$iPF_{2\alpha}$-VI EIA Kit

Item No. 516301

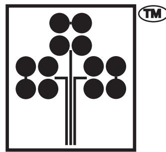


TABLE OF CONTENTS

GENERAL INFORMATION
3 Materials Supplied
4 Precautions
4 If You Have Problems
4 Storage and Stability
4 Materials Needed but Not Supplied

INTRODUCTION
5 Background
5 About This Assay
7 Description of ACE™ Competitive EIAs
8 Biochemistry of Acetylcholinesterase
10 Definition of Key Terms

PRE-ASSAY PREPARATION
11 Buffer Preparation
12 Sample Preparation

ASSAY PROTOCOL
15 Preparation of Assay-Specific Reagents
17 Plate Set Up
18 Performing the Assay

ANALYSIS
21 Calculations
24 Performance Characteristics

RESOURCES
28 Troubleshooting
28 Additional Reading
29 References
29 Related Products
30 Warranty and Limitation of Remedy
31 Plate Template
32 Notes

Materials Supplied

Item Number	Item	96 wells Quantity/Size	480 wells Quantity/Size
416302	iPF$_{2\alpha}$-VI EIA Antiserum	1 vial/100 dtn	1 vial/500 dtn
416300	iPF$_{2\alpha}$-VI AChE Tracer	1 vial/100 dtn	1 vial/500 dtn
416304	iPF$_{2\alpha}$-VI EIA Standard	1 vial	1 vial
400060	EIA Buffer Concentrate (10X)	2 vials/10 ml	4 vials/10 ml
400062	Wash Buffer Concentrate (400X)	1 vial/5 ml	1 vial/12.5 ml
400035	Tween 20	1 vial/3 ml	1 vial/3 ml
400004/400006	Mouse Anti-Rabbit IgG Coated Plate	1 plate	5 plates
400012	96-Well Cover Sheet	1 cover	5 covers
400050	Ellman's Reagent	3 vials/100 dtn	6 vials/250 dtn
400040	EIA Tracer Dye	1 vial	1 vial
400042	EIA Antiserum Dye	1 vial	1 vial

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 975-3999. We cannot accept any returns without prior authorization.

WARNING: This product is for laboratory research use only: not for administration to humans. Not for human or veterinary diagnostic or therapeutic use.

Precautions

Please read these instructions carefully before beginning this assay.

The reagents in this kit have been tested and formulated to work exclusively with Cayman Chemical's ACE™ EIA Kits. This kit may not perform as described if any reagent or procedure is replaced or modified.

For research use only. Not for human or diagnostic use.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Fax: 734-971-3641

Email: techserv@caymanchem.com

Hours: M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed at -20°C and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader capable of measuring absorbance between 405-420 nm.
2. Adjustable pipettes and a repeat pipettor.
3. A source of 'UltraPure' water. Water used to prepare all EIA reagents and buffers must be deionized and free of trace organic contaminants ('UltraPure'). Use activated carbon filter cartridges or other organic scavengers. Glass distilled water (even if double distilled), HPLC-grade water, and sterile water (for injections) are not adequate for EIA. *NOTE: UltraPure water is available for purchase from Cayman (Item No. 400000).*
4. Materials used for **Sample Preparation** (see page 12).

INTRODUCTION

Background

The isoprostanes (iPs) are a family of eicosanoids of non-enzymatic origin produced by the random oxidation of tissue phospholipids by oxygen radicals. Isoprostanes appear as artifacts in tissue and plasma samples which have undergone oxidative degradation during prolonged or improper storage. They also appear in the plasma and urine under normal conditions and are elevated by the ingestion of alcohol, carbon tetrachloride, and cigarette smoke.

There are four main families or classes of isoprostanes (Classes III, IV, V, and VI).[1-3] It is ironic that the best studied class, the $iPF_{2\alpha}$-III series, is also the least abundant. This assay is the first simple, reliable EIA method for the measurement of the more prominent $iPF_{2\alpha}$-VI isoprostane. Normal urinary levels of $iPF_{2\alpha}$-VI are 500-700 pg/mg creatinine, whereas the III series compound 8-isoprostane ($iPF_{2\alpha}$-III) is present at about 150 pg/mg creatinine.

In addition to a more robust signal, the Class VI family of isoprostanes can be readily interconverted between the open, free acid form, and a neutral, internal lactone.[4] This can facilitate the purification and separation of $iPF_{2\alpha}$-VI from interfering matrix components.

About This Assay

Cayman's $iPF_{2\alpha}$-VI EIA Kit is a competitive assay that can be used for quantification of $iPF_{2\alpha}$-VI in tissue culture supernatants and other sample matrices. The EIA typically displays an IC_{50} (50% B/B_0) of aproximately 250 pg/ml and a detection limit (80% B/B_0) of approximately 50 pg/ml.

