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Northwest
Life Science Specialties, LLC

Premier Products for Superior Life Science Research

NWLSS™ Urinary Isoprostane ELISA

Product NWK-ISO02

For Research Use Only

Assay system for measurement of 15-isoprostane F_{2t} in urine without the need to extract the samples prior to testing

New, simpler format allows application of diluted samples directly to microwells without any pretreatment.

nwkiso02rev011008

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Introduction:
Isoprostanes are prostaglandin-like compounds that are produced by free radical mediated peroxidation of lipoproteins. This kit is for the quantification of 15-isoprostane F_{2t} (also known as 8-epi-$PGF_{2\alpha}$ or 8-iso-$PGF_{2\alpha}$) in urine samples. Levels of 15-isoprostane F_{2t} in urine are useful for the non-invasive assessment of oxidant stress *in vivo*. 15-isoprostane F_{2t} has also been shown to be a potent vasoconstrictor in rat kidneys and rabbit lungs, and plays a causative role in atherogenesis. Elevated isoprostane levels are associated with hepatorenal syndrome, rheumatoid arthritis, atherosclerosis, and carcinogenesis. This kit can be used for the quantification of free 15-isoprostane F_{2t} in urine serum samples without the need for prior purification or extraction.

Intended Use:
This kit is intended for the quantification of free 15-isoprostane F_{2t} in urine, the best characterized Isoprostane, without the need to extract the samples prior to testing.

NOTE: Plasma, tissue or cell lysates may be tested using NWLSS product number NWK-ISO01 assay for 8-isoprostane. Detailed extraction procedures are included with the product.

Test Principle:
This assay utilizes a competitive enzyme-linked immunoassay (ELISA) strategy. Samples are first diluted with an Enhancer/Dilution Buffer that works to minimize nonspecific binding. After dilution, 15-isoprostane F_{2t} in the samples or standards is allowed to compete with 15-isoprostane F_{2t} conjugated to horseradish peroxidase (HRP) for binding to a polyclonal antibody specific for 15-isoprostane F_{2t} coated on a microplate. TMB substrate addition results in a blue color development that is inversely proportional to the quantity of 15-isoprostane F_{2t} in the original samples or standards. Addition of an acid stop solution causes a color change to yellow where absorbance is read at 450 nM.

General Specifications:

Format:	96 well competitive ELISA
Number of tests:	Triplicate = 24 Duplicate= 40
Specificity:	15-isoprostane F_{2t} (8-Isoprostane)
Sensitivity:	0.05 ng (50 pg)
Effective Range:	0.1ng/mL - 10 ng/mL

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Kit Contents

Microwells precoated with Anti-15-isoprostane F_{2t}:	1 X 96 wells
15-isoprostane F_{2t} Standard:	2 X 60 µL
Enhancer/Dilution Buffer	1 X 100 mL
5X Wash Buffer:	1 X 40 mL
TMB Substrate:	1 X 25 mL
15-isoprostane F_{2t} HRP Conjugate:	1 X 250 µL
Reagent Trough:	2 Each

Required Materials Not Provided:

Adjustable pipettes with a range of 10 µL to 1,000 µL with disposable tips.

Glassware for preparation of reagents.

Reagents for quantification of Creatinine to normalize 15-isoprostane F_{2t} values obtained. NWLSS offers product number NWK-CRT01 for this purpose

Deionized water.

3M sulfuric acid.

Required Instrumentation:

Microtiter plate reader with 450 nm capability.

Warnings, Limitations, Precautions:

Individual components may be harmful if swallowed, inhaled or absorbed through the skin. Contact should be minimized through the use of gloves and standard good laboratory practices. If contact with skin or eyes occurs, rinse the site immediately with water and consult a physician.

Storage Instructions:

Store all components at 4°C until immediately before use. Do not freeze.

Assay Preparation

1. Determine the number of wells required to assay standards, samples and controls for the appropriate replicate.

2. Create an assay template showing positioning of standards, controls and samples. Include blank wells also.

3. Bring all samples and reagents to room temperature before use. To avoid condensation, do not open foil pouches containing the microtiter strips until after they have reached room temperature.

REFERENCES

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3. Morrow, et. al., The Isoprostanes: Unique Bioactive Products of Lipid Peroxidation. *Prog. Lipid Res.* 36:1-21, 1997.

