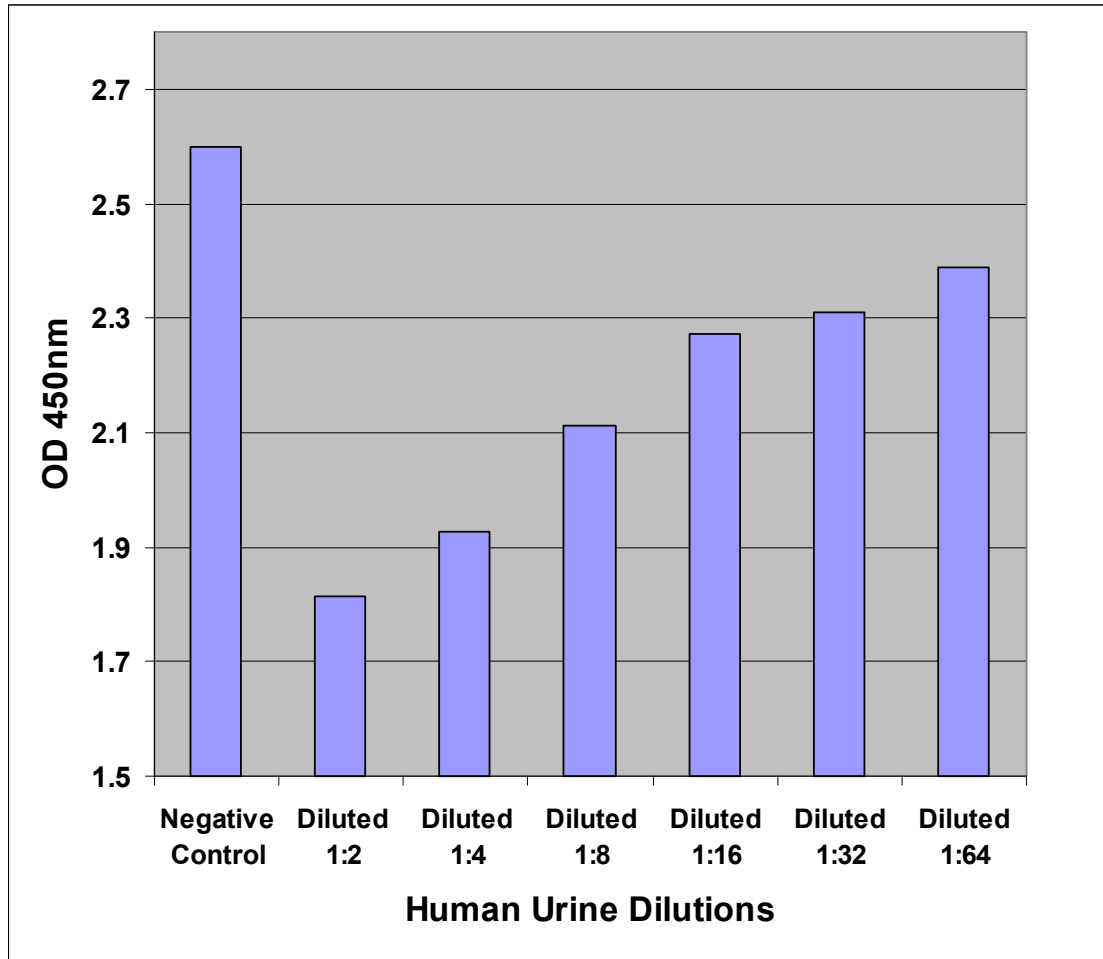


Figure 2: Dilutions of Human Urine tested with 8-iso-PGF2 α ELISA.

References

1. Banerjee, M., Kang, K.H., Morrow, J.D., et al. (1992) *Am. J. Physiol.* 263: H660-H663.
2. Morrow, J.D., Hill, K.E., Burk, R.F., et al. (1990) *Proc. Natl. Acad. Sci. USA.* 87: 9383-9387.
3. Morrow, J.D., Harris, T.M., Roberts, L.J. (1990) *Anal. Biochem.* 184: 1-10.
4. Vacchiano, C.A., and Tempel, G.E. (1994) *J. Appl. Physiol.* 77: 2912-2917.
5. Wang, Z., Ciabattini, G., Cre'minon, C., et al. (1995) *Pharmacol. Exp. Ther.* 275: 94-100.

Warranty

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