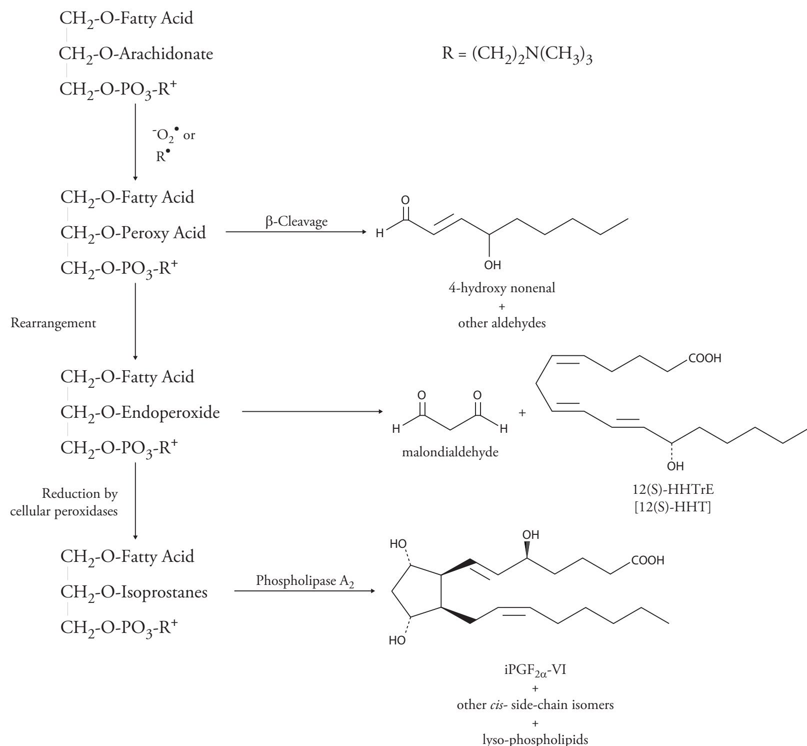


Figure 1. The formation of $iPF_{2\alpha}$-VI

Description of ACE™ Competitive EIAs[5,6]

This assay is based on the competition between $iPF_{2\alpha}$-VI and an $iPF_{2\alpha}$-VI-acetylcholinesterase (AChE) conjugate ($iPF_{2\alpha}$-VI Tracer) for a limited number of $iPF_{2\alpha}$-VI-specific rabbit antiserum binding sites. Because the concentration of the $iPF_{2\alpha}$-VI Tracer is held constant while the concentration of $iPF_{2\alpha}$-VI varies, the amount of $iPF_{2\alpha}$-VI Tracer that is able to bind to the rabbit antiserum will be inversely proportional to the concentration of $iPF_{2\alpha}$-VI in the well. This rabbit antiserum-$iPF_{2\alpha}$-VI (either free or tracer) complex binds to the mouse monoclonal anti-rabbit IgG that has been previously attached to the well. The plate is washed to remove any unbound reagents and then Ellman's Reagent (which contains the substrate to AChE) is added to the well. The product of this enzymatic reaction has a distinct yellow color and absorbs strongly at 412 nm. The intensity of this color, determined spectrophotometrically, is proportional to the amount of $iPF_{2\alpha}$-VI Tracer bound to the well, which is inversely proportional to the amount of free $iPF_{2\alpha}$-VI present in the well during the incubation; or

$$\textbf{Absorbance} \propto [\textbf{Bound iPF}_{2\alpha}\textbf{-VI Tracer}] \propto 1/[\textbf{iPF}_{2\alpha}\textbf{-VI}]$$

A schematic of this process is shown in Figure 2, below.

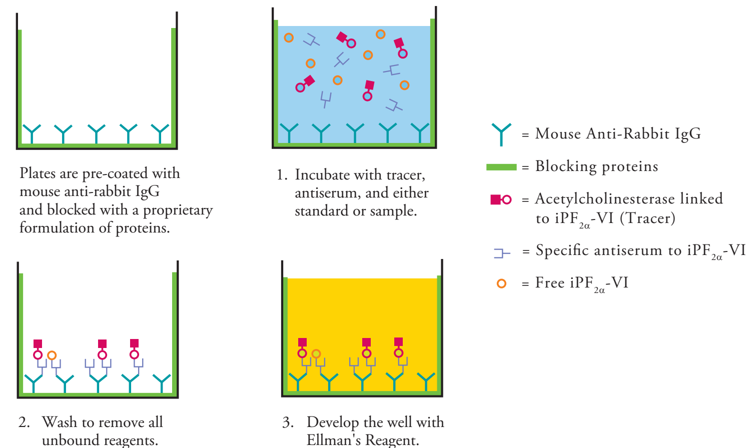


Figure 2. Schematic of the ACE™ EIA

Biochemistry of Acetylcholinesterase

The electric organ of the electric eel, *Electrophorus electricus*, contains an avid acetylcholinesterase (AChE) capable of massive catalytic turnover during the generation of its electrochemical discharges. The electric eel AChE has a clover leaf-shaped tertiary structure consisting of a triad of tetramers attached to a collagen-like structural fibril. This stable enzyme is capable of high turnover (64,000 s^{-1}) for the hydrolysis of acetylthiocholine.

A molecule of the analyte covalently attached to a molecule of AChE serves as the tracer in ACE™ enzyme immunoassays. Quantification of the tracer is achieved by measuring its AChE activity with Ellman's Reagent. This reagent consists of acetylthiocholine and 5,5'-dithio-*bis*-(2-nitrobenzoic acid). Hydrolysis of acetylthiocholine by AChE produces thiocholine (see Figure 3, on page 9). The non-enzymatic reaction of thiocholine with 5,5'-dithio-*bis*-(2-nitrobenzoic acid) produces 5-thio-2-nitrobenzoic acid, which has a strong absorbance at 412 nm (ε = 13,600).

AChE has several advantages over other enzymes commonly used for enzyme immunoassays. Unlike horseradish peroxidase, AChE does not self-inactivate during turnover. This property of AChE also allows re-development of the assay if it is accidentally splashed or spilled. In addition, the enzyme is highly stable under the assay conditions, has a wide pH range (pH 5-10), and is not inhibited by common buffer salts or preservatives. Since AChE is stable during the development step, it is unnecessary to use a 'stop' reagent, and the plate may be read whenever it is convenient.