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7. Roberts II, L.J., Morrow, J.D., Measurement of F2-isoprostanes as an index of oxidative stress in vivo, Free *Radical. Biol. Med.* 28:505-513 (2000).

8. Morrow, J.D., Roberts II, L.J., Mass Specrometric Quantification of F2-Isoprostanes in Biological Fluids and Tissues as Measure of Oxidant Stress. Meth. Enz. 300: 3-12.

Statement of Limited Warranty:

Northwest Life Science Specialties, LLC (NWLSS) makes no guarantee of any kind, expressed or implied, that extends beyond the description of the material in this kit, except that they will meet our specifications at the time of delivery. Customer's remedy and NWLSS' sole liability is limited to, at NWLSS' option, refund of the purchase price, or the replacement of material not meeting our specification. By acceptance of our product, customer assumes all liability and will indemnify and hold NWLSS harmless for the consequence of this product's use or misuse by the customer, its employees, or others. Refund or replacement is conditioned of customer notifying NWLSS within twenty-one (21) days of the receipt of product. Failure to give notice within 21 days shall constitute a waiver by the customer of all claims hereunder with respect to said product.

Performance Details:

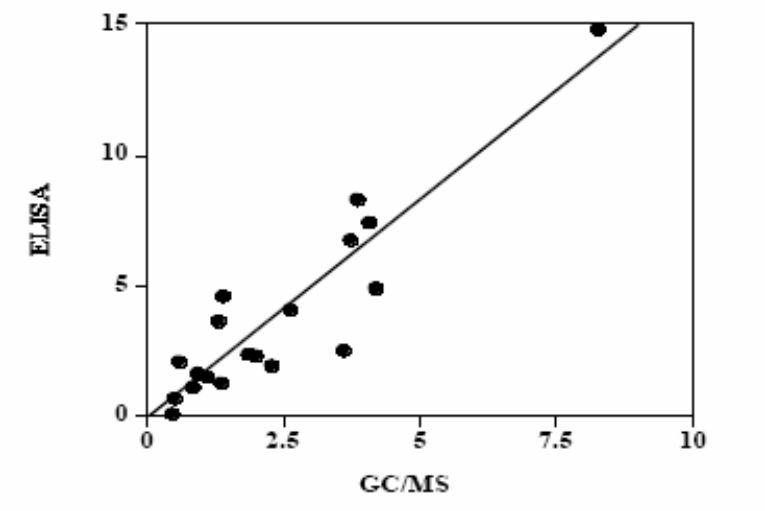
Cross reactivity at 50% B/B0

15-isoprostane F_{2t}	**100.0%**
9α,11β-Prostaglandin$F_{2\alpha}$	4.1%
13,14-Dihydro-15-Keto-$PGF_{2\alpha}$	3.0%
9β,11α-Prostaglandin $F_{2\alpha}$	< 0.01%
Prostaglandin $F_{2\alpha}$	< 0.01%
6-Keto-Prostaglandin F□$_{\alpha}$	< 0.01%
Prostaglandin E2	< 0.01%
Prostaglandin D2	< 0.01%
Arachidonic Acid	< 0.01%

Method Comparison:

The concentrations of 15-isoprostane F_{2t} in several human urine samples were determined by immunoassay and by GC/MS following solid phase extraction of separate aliquots, and a correlation (r^2) of > 0.8 was obtained. See Figure 2.

Figure 2.



Reagent Preparation:

The following instructions are based on the user using the entire kit at one time. If portions of the kit are to be used at a later time, smaller quantities may be prepared saving the remaining stock for later use.

Enhancer/Dilution Buffer is supplied ready to use.

TMB Substrate is supplied ready to use.

5X Wash Buffer

Add the contents of the 5X Wash Buffer to 160 mL deionized H_2O, mix well and label as **Working Wash Buffer.**

50X 15-isoprostane F_{2t} HRP-Enzyme Conjugate

Briefly centrifuge the vial to remove all liquid from the cap and vial walls. Pre-wet the pipet tip in the conjugate then add 240 µL conjugate to 11.76 mL Working Dilution Buffer. Label as **Diluted HRP-Conjugate.**

Standard Preparation:

Standard Supplied: 15-isoprostane F_{2t}: 2 X 60 µL at 1 µg/mL in ETOH.

