RayBio® Human/Mouse/Rat Isoprostane (8-iso PGF2α) Enzyme Immunoassay Kit

Catalog #: EIA-IPF2A, EIAM-IPF2A, EIAR-IPF2A

User Manual
Last revised April 30th, 2019

Caution:
Extraordinarily useful information enclosed

RayBiotech
Empowering your proteomics

ISO 13485 Certified

3607 Parkway Lane, Suite 100
Norcross, GA 30092
Tel: 1-888-494-8555 (Toll Free) or 770-729-2992, Fax:770-206-2393
Web: www.RayBiotech.com, Email: info@raybiotech.com

Table of Contents

Please read the entire manual carefully before starting your experiment

I. Introduction

Isoprostane (8-iso PGF2α) is prostaglandin-like compound formed in vivo from the free radical-catalyzed peroxidation of essential fatty acids without the direct action of cyclooxygenase enzymes. Measurement of the concentration of isoprostane in blood or urine is now a well-established method for the diagnostic assessment of oxidative stress.

Synonyms

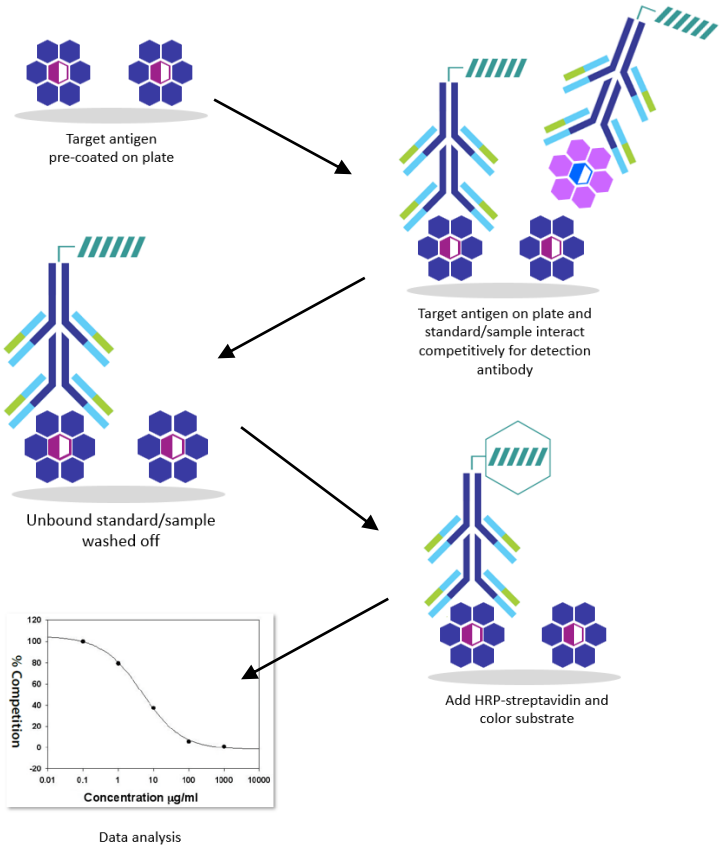
- iPF2α-III
- 8-iso-15(S)-Prostaglandin F2α
- 8-Isoprostane
- 8-epi PGF2α
- 15-F2t-Isoprostane
- 8-iso-PGF2a

II. General Description

The RayBio® Isoprostane Enzyme Immunoassay (EIA) Kit is an in vitro quantitative assay for detecting Isoprostane based on the competitive enzyme immunoassay principle.

In this assay, the samples and standards are added to the plate, where endogenous Isoprostane or the standards competes with the pre-coated Isoprostane for binding to the anti-Isoprostane antibody. After a wash step, any bound Isoprostane antibody then interacts with horseradish peroxidase (HRP)-secondary antibody, which catalyzes a color development reaction. The intensity of the colorimetric signal is directly proportional to the amount of bound Isoprostane antibody and inversely proportional to the amount of endogenous Isoprostane in the standard or samples. A standard curve of known concentration of Isoprostane can be established and the concentration of Isoprostane in the samples can be calculated accordingly.

III. How It Works



IV. Storage

The entire kit may be stored at -20°C to -80°C for up to 6 months from the date of shipment. For extended storage, it is recommended to store at -80°C. **Avoid repeated freeze-thaw cycles.** For prepared reagent storage, see table below.