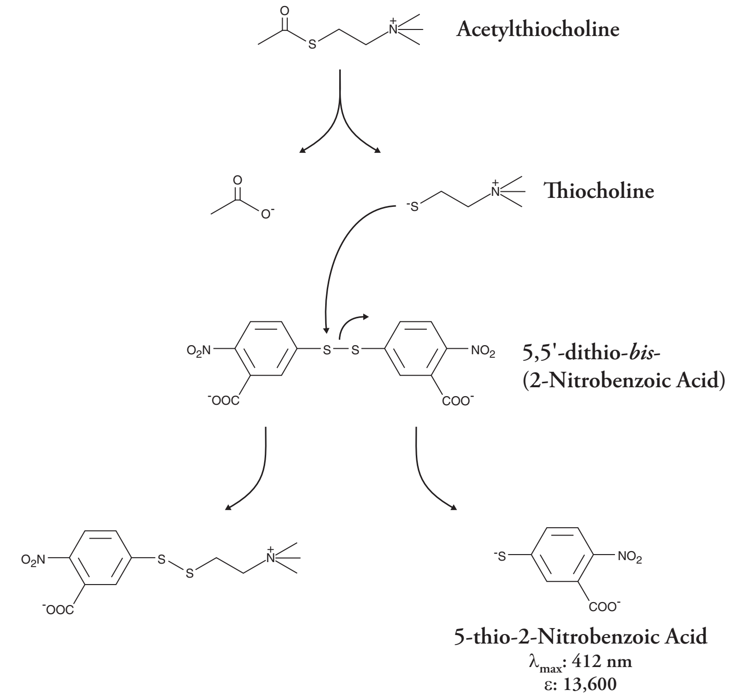


Figure 3. Reaction catalyzed by acetylcholinesterase

Definition of Key Terms

Blank: background absorbance caused by Ellman's Reagent. The blank absorbance should be subtracted from the absorbance readings of all the other wells.

Total Activity: total enzymatic activity of the AChE-linked tracer. This is analogous to the specific activity of a radioactive tracer.

NSB (Non-Specific Binding): non-immunological binding of the tracer to the well. Even in the absence of specific antibody a very small amount of tracer still binds to the well; the NSB is a measure of this low binding.

B_0 (Maximum Binding): maximum amount of the tracer that the antibody can bind in the absence of free analyte.

%B/B_0 (%Bound/Maximum Bound): ratio of the absorbance of a particular sample or standard well to that of the maximum binding (B_0) well.

Standard Curve: a plot of the %B/B_0 values *versus* concentration of a series of wells containing various known amounts of analyte.

Dtn: determination, where one dtn is the amount of reagent used per well.

PRE-ASSAY PREPARATION

NOTE: Water used to prepare all EIA reagents and buffers must be deionized and free of trace organic contaminants ('UltraPure'). Use activated carbon filter cartridges or other organic scavengers. Glass distilled water (even if double distilled), HPLC-grade water, and sterile water (for injections) are not adequate for EIA. UltraPure water may be purchased from Cayman (Item No. 400000).

Buffer Preparation

Store all diluted buffers at 4°C; they will be stable for about two months.

1. EIA Buffer Preparation

Dilute the contents of one vial of EIA Buffer Concentrate (10X) (Item No. 400060) with 90 ml of UltraPure water. Be certain to rinse the vial to remove any salts that may have precipitated. *NOTE: It is normal for the concentrated buffer to contain crystalline salts after thawing. These will completely dissolve upon dilution with water.*

2. Wash Buffer Preparation

5 ml vial Wash Buffer Concentrate (400X) (96-well kit; Item No. 400062): Dilute to a total volume of 2 liters with UltraPure water and add 1 ml of Tween 20 (Item No. 400035).

OR

12.5 ml vial Wash Buffer Concentrate (400X) (480-well kit; Item No. 400062): Dilute to a total volume of 5 liters with UltraPure water and add 2.5 ml of Tween 20 (Item No. 400035).

Smaller volumes of Wash Buffer can be prepared by diluting the Wash Buffer Concentrate 1:400 and adding Tween 20 (0.5 ml/liter of Wash Buffer).

NOTE: Tween 20 is a viscous liquid and cannot be measured by a regular pipette. A positive displacement pipette or a syringe should be used to deliver small quantities accurately.

Sample Preparation

This assay has been validated for urine. Proper sample storage and preparation are essential for consistent and accurate results. Please read this section thoroughly before beginning the assay.

In general, tissue culture supernatant samples may be diluted with EIA Buffer and added directly to the assay well. Plasma, serum, urine, whole blood, as well as other heterogeneous mixtures such as lavage fluids and aspirates often contain contaminants which can interfere in the assay. It is best to check for interference before embarking on a large number of sample measurements. To test for interference, dilute one or two test samples to obtain at least two different dilutions of each sample between 50 and 2,500 pg/ml (*i.e.*, 20-80% B/B_0). If the two different dilutions of the sample show good correlation (differ by 20% or less) in the final calculated $iPF_{2\alpha}$-VI concentration, purification is not required. If you do not see good correlation of the different dilutions, purification is advised.

General Precautions

- All samples must be free of organic solvents prior to assay.
- Samples should be assayed immediately after collection; samples that cannot be assayed immediately should be stored at -80°C in the presence of 0.005% BHT. Storage at -20°C is insufficient to prevent oxidative formation of $iPF_{2\alpha}$-VI.[7]
- Samples of rabbit origin may contain antibodies which interfere with the assay by binding to the mouse anti-rabbit plate. We recommend that all rabbit samples be purified prior to use in this assay.