Standard 7 (S_7): Add 540 µL of Enhancer/Dilution Buffer to **one vial** of the Standard supplied. *Label as S_7 with a concentration of* ***100 ng/mL.***

Standard 6 (S_6): Add 250 µL of S_7 to 250 µL Enhancer/Dilution Buffer and vortex. *The S_6 concentration is now* ***50 ng/mL.***

Standard 5 (S_5): Add 150 µL of S_6 to 600 µL Enhancer/Dilution Buffer and vortex. *The S_5 concentration is now* ***10 ng/mL.***

Standard 4 (S_4): Add 250 µL of S_5 to 250 µL Enhancer/Dilution Buffer and *vortex. The S_4 concentration is now* ***5 ng/mL.***

Standard 3 (S_3): Add 100 µL of S_4 to 400 µL Enhancer/Dilution Buffer and vortex. *The S_3 concentration is now* ***1 ng/mL.***

Standard 2 (S_2): Add 75 µL of S_3 to 675 µL Enhancer/Dilution Buffer and vortex. *The S_2 concentration is now* ***0.1 ng/mL****.*

Standard 1 (S_1): Add 200 µL of S_2 to 200 µL Enhancer/Dilution Buffer and vortex. *The S_1 concentration is now* ***0.05 ng/mL.***

Standard 0 (S_0): Add 400 µL Enhancer/Dilution Buffer only to this tube. It will be used to determine maximal color development.

Sample Handling/Preparation

Dilute urine samples 1:4 or 1: 8 with Enhancer/Dilution Buffer. The extent of dilution required for accurate quantification will vary depending on the 15-isoprostane F_{2t} levels in the samples and will have to be determined by the individual user.

Assay Protocol:

1. Add 100 µL of Standard or Diluted Urine Sample to each well.

2. Add 100 µL of Diluted HRP-Conjugate to each well except reagent blank. Add 100 µL Enhancer/Dilution Buffer to reagent blank wells. Allow plate to stand for 2 hours at Room Temperature.

3. Invert plate and empty contents. Pat dry upside-down on a lint free towel.

4. Wash plate with 300µL Working Wash Buffer 3 times allowing plate to stand 2 minutes per wash. Empty plate again by inversion and pat dry upside-down on a lint free towel after final wash.

5. Add 200 µL TMB Substrate to each well, allow to stand for 20-40 minutes allowing blue color to develop maximally in S_0 wells.

6. Add 50 µL of 3M sulfuric acid to each well to stop the reaction. This changes the color to yellow.

6. Record the absorbance at 450 nm using a plate reader.

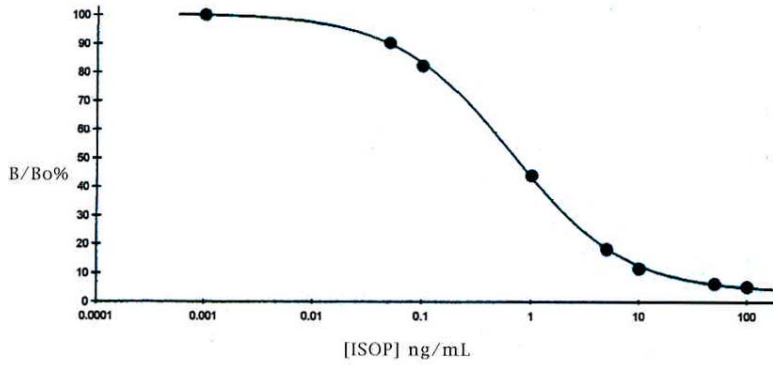
Data Analysis

1. Average the reagent blank absorbance values and subtract this average from each well. Most modern microplate readers are capable of doing this automatically.

2. Average standard replicates (S_1 through S_7) and divide by the average obtained for S_0 (Zero Bound or B_0) to express data as a percent of B_0.

3. Graph $\%B_0$ values (y-axis-linear) vs. standard concentration (x-axis-logarithmic) to obtain a standard curve. Figure 1 shows a typical curve obtained when plotting concentration vs. percent bound in this fashion.

Figure 1.



4. Average the replicates of each unknown and divide by the average S_0 (B_0) value to express data in terms of $\%B_0$, then determine corresponding concentration using the standard curve. Remember to account for any dilution factor incurred by samples prior to assay.