V. Reagents

Component	**Size / Description**	**Storage / Stability <u>After Preparation</u>**
EIA Microplate (Item A)	96 wells (12 strips x 8 wells) coated with Isoprostane.	1 month at 4°C*
Wash Buffer Concentrate (20X) (Item B)	25 ml of 20X concentrated solution.	1 month at 4°C
Standard Isoprostane (Item C)	2 vials of Lyophilized Isoprostane . 1 vial is enough to run each standard in duplicate.	The first standard: 2-3 days at 4°C Additional dilutions: Do not store
Anti-Isoprostane Polyclonal Antibody (Item N)	2 vials of Lyophilized anti-Isoprostane.	1 month at 4°C
Assay Diluent B (Item E)	15 ml of 5X concentrated buffer. Diluent for standards, cell culture media or other sample types, and HRP-Secondary antibody.	1 month at 4°C
HRP-conjugated secondary antibody (Item D3)	25 µl 500X concentrated HRP-conjugated secondary antibody.	Do not store and reuse
Positive Control (Item M)	1 vial of Lyophilized Positive Control.	2-3 days at 4°C
TMB One-Step Substrate Reagent (Item H)	12 ml of 3,3,5,5'-tetramethylbenzidine (TMB) in buffer solution.	N/A
Stop Solution (Item I)	8 ml of 0.2 M sulfuric acid.	N/A

*Return unused wells to the pouch containing desiccant pack, reseal along entire edge.

VI. Additional Materials Required

1. Microplate reader capable of measuring absorbance at 450 nm
2. Precision pipettes to deliver 2 µl to 1 ml volumes
3. Adjustable 1-25 ml pipettes for reagent preparation
4. 100 ml and 1 liter graduated cylinders
5. Absorbent paper
6. Distilled or deionized water
7. SigmaPlot software (or other software which can perform four-parameter logistic regression models)
8. Tubes to prepare standard or sample dilutions
9. Orbital shaker
10. Aluminum foil
11. Plastic wrap

VII. Reagent Preparation

Keep kit reagents on ice during reagent preparation steps.

A. **Preparation of Plate and Anti-Isoprostane Antibody**

1. Equilibrate plate to room temperature before opening the sealed pouch.

2. Label removable 8-well strips as appropriate for your experiment.

3. 5X Assay Diluent B (Item E) should be diluted 5-fold with deionized or distilled water.

4. Briefly centrifuge the anti-Isoprostane antibody vial (Item N) and reconstitute with 30 µl of 1X Assay Diluent B to prepare the antibody concentrate. Pipette up and down to mix gently.

5. The antibody concentrate should then be diluted 100-fold with 1X Assay Diluent B This is your anti-Isoprostane antibody working solution, which will be used in step 2 of Assay Procedure (Section VIII).

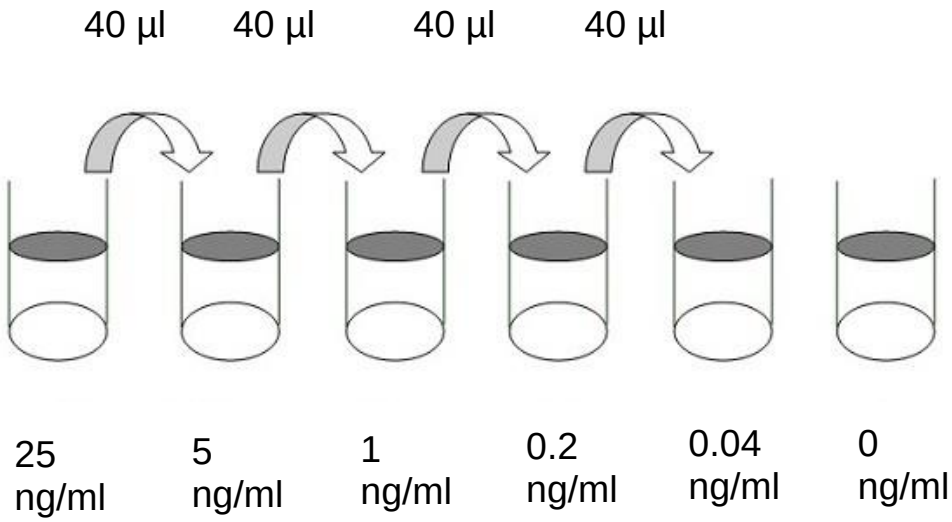
B. Preparation of Standards

6. Label 5 microtubes with the following concentrations: 5 ng/ml, 1 ng/ml, 0.2 ng/ml, 0.04 ng/ml and 0 ng/ml. Pipette 160 µl of 1x Assay Diluent B into each tube.