Urine

Urine samples give excellent correlation to GC-MS if purified as described on page 13.

Purification Protocol

1. Aliquot an equal amount of each sample into two clean test tubes (1 ml is recommended). If your samples need to be concentrated, a larger volume should be used (*e.g.*, a 5 ml aliquot will be concentrated by a factor of 5, a 10 ml aliquot will be concentrated by a factor of 10, etc.). Label the first tube "sample #" and the second "sample # + spike".
2. Add a cold spike of $iPF_{2\alpha}$-VI (5 ng/ml is recommended) to the "sample + spike" tube. Follow steps 3-7 for both the unspiked and spiked tubes.
3. Add acetone (4X the sample volume) to the sample and vortex 2 x 10 seconds. Incubate at 4°C for five minutes, and centrifuge at 3,000 x g for 10 minutes to remove precipitated proteins. Decant the supernatant into a clean test tube. Evaporate the acetone by either vacuum centrifugation or under a gentle stream of nitrogen.
4. Resuspend each sample in 2 ml of extraction buffer (1 M sodium citrate, pH 4.0, containing 10% NaCl). Incubate 90 minutes at room temperature.
5. Add 5 ml of methylene chloride and vortex 2 x 10 seconds. Transfer the lower (methylene chloride) fraction to a clean test tube. Add another 5 ml methylene chloride to the original sample, vortex, and combine the lower fraction with the previous extract.*
6. Evaporate to dryness by either vacuum centrifugation or by evaporation under a stream of dry nitrogen. It is imperative that all of the organic solvent be removed as even trace quantities will adversely affect the EIA.
7. Resuspend each sample in 1 ml of EIA Buffer, vortex, and incubate at room temperature for 90 minutes. Use this for EIA analysis.

*If it is necessary to stop during this purification, samples may be stored in the methylene chloride solution at -20°C or -80°C.

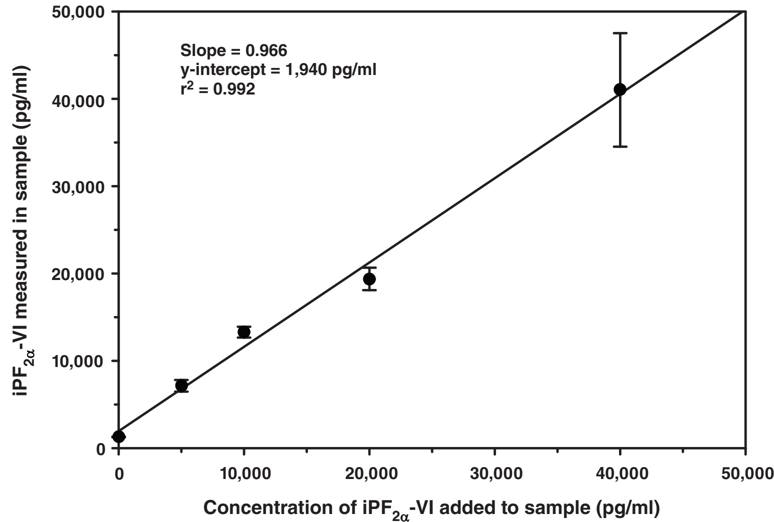


Figure 4. Recovery of $iPF_{2\alpha}$-VI from urine
Urine samples were spiked with $iPF_{2\alpha}$-VI, purified as described in the **Sample Preparation** section and analyzed using the $iPF_{2\alpha}$-VI EIA Kit. The y-intercept corresponds to the amount of $iPF_{2\alpha}$-VI in unspiked urine. Error bars represent standard deviations obtained from multiple dilutions of each sample.

Preparation of Assay-Specific Reagents

$iPF_{2\alpha}$-VI EIA Standard

Equilibrate a pipette tip in ethanol by repeatedly filling and expelling the tip with ethanol several times. Using the equilibrated pipette tip, transfer 100 µl of the $iPF_{2\alpha}$-VI EIA Standard (Item No. 416304) into a clean test tube, then dilute with 900 µl UltraPure water. The concentration of this solution (the bulk standard) will be 50 ng/ml.

NOTE: If assaying culture medium samples that have not been diluted with EIA Buffer, culture medium should be used in place of EIA Buffer for dilution of the standard curve.

To prepare the standard for use in EIA: obtain eight clean test tubes and number them #1 through #8. Aliquot 900 µl EIA Buffer to tube #1 and 600 µl EIA Buffer to tubes #2-8. Transfer 100 µl of the bulk standard (50 ng/ml) to tube #1 and mix thoroughly. Serially dilute the standard by removing 300 µl from tube #1 and placing in tube #2; mix thoroughly. Next, remove 300 µl from tube #2 and place it into tube #3; mix thoroughly. Repeat this process for tubes #4-8. These diluted standards should not be stored for more than 24 hours.

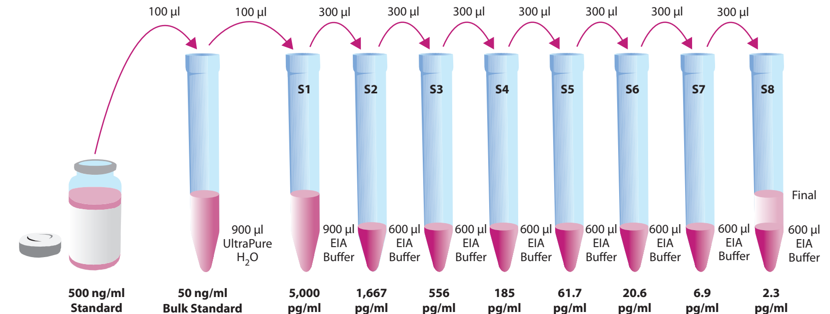


Figure 5. Preparation of the $iPF_{2\alpha}$-VI standards

iPF$_{2\alpha}$-VI AChE Tracer

Reconstitute the iPF$_{2\alpha}$-VI AChE Tracer as follows:

100 dtn iPF$_{2\alpha}$-VI AChE Tracer (96-well kit; Item No. 416300): Reconstitute with 6 ml EIA Buffer.

