8-Epi-PGF2-alpha ELISA Kit

Catalog Number KA0443

96 assays

Version: 03

Intended for research use only

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Introduction

Intended Use

Isoprostanes are prostanglandin-like compounds that are produced by peroxidation of lipoproteins (1,2). 8-epi-prostaglandin-$F_{2\alpha}$ has been shown to be a potent vasoconstrictor in rat kidneys (3) and rabbit lungs (4). Isoprostanes may also play a role in atherosclerosis (5,6). Measurement of isoprostanes concentration may be helpful in assessment of oxidative stress, hepatorenal syndrome, rheumatoid arthritis, atherosclerosis and carcinogenesis (7). This kit can be used for the quantitation of free 8-epi-prostaglandin-$F_{2\alpha}$ in urine samples without the need for prior purification or extraction.

Principle of the Assay

This 8-Epi-PGF2-alpha ELISA Kit is a competitive enzyme-linked immunoassay (ELISA) for determining levels of 8-epi-prostaglandin-$F_{2\alpha}$ in urine samples. Briefly, the samples are mixed with an enhancing reagent that essentially eliminates interferences due to non-specific binding. The 8-epi-prostaglandin-$F_{2\alpha}$ in the sample or standard then competes with 8-epi-prostaglandin-$F_{2\alpha}$ conjugated to horseradish peroxidase (HRP Conjugate) for binding to a polyclonal antibody specific for 8-epi-prostaglandin-$F_{2\alpha}$ coated on the microplate. Following substrate addition, the intensity of the color is inversely proportional to the amount of unconjugated 8-epi-prostaglandin-$F_{2\alpha}$ in the sample or standard.

General Information

Materials Supplied

List of component

Component	Amount
96-well micortiter plate, pre-coated with 8-epi-prostaglandin-F2α antibody	1
8-epi-prostaglandin-$F_{2\alpha}$ Standards (1µg/mL)	2 x 60 µL
Enhanced Dilution Buffer	100 mL
Wash Buffer (5x)	40 mL
TMB Substrate (Tetramethylbenzidine)	25 mL
HRP Conjugate	250 µL
Disposable reagent troughs for a multichannel pipettor	2
Stop Solution	5 mL

Storage Instruction

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

Materials Required but Not Supplied

- ✓ Precision pipettes with disposable tips. A multichannel pipette is helpful, but not required.
- ✓ 96-well microplate reader for measurement of absorbance at 450 nm.
- ✓ Reagents for the quantification of creatinine for normalization.
- ✓ Deionized water.

Precautions for Use

- ✓ Do not smoke, eat or drink in areas where samples and reagents are handled.
- ✓ Wear disposable gloves when handling samples and reagents.
- ✓ Do not pipette reagents or samples by mouth.
- ✓ In case of accidental exposure of skin, mucous membranes or eyes to the components of this kit, thoroughly wash the exposed area with water.
- ✓ For in vitro use only. For research purposes only. Not for use in diagnostic procedures.

Assay Protocol

Note: This product is intended for use with urine samples and has not been validated for use with serum, tissue culture supernatants or tissue extracts.

The following instructions are based on using the entire kit (all of the wells at one time). If portions of the kit are to be used at a later time, one may desire to prepare smaller quantities and save the remaining stock for later use.

Reagent Preparation

1. Substrate and Enhanced Dilution Buffer: Ready to use.
2. Add the 5x Wash Buffer (40 mL) to 160 ml of deionized water, mix well.
3. HRP Conjugate:
   a. Centrifuge vial before removing the cap.
   b. Prepare a 1/50 dilution of the HRP conjugate of sufficient volume for the amount of samples to be run that day. For the performance of the entire assay, add 240 µL of conjugate to 11.760 ml of Enhanced Dilution Buffer.

Preparation of Standards

Prepare a series of standards by diluting the 8-epi-prostaglandin-$F_{2\alpha}$ Standards (1µg/ml) to the following concentrations: 100, 50, 10, 5, 1, 0.1 and 0.05 ng/ml.

