2,3-dinor-6-keto Prostaglandin $F_{1\alpha}$ EIA Kit

Catalog No. 515121 (Strip Plate)
Catalog No. 10008826 (Solid Plate)

ACE™

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GENERAL INFORMATION

Materials Supplied

Catalog Number	Item	96 wells Quantity/Size	480 wells Quantity/Size
10008831	2,3-dinor-6-keto $PGF_{1\alpha}$ EIA Monoclonal Antibody	1 vial/100 dtn	1 vial/500 dtn
10008830	2,3-dinor-6-keto $PGF_{1\alpha}$ AChE Tracer	1 vial/100 dtn	1 vial/500 dtn
10008828	2,3-dinor-6-keto $PGF_{1\alpha}$ EIA Standard	1 vial	1 vial
10008829	2,3-dinor-6-keto $PGF_{1\alpha}$ Assay Buffer Concentrate	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
400009	Goat Anti-Mouse IgG Coated Plate	1 plate	5 plates
400012	96-Well Cover Sheets	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

E-Mail: 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 (Catalog No. 400000).*
4. Materials used for **Sample Preparation** (see page 12).

INTRODUCTION

Background

Prostacyclin (Prostaglandin I_2; PGI_2) is formed from arachidonic acid primarily in the vascular endothelium and renal cortex by sequential activities of cyclooxygenase (COX) and PGI_2 synthase.[1,2] PGI_2 is a potent vasodilator and inhibitor of platelet aggregation.[2] PGI_2 was once thought to be a circulating hormone that regulated platelet-vasculature interactions, but the rate of secretion into circulation coupled with the short half-life indicate that PGI_2 functions locally.[3] PGI_2 is non-enzymatically hydrated to 6-keto $PGF_{1\alpha}$ ($t_{1/2}$ = 2-3 minutes), and then quickly converted to the major metabolite, 2,3-dinor-6-keto $PGF_{1\alpha}$ ($t_{1/2}$ = 30 minutes).[4,5,6]

Although 6-keto $PGF_{1\alpha}$ is commonly measured in plasma and urine as an estimate of PGI_2 synthesis, it should be noted that there may be more than one source of PGI_2 in these samples. For instance, venipuncture may cause the release of PGI_2 which will artifactually increase the 6-keto $PGF_{1\alpha}$ concentration in plasma.[7] Urinary concentrations of 6-keto $PGF_{1\alpha}$ are confounded by the fact that approximately 14% originates from plasma and the remainder is produced by the kidney.[3,7] These problems are circumvented by measuring urinary 2,3-dinor-6-keto $PGF_{1\alpha}$ rather than 6-keto $PGF_{1\alpha}$ as an indicator of systemic PGI_2 production.

Evidence has emerged for an elevated risk of myocardial infarction and stroke associated with the use of COX-2 selective inhibitors.[8,9] A theory explaining this association suggests that COX-2 couples with PGI_2 synthase in endothelial cells for production of vasoprotective PGI_2 whereas COX-1/thromboxane synthase (TXS) in platelets produce TXA_2, an eicosanoid with potent platelet aggregation and vasoconstriction activity. Celecoxib™ and rofecoxib both decrease urinary excretion of 2,3-dinor-6-keto $PGF_{1\alpha}$ (288 pg/mg creatinine