OR

500 dtn iPF$_{2\alpha}$-VI AChE Tracer (480-well kit; Item No. 416300): Reconstitute with 30 ml EIA Buffer.

Store the reconstituted iPF$_{2\alpha}$-VI AChE Tracer at 4°C *(do not freeze!)* and use within four weeks. A 20% surplus of tracer has been included to account for any incidental losses.

Tracer Dye Instructions (optional)

This dye may be added to the tracer, if desired, to aid in visualization of tracer-containing wells. Add the dye to the reconstituted tracer at a final dilution of 1:100 (add 60 µl of dye to 6 ml tracer or add 300 µl of dye to 30 ml of tracer). *NOTE: Do not store tracer with dye for more than 24 hours.*

iPF$_{2\alpha}$-VI EIA Antiserum

Reconstitute the iPF$_{2\alpha}$-VI EIA Antiserum as follows:

100 dtn iPF$_{2\alpha}$-VI EIA Antiserum (96-well kit; Item No. 416302): Reconstitute with 6 ml EIA Buffer.

OR

500 dtn iPF$_{2\alpha}$-VI EIA Antiserum (480-well kit; Item No. 416302): Reconstitute with 30 ml EIA Buffer.

Store the reconstituted iPF$_{2\alpha}$-VI Antiserum at 4°C. It will be stable for at least four weeks. A 20% surplus of antiserum has been included to account for any incidental losses.

Antiserum Dye Instructions (optional)

This dye may be added to the antiserum, if desired, to aid in visualization of antiserum-containing wells. Add the dye to the reconstituted antiserum at a final dilution of 1:100 (add 60 µl of dye to 6 ml antiserum or add 300 µl of dye to 30 ml of antiserum). *NOTE: Do not store antiserum with dye for more than 24 hours.*

Plate Set Up

The 96-well plate(s) included with this kit is supplied ready to use. It is not necessary to rinse the plate(s) prior to adding the reagents. *NOTE: If you do not need to use all the strips at once, place the unused strips back in the plate packet and store at 4°C. Be sure the packet is sealed with the desiccant inside.*

Each plate or set of strips must contain a minimum of two blanks (Blk), two non-specific binding wells (NSB), two maximum binding wells (B_0), and an eight point standard curve run in duplicate. *NOTE: Each assay must contain this minimum configuration in order to ensure accurate and reproducible results.* Each sample should be assayed at two dilutions and each dilution should be assayed in duplicate. For statistical purposes, we recommend assaying samples in triplicate.

A suggested plate format is shown in Figure 6, below. The user may vary the location and type of wells present as necessary for each particular experiment. The plate format provided below has been designed to allow for easy data analysis using a convenient spreadsheet offered by Cayman (see page 21, for more details). We suggest you record the contents of each well on the template sheet provided (see page 31).

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blk	S1	S1	1	1	1	9	9	9	17	17	17
B	Blk	S2	S2	2	2	2	10	10	10	18	18	18
C	NSB	S3	S3	3	3	3	11	11	11	19	19	19
D	NSB	S4	S4	4	4	4	12	12	12	20	20	20
E	B_0	S5	S5	5	5	5	13	13	13	21	21	21
F	B_0	S6	S6	6	6	6	14	14	14	22	22	22
G	B_0	S7	S7	7	7	7	15	15	15	23	23	23
H	TA	S8	S8	8	8	8	16	16	16	24	24	24

Blk - Blank
TA - Total Activity
NSB - Non-Specific Binding
B_0 - Maximum Binding
S1-S8 - Standards 1-8
1-24 - Samples

Figure 6. Sample plate format

Performing the Assay

Pipetting Hints

- Use different tips to pipette each reagent.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

Addition of the Reagents

1. EIA Buffer

Add 100 µl EIA Buffer to Non-Specific Binding (NSB) wells. Add 50 µl EIA Buffer to Maximum Binding (B_0) wells. If culture medium was used to dilute the standard curve, substitute 50 µl of culture medium for EIA Buffer in the NSB and B_0 wells (*i.e.*, add 50 µl culture medium to NSB and B_0 wells and 50 µl EIA Buffer to NSB wells).

2. iPF$_{2\alpha}$-VI EIA Standard

Add 50 µl from tube #8 to both of the lowest standard wells (S8). Add 50 µl from tube #7 to each of the next two standard wells (S7). Continue with this procedure until all the standards are aliquoted. The same pipette tip should be used to aliquot all the standards. Before pipetting each standard, be sure to equilibrate the pipette tip in that standard.

3. Samples

Add 50 µl of sample per well. Each sample should be assayed at a minimum of two dilutions. Each dilution should be assayed in duplicate (triplicate recommended).

4. iPF$_{2\alpha}$-VI AChE Tracer

Add 50 µl to each well *except* the Total Activity (TA) and the Blank (Blk) wells.

5. iPF$_{2\alpha}$-VI EIA Antiserum

Add 50 µl to each well *except* the Total Activity (TA), the Non-Specific Binding (NSB), and the Blank (Blk) wells.

