Datasheet for ABIN2344990

8isoPGF2a ELISA Kit

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Overview

Quantity:	96 tests
Target:	8isoPGF2a
Reactivity:	Others
Method Type:	Cell ELISA
Application:	ELISA

Product Details

Purpose:	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.
Brand:	OxiSelect™
Sample Type:	Plasma, Serum
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Characteristics:	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.
Components:	1. Goat Anti-Rabbit Antibody Coated Plate : One 96-well strip plate. 2. Anti-8-iso-PGF2α Antibody : One 20 µL tube of anti-8-iso-PGF2α rabbit IgG. 3. Sample Diluent : One 50 mL bottle.

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Product Details

	4. Neutralization Solution : One 20 mL bottle. 5. 10X Wash Buffer : One 100 mL bottle. 6. Substrate Solution : One 12 mL amber bottle. 7. Stop Solution (Part. No. 310808): One 12 mL bottle. 8. 8-iso-PGF2α Standard : One 25 µL tube of 200 µg/mL 8-iso-PGF2α in DMSO. 9. 8-iso-PGF2α-HRP Conjugate : One 70 µL tube of 8-iso-PGF2α-HRP conjugate.
Material not included:	1. Protein samples such as purified protein, plasma, serum, cell lysate 2. Deionized water 3. 5 µL to 1000 µL adjustable single channel precision micropipettes with disposable tips 4. 50 µL to 300 µL 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 3 9. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Target Details

Target:	8isoPGF2a
Abstract:	8isoPGF2a Products
Background:	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

Target Details

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)

Application Details

Application Notes:	Optimal working dilution should be determined by the investigator.
Comment:	• Quantify 8-isoprostane in about 3 hours • Suitable for use with urine, plasma, serum, or cell lysates • 8-iso-PGF2alpha included as positive control
Plate:	Uncoated
Protocol:	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 2 sample is determined by comparing with the known predetermined standard curve. Please read the complete kit insert prior to performing the assay.
Reagent Preparation:	• 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.
Sample Preparation:	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 p er 5 0 0µ 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 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. 4 Preparation of 8-iso-PGF2α Standards

1. Prepare fresh standards by diluting the 8-iso-PGF2α Standard from 200 µg/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 8-iso-PGF2α Standard 8-iso-PGF2α Standard Tubes (µL) Sample Diluent (µL) (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 Procedure:

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 25 °C 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 25 °C 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
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

Application Details

	length.
Restrictions:	For Research Use only

Handling

Precaution of Use:	• 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.
Handling Advice:	Avoid multiple freeze/thaw cycles.
Storage:	4 °C/-20 °C
Storage Comment:	Upon receipt, store the Anti-8-iso-PGF2α Antibody, 8-iso-PGF2α-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. Any partial or unused components should return to their proper storage temperatures.

Publications

Product cited in:	Konstantinou, Gaengler, Oikonomou, Delplancke, Charisiadis, Makris et al.: "Use of metabolomics in refining the effect of an organic food intervention on biomarkers of exposure to pesticides and biomarkers of oxidative damage in primary school children in Cyprus: A ..." in: **Environment international**, Vol. 158, pp. 107008, (2022) (PubMed). Makris, Efthymiou, Konstantinou, Anastasi, Schoeters, Kolossa-Gehring, Katsonouri: "Oxidative stress of glyphosate, AMPA and metabolites of pyrethroids and chlorpyrifos pesticides among primary school children in Cyprus." in: , Vol. 212, Issue Pt B, pp. 113316, (2022) (PubMed). Makris, Konstantinou, Andrianou, Charisiadis, Kyriacou, Gribble, Christophi: "A cluster-randomized crossover trial of organic diet impact on biomarkers of exposure to pesticides and biomarkers of oxidative stress/inflammation in primary school children." in: **PloS one**, Vol. 14, Issue 9, pp. e0219420, (2019) (PubMed). Fukuhara, Nakashima, Abe, Masuda, Hamada, Iwamoto, Fujitaka, Kohno, Hattori: "Suplatast tosilate protects the lung against hyperoxic lung injury by scavenging hydroxyl radicals." in: **Free radical biology & medicine**, Vol. 106, pp. 1-9, (2017) (PubMed).

Publications

Bironneau, Goupil, Ducluzeau, Le Vaillant, Abraham, Henni, Dubois, Paris, Priou, Meslier, Sanguin, Trzépizur, Andriantsitohaina, Martinez, Gagnadoux: "Association between obstructive sleep apnea severity and endothelial dysfunction in patients with type 2 diabetes." in: **Cardiovascular diabetology**, Vol. 16, Issue 1, pp. 39, (2017) (PubMed).

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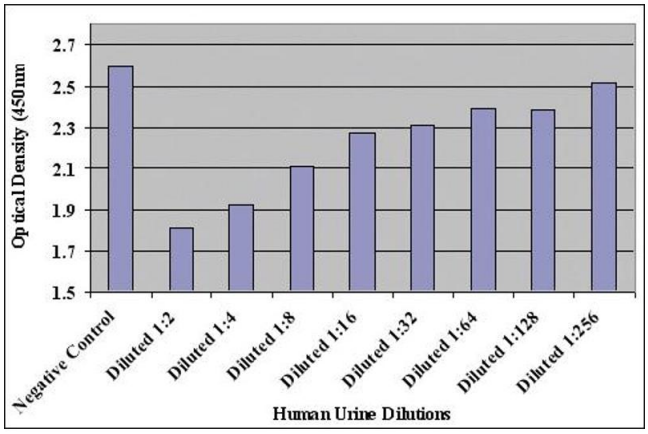
Images



ELISA

Image 1. Dilutions of Human Urine Tested with the OxiSelect™ 8-iso-Prostaglandin F2alpha ELISA Kit.