- ✓ S7: Add 50 µL of Standard to 450 µL of Dilution buffer (1x) = 100 ng/ml
- ✓ S6: Add 200 µL of S7 to 200 µL of Dilution Buffer (1x) = 50 ng/ml
- ✓ S5: Add 100 µL of S6 to 400 µL of Dilution Buffer (1x) = 10 ng/ml
- ✓ S4: Add 200 µL of S5 to 200 µL of Dilution Buffer (1x) = 5 ng/ml
- ✓ S3: Add 100 µL of S4 to 400 µL of Dilution Buffer (1x) = 1 ng/ml
- ✓ S2: Add 100 µL of S3 to 900 µL of Dilution Buffer (1x) = 0.1 ng/ml
- ✓ S1: Add 500 µL of S2 to 500 µL of Dilution Buffer (1x) = 0.05 ng/ml
- ✓ S0: Dilution Buffer (1x) only.

Sample Preparation

Make multiple dilutions of each sample, e.g.1:2; 1:4; 1:8 using Dilution Buffer (1x).Optimal dilution will need to be determined by the researcher.

Assay Procedure

1. Remove microplate from foil pouch.
2. Pipet 100 µL of the prepared standard or sample mixture into each well.

3. Pipet 100 µL of diluted HRP Conjugate to each well, except for the reagent blank (S0). Add 100 µL of Dilution Buffer to blank, instead.
4. Seal plate or place in humidity chamber.
5. Allow the plate to stand at room temperature for 2 hours.
6. Empty the contents and blot the plate on a lint free towel.
7. Add 300 µL of diluted Wash Buffer (1x) and let stand 2-3 minutes.
8. Repeat step 6 and 7 two additional times.
9. Empty the contents and blot the plate on a lint free towel.
10. Add 200 µL TMB Substrate to each well. Incubate for 20-40 minutes at room temperature, until a blue hue can be seen for the blank standard, S0.
11. Add 50 µL of Stop Solution to each well. The color in each well should change from blue to yellow. Read absorbance at 450 nm.

Data Analysis

Calculation of Results

An example of what the Standard Cure should look like is pictured below. The Standard Curve is obtained by fitting the Standard absorbances at 450 nm to the concentration of 8-epi-prostaglandin-$F_{2\alpha}$ by a logistic curve fit method. Average absorbance values obtained for the Blanks and subtract this value from all other wells.

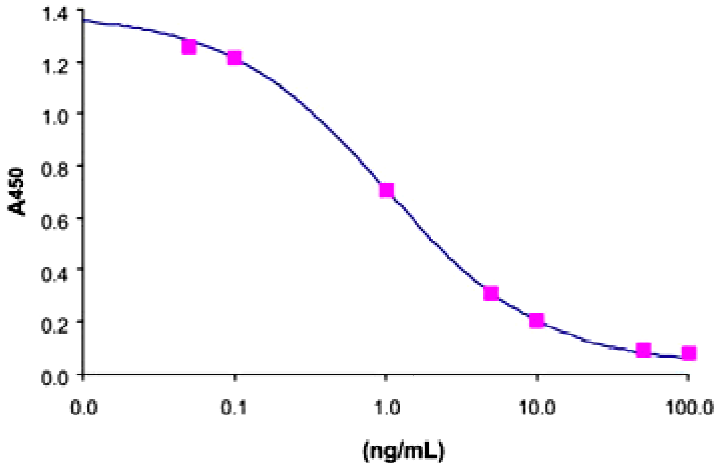

Figure 1: 8-epi-Prostaglandin-$F_{2\alpha}$ Standard Curve

Performance Characteristics

- Specificity*

8-epi-Prostaglandin $F_{2\alpha}$	100.0%
9_{α},11β-Prostaglandin $F_{2\alpha}$	4.1%
13,14-Dihydro-15-Keto-$PGF_{2\alpha}$	3.0%
9_{α},11β-Prostaglandin $F_{2\alpha}$	<0.01%
Prostaglandin $F_{2\alpha}$	<0.01%
6-Keto-Prostaglandin $F_{1\alpha}$	<0.01%
Prostaglandin E_2	<0.01%
Prostaglandin D_2	<0.01%
Arachidonic Acid	<0.01%

*Cross reactivity at mid-point of the Standard Curve.

Resources

References

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Plate Layout

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