Well	EIA Buffer	Standard/ Sample	Tracer	Antibody
Blk	-	-	-	-
TA	-	-	5 µl (at devl. step)	-
NSB	100 µl	-	50 µl	-
B_0	50 µl	-	50 µl	50 µl
Std/Sample	-	50 µl	50 µl	50 µl

Table 1. Pipetting summary

Incubation of the Plate

Cover each plate with plastic film (Item No. 400012) and incubate 18 hours at 4°C.

Development of the Plate

1. Reconstitute Ellman's Reagent immediately before use (20 ml of reagent is sufficient to develop 100 wells):

   100 dtn vial Ellman's Reagent (96-well kit; Item No. 400050): Reconstitute with 20 ml of UltraPure water.

   OR

   250 dtn vial Ellman's Reagent (480-well kit; Item No. 400050): Reconstitute with 50 ml of UltraPure water.

NOTE: Reconstituted Ellman's Reagent is unstable and should be used the same day it is prepared; protect the Ellman's Reagent from light when not in use. Extra vials of the reagent have been provided should a plate need to be re-developed or multiple assays run on different days.

2. Empty the wells and rinse five times with Wash Buffer.
3. Add 200 µl of Ellman's Reagent to each well.
4. Add 5 µl of tracer to the Total Activity wells.
5. Cover the plate with plastic film. Optimum development is obtained by using an orbital shaker equipped with a large, flat cover to allow the plate(s) to develop in the dark. This assay typically develops (*i.e.*, B_0 wells ≥0.3 A.U. (blank subtracted)) in 90-120 minutes.

Reading the Plate

1. Wipe the bottom of the plate with a clean tissue to remove fingerprints, dirt, etc.
2. Remove the plate cover being careful to keep Ellman's Reagent from splashing on the cover. *NOTE: Any loss of Ellman's Reagent will affect the absorbance readings. If Ellman's Reagent is present on the cover, use a pipette to transfer the Ellman's Reagent into the well. If too much Ellman's Reagent has splashed on the cover to easily redistribute back into the wells, wash the plate three times with wash buffer and repeat the development with fresh Ellman's Reagent.*
3. Read the plate at a wavelength between 405 and 420 nm. The absorbance may be checked periodically until the B_0 wells have reached a minimum of 0.3 A.U. (blank subtracted). The plate should be read when the absorbance of the B_0 wells are in the range of 0.3-1.0 A.U. (blank subtracted). If the absorbance of the wells exceeds 1.5, wash the plate, add fresh Ellman's Reagent and let it develop again.

ANALYSIS

Many plate readers come with data reduction software that plot data automatically. Alternatively a spreadsheet program can be used. The data should be plotted as either $\%B/B_0$ *versus* log concentration using a four-parameter logistic fit or as logit B/B_0 *versus* log concentration using a linear fit. *NOTE: Cayman has a computer spreadsheet available for data anaylsis. Please contact Technical Service or visit our website (www.caymanchem.com/analysis/eia) to obtain a free copy of this convenient data analysis tool.*

Calculations

Preparation of the Data

The following procedure is recommended for preparation of the data prior to graphical analysis.

NOTE: If the plate reader has not subtracted the absorbance readings of the blank wells from the absorbance readings of the rest of the plate, be sure to do that now.

1. Average the absorbance readings from the NSB wells.
2. Average the absorbance readings from the B_0 wells.
3. Subtract the NSB average from the B_0 average. This is the corrected B_0 or corrected maximum binding.
4. Calculate the B/B_0 (Sample or Standard Bound/Maximum Bound) for the remaining wells. To do this, subtract the average NSB absorbance from the S1 absorbance and divide by the corrected B_0 (from Step 3). Repeat for S2-S8 and all sample wells. (To obtain $\%B/B_0$ for a logistic four-parameter fit, multiply these values by 100.)

NOTE: The total activity (TA) values are not used in the standard curve calculations. Rather, they are used as a diagnostic tool; the corrected B_0 divided by the actual TA (10X measured absorbance) will give the % Bound. This value should closely approximate the % Bound that can be calculated from the ***Sample Data*** *(see page 24). Erratic absorbance values and a low (or no) % Bound could indicate the presence of organic solvents in the buffer or other technical problems (see page 28 for* ***Troubleshooting****).*

Plot the Standard Curve

Plot %B/B_0 for standards S1-S8 *versus* iPF$_{2\alpha}$-VI concentration using linear (y) and log (x) axes and perform a 4-parameter logistic fit.

Alternative Plot - The data can also be lineraized using a logit transformation. The equation for this conversion is shown below. *NOTE: Do not use %B/B_0 in this calculation.*

$$\textbf{logit}\ (B/B_0) = \ln\ [B/B_0/(1 - B/B_0)]$$

Plot the data as logit (B/B_0) *versus* log concentrations and perform a linear regression fit.

Determine the Sample Concentration

Calculate the B/B_0 (%B/B_0) value for each sample. Determine the concentration of each sample using the equation obtained from the standard curve plot. *NOTE: Remember to account for any concentration or dilution of the sample prior to the addition to the well.* Samples with %B/B_0 values greater than 80% or less than 20% should be re-assayed as they generally fall out of the linear range of the standard curve. A 20% or greater disparity between the apparent concentration of two different dilutions of the same sample indicates interference which could be eliminated by purification.