7. Briefly centrifuge the vial of Isoprostane Standard (Item C). Reconstitute with 200ul µl of 1x Assay Diluent B. Mix thoroughly. This solution serves as the first standard (25 ng/ml).

8. To make the 5 ng/ml standard, pipette 40 µl of the 25 ng/ml Isoprostane standard into the tube labeled 5 ng/ml. Mix thoroughly.

9. Repeat this step with each successive concentration, preparing a dilution series as shown in the illustration below. Each time, use 160 µl of 1x Assay Diluent B and 40 µl of the prior concentration until the 0.04 ng/ml is reached. Mix each tube thoroughly before the next transfer.



C. Positive Control Preparation

10. Briefly centrifuge the Positive Control vial (Item M) and reconstitute with 100 µl of ddH_2O. The Positive Control is a mouse serum sample that serves as a system control to verify that the kit components are working. The resulting OD will not be used in any calculations; if no positive competition is observed please contact RayBiotech Technical Support. The Positive Control may be diluted further if desired.

D. Sample Preparation

11. If you wish to perform a dilution of your sample, dilute your sample with the 1x Assay Diluent B.

    Note: Optimal sample dilution factors should be determined empirically, however you may reference below for recommended dilution factors for serum:
    Human=2X Mouse=4X Rat=4X.
    If you have any questions regarding the recommended dilutions you may contact technical support at 888-494-8555 or techsupport@raybiotech.com.

E. Preparation of Wash Buffer and HRP

12. If Item B (20X Wash Concentrate) contains visible crystals, warm to room temperature and mix gently until dissolved.

13. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1X Wash Buffer.

14. Briefly centrifuge the HRP-Secondary Antibody vial (Item D3) before use.

15. Dilute the HRP-Secondary Antibody concentrate 500-fold with 1X Assay Diluent B.

VIII. Assay Procedure

1. Keep kit reagents on ice during reagent preparation steps. It is recommended that all standards and samples be run at least in duplicate.

2. Add 50 µl of Anti-Isoprostane Antibody (Item N) (See Reagent Preparation step 5) to each well except blank wells. Add 50 µl of each standard (see Reagent Preparation Section B), Positive Control (see Reagent Preparation Section C) and sample (see Reagent Preparation Section D) to appropriate wells. Be sure to include a blank well (Assay Diluent only). Cover wells and incubate for 2.5 hours at room temperature with gentle shaking (1-2 cycles/sec) or overnight at 4ºC.

3. Discard the solution and wash wells 4 times with 1X Wash Solution Buffer (200-300 µl each). Washing may be done with a multichannel pipette or an automated plate washer. Complete removal of liquid at each step is essential to good assay performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

4. Add 100 µl of prepared HRP-Secondary antibody solution (see Reagent Preparation step 17) to each well. Incubate for 45 minutes at room temperature with gentle shaking. It is recommended that incubation time should not be shorter or longer than 45 minutes.

5. Discard the solution and wash 4 times as directed in Step 3.

6. Add 100 µl of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking (1-2 cycles/sec).

7. Add 50 µl of Stop Solution (Item I) to each well. Read at 450 nm immediately.

IX. Assay Procedure Summary

1. Prepare all reagents, samples and standards as instructed.

2. Add 50 µl anti-Isoprostane to each well.

3. Add 50 µl standard or sample to appropriate well. Incubate 2.5 hours at room temperature or overnight at 4ºC.

4. Add 100 µl prepared Secondary antibody solution. Incubate 45 minutes at room temperature.

5. Add 100 µl TMB One-Step Substrate Reagent to each well. Incubate 30 minutes at room temperature.

6. Add 50 µl Stop Solution to each well. Read at 450 nm immediately.

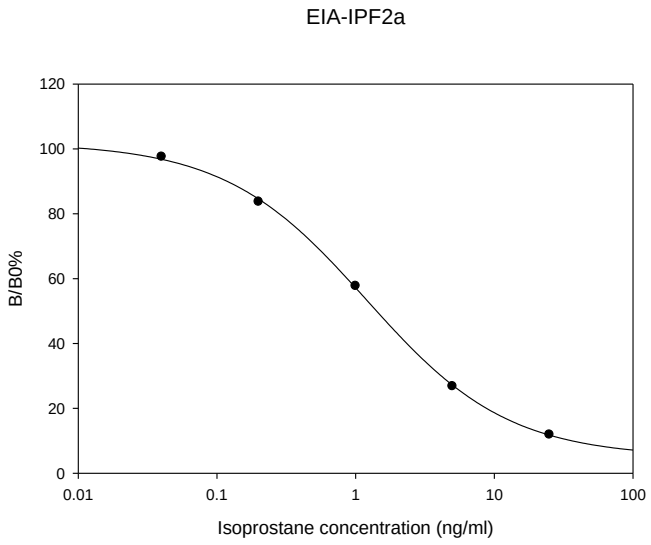
X. Calculation of Results

Calculate the mean absorbance for each set of duplicate stands, controls, and samples and subtract the blank optical density. Plot the standard curve using SigmaPlot software (or other software which can perform four-parameter logistic regression models), with standard concentration on the x-axis and percentage of absorbance (see calculation below) on the y-axis. Draw the best-fit curve through the standard points.