Cold Spike Recovery

The original concentration of the sample and recovery factor can be determined by the following method:

V = EIA determined concentration of the unspiked sample (pg/ml)

S = concentration of the spike (pg/ml)

Y = EIA determined concentration of the spiked sample (pg/ml)

Z = recovery factor (%)

$$Z = \left[\frac{Y - V}{S}\right]$$

$$\text{iPF}_{2\alpha}\text{-VI (pg) in purified sample} = \left[\frac{V}{Z}\right] \times 1\ \text{ml}$$

$$\text{iPF}_{2\alpha}\text{-VI in unpurified sample (pg/ml)} = \frac{\text{iPF}_{2\alpha}\text{-VI (pg) in purified sample}}{\text{Volume of sample used for purification (ml)}}$$

Performance Characteristics

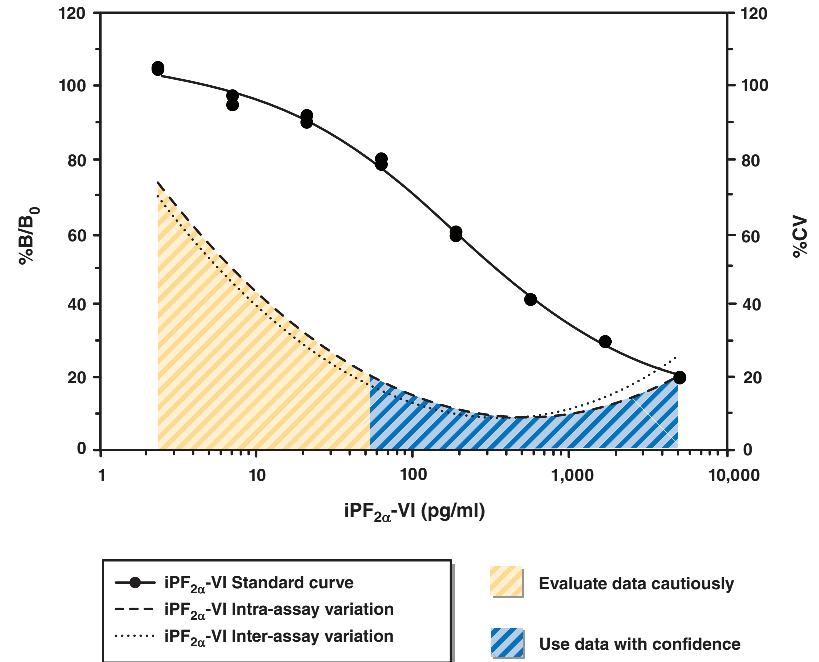
Sample Data

The standard curve presented here is an example of the data typically produced with this kit; however, your results will not be identical to these. You **must** run a new standard curve. Do not use the data below to determine the values of your samples. Your results could differ substantially.

	Raw Data		Average	Corrected
Total Activity	1.781	1.798	1.790	
NSB	0	0	0	
B_0	0.352	0.339		
	0.342	0.358	0.348	0.348

Dose (pg/ml)	Raw Data		Corrected		%B/B_0	
5,000	0.069	0.068	0.069	0.068	19.8	19.6
1,667	0.103	0.103	0.103	0.103	29.6	29.6
556	0.143	0.143	0.143	0.143	41.1	41.1
185	0.204	0.208	0.204	0.208	58.7	59.9
61.7	0.278	0.272	0.278	0.272	80.0	78.2
20.6	0.312	0.319	0.312	0.319	89.7	91.7
6.9	0.329	0.338	0.329	0.338	94.6	97.2
2.3	0.365	0.363	0.365	0.363	105.0	104.4

Table 2. Typical results



50% B/B_0 - 250 pg/ml

Detection Limit (80% B/B_0) - 50 pg/ml

Figure 7. Typical standard curve

Precision:

The intra- and inter-assay CVs have been determined at multiple points on the standard curve. These data are summarized in the graph on page 25 and in the table below.

Dose (pg/ml)	%CV* Intra-assay variation	%CV* Inter-assay variation
5,000	20.7	18.8
1,667	12.1	19.4
556	10.2	18.2
185	9.9	9.6
61.7	16.2	16.6
20.6	†	†
6.9	†	†
2.3	†	†

Table 3. Intra- and inter-assay variation
*%CV represents the variation in concentration (not absorbance) as determined using a reference standard curve.
†Outside of the recommended usable range of the assay.

Specificity:

Compound	Cross-reactivity	Compound	Cross-reactivity
iPF$_{2\alpha}$-VI	100%	Prostaglandin F$_{2\alpha}$	<0.01%
8,12-*epi* iPF$_{2\alpha}$-VI	0.5%	11β-Prostaglandin F$_{2\alpha}$	<0.01%
Prostaglandin D$_2$	0.01%	8-*iso* Prostaglandin F$_{2\alpha}$	<0.01%
8,12-*epi* iPF$_{2\alpha}$-III	<0.01%	8-*iso*-15(R)-Prostaglandin F$_{2\alpha}$	<0.01%
Prostaglandin E$_1$	<0.01%	8-*iso*-15-keto Prostaglandin F$_{2\alpha}$	<0.01%
8-*iso* Prostaglandin E$_1$	<0.01%	8-*iso*-13,14-dihydro-15-keto-Prostaglandin F$_{2\alpha}$	<0.01%
Prostaglandin E$_2$	<0.01%	13,14-dihydro-15-keto-Prostaglandin F$_{2\alpha}$	<0.01%
8-*iso* Prostaglandin E$_2$	<0.01%	2,3-dinor-8-*iso* Prostaglandin F$_{2\alpha}$	<0.01%
8-*iso*-15-keto Prostaglandin E$_2$	<0.01%	8-*iso* Prostaglandin F$_{2\beta}$	<0.01%
Prostaglandin F$_{1\alpha}$	<0.01%	Prostaglandin F$_{3\alpha}$	<0.01%
2,3-dinor-8-*iso* Prostaglandin F$_{1\alpha}$	<0.01%	Thromboxane B$_2$	<0.01%
8-*iso* Prostaglandin F$_{1\alpha}$	<0.01%	11-dehydro Thromboxane B$_2$	<0.01%
6-keto Prostaglandin F$_{1\alpha}$	<0.01%	tetranor-PGEM	<0.01%
8-*iso* Prostaglandin F$_{1\beta}$	<0.01%	tetranor-PGFM	<0.01%

Table 4. Specificity of the iPF$_{2\alpha}$-VI EIA Antiserum

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates	A. Trace organic contaminants in the water source B. Poor pipetting/technique	A. Replace activated carbon filter or change source of UltraPure water
High NSB (>0.035)	A. Poor washing B. Exposure of NSB wells to specific antibody	A. Rewash plate and redevelop
Very low B_0	A. Trace organic contaminants in the water source B. Plate requires additional development time C. Dilution error in preparing reagents	A. Replace activated carbon filter or change source of UltraPure water B. Return plate to shaker and re-read later
Low sensitivity (shift in dose response curve)	Standard is degraded	Replace standard
Analyses of two dilutions of a biological sample do not agree (*i.e.*, more than 20% difference)	Interfering substances are present	Purify sample prior to analysis by EIA[8]
Only Total Activity (TA) wells develop	Trace organic contaminants in the water source	Replace activated carbon filter or change source of UltraPure water

Additional Reading

Go to **www.caymanchem.com/516301/references** for a list of publications citing the use of Cayman's iPF$_{2\alpha}$-VI EIA Kit.

References

1. Li, H., Lawson, J.A., Reilly, M., *et al.* Quantitative high performance liquid chromatography/tandem mass spectrometric analysis of the four classes of F$_2$-isoprostanes in human urine. *Proc. Natl. Acad. Sci. USA* **96(23)**, 13381-13386 (1999).
2. Morrow, J.D., Hill, K.E., Burk, R.F., *et al.* A series of prostaglandin F$_2$-like compounds are produced *in vivo* in humans by a non-cyclooxygenase, free radical-catalyzed mechanism. *Proc. Natl. Acad. Sci. USA* **87**, 9383-9387 (1990).
3. Waugh, R.J. and Murphy, R.C. Mass spectrometric anaysis of four regioisomers of F$_2$-isoprostanes formed by free radical oxidation of arachidonic acid. *J. Am. Soc. Mass Spectrom.* **7**, 490-499 (1996).
4. Praticò, D., Barry, O.P., Lawson, J.A., *et al.* IPF$_{2\alpha}$-I: An index of lipid peroxidation in humans. *Proc. Natl. Acad. Sci. USA* **95**, 3449-3454 (1998).
5. Maclouf, J., Grassi, J., and Pradelles, P. Development of enzyme-immunoassay techniques for the measurement of eicosanoids, Chapter 5, *in* Prostaglandin and Lipid Metabolism in Radiation Injury. Walden, T.L., Jr. and Hughes, H.N., editors, Plenum Press, Rockville, 355-364 (1987).
6. Pradelles, P., Grassi, J. and Maclouf, J. Enzyme immunoassays of eicosanoids using AChE as label: An alternative to radioimmunoassay. *Anal. Chem.* **57**, 1170-1173 (1985).
7. Morrow, J.D., Harris, T.M., and Roberts, L.J., II Noncyclooxygenase oxidative formation of a series of novel prostaglandins: Analytical ramifications for measurement of eicosanoids. *Anal. Biochem.* **184**, 1-10 (1990).
8. Maxey, K.M., Maddipati, K.R. and Birkmeier, J. Interference in enzyme immunoassays. *J. Clin. Immunoassay* **15**,116-120 (1992).

Related Products

8-*iso* Prostaglandin F$_{2\alpha}$ - Item No. 16350
[^{3}H]-8-*iso* Prostaglandin F$_{2\alpha}$ - Item No. 216350
8-Isoprostane EIA Kit - Item No. 516351
UltraPure Water - Item No. 400000

Warranty and Limitation of Remedy

Cayman Chemical Company makes **no warranty or guarantee** of any kind, whether written or oral, expressed or implied, including without limitation, any warranty of fitness for a particular purpose, suitability and merchantability, which extends beyond the description of the chemicals hereof. Cayman **warrants only** to the original customer that the material will meet our specifications at the time of delivery. Cayman will carry out its delivery obligations with due care and skill. Thus, in no event will Cayman have **any obligation or liability**, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if Cayman is informed about their possible existence. This limitation of liability does not apply in the case of intentional acts or negligence of Cayman, its directors or its employees.

Buyer's **exclusive remedy** and Cayman's sole liability hereunder shall be limited to a refund of the purchase price, or at Cayman's option, the replacement, at no cost to Buyer, of all material that does not meet our specifications.

Said refund or replacement is conditioned on Buyer giving written notice to Cayman within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

For further details, please refer to our Warranty and Limitation of Remedy located on our website and in our catalog.

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

NOTES

This document is copyrighted. All rights are reserved. This document may not, in whole or part, be copied, photocopied, reproduced, translated, or reduced to any electronic medium or machine-readable form without prior consent, in writing, from Cayman Chemical Company.

©08/12/2008, Cayman Chemical Company, Ann Arbor, MI, All rights reserved. Printed in U.S.A.