Percentage absorbance = (B-blank OD)/(B_0-blank OD) where
B = OD of sample or standard and
B_0 = OD of zero standard (total binding)

A. Typical Data

These standard curves are for demonstration only. A standard curve must be run with each assay.



B. Sensitivity

The minimum detectable concentrations of Isoprostane is 0.3 ng/ml.

C. Standard Curve Range

1-10,000 pg/ml

D. Reproducibility

Intra-Assay: CV<10%
Inter-Assay: CV<15%

E. Assay Diagram

Recommended Plate Layout:

Blank	Blank	SA1	SA1	SA9	SA9	SA17	SA17	SA25	SA25	SA33	SA33
Total Binding	Total Binding	SA2	SA2	SA10	SA10	SA18	SA18	SA26	SA26	SA34	SA34
Standard1	Standard1	SA3	SA3	SA11	SA11	SA19	SA19	SA27	SA27	SA35	SA35
Standard2	Standard2	SA4	SA4	SA12	SA12	SA20	SA20	SA28	SA28	SA36	SA36
Standard3	Standard3	SA5	SA5	SA13	SA13	SA21	SA21	SA29	SA29	SA37	SA37
Standard4	Standard4	SA6	SA6	SA14	SA14	SA22	SA22	SA30	SA30	SA38	SA38
Standard5	Standard5	SA7	SA7	SA15	SA15	SA23	SA23	SA31	SA31	SA39	SA39
Pos Control	Pos Control	SA8	SA8	SA16	SA16	SA24	SA24	SA32	SA32	SA40	SA40

Key:

Blank = Buffer Only

Total Binding = Isoprostane only

Standard 1 = 25 ng/ml

Standard 2 = 5 ng/ml

Standard 3 = 1 ng/ml

Standard 4 = 0.2 ng/ml

Standard 5 = 0.04 ng/ml

Pos Control = Item M

XI. Specificity

This EIA kit is designed to detect human, mouse, and rat Isoprostane.

XIV. Publications Citing This Product

1. KyrÃ¶nlahti A, et al. GATA4 regulates Sertoli cell function and fertility in adult male mice. Molecular and Cellular Endocrinology Volume 333, Issue 1, 10 February 2011, Pages 85Â–95
   Species: Mouse
   Sample Type:
2. Satie AP., et al. Excess Type I Interferon Signaling in the Mouse Seminiferous Tubules Leads to Germ Cell Loss and Sterility. J Biol Chem. 2011 Jul 1;286(26):23280-95. doi: 10.1074/jbc.M111.229120
   Species: Mouse
   Sample Type:

XIII. Troubleshooting Guide

Problem	Cause	Solution
Poor standard curve	○ Inaccurate pipetting ○ Improper standard dilution	○ Check pipettes ○ Briefly centrifuge Item C and dissolve the powder thoroughly by gently mixing
Low signal	○ Improper preparation of standard and/or biotinylated antibody ○ Too brief incubation times ○ Inadequate reagent volumes or improper dilution	○ Briefly spin down vials before opening. Dissolve the powder thoroughly. ○ Ensure sufficient incubation time; assay procedure step 2 may be done overnight ○ Check pipettes and ensure correct preparation
Large CV	○ Inaccurate pipetting ○ Air bubbles in wells	○ Check pipettes ○ Remove bubbles in wells
High background	○ Plate is insufficiently washed ○ Contaminated wash buffer	○ Review the manual for proper wash. If using a plate washer, ensure that all ports are unobstructed. ○ Make fresh wash buffer
Low sensitivity	○ Improper storage of the ELISA kit ○ Stop solution	○ Follow storage recomendations in sections IV and V. Keep substrate solution protected from light. ○ Add stop solution to each well before reading plate

RayBio® ELISA Kits

Over 3,000 ELISA kits available, visit www.RayBiotech.com/ELISA-Kits.html for details.

This product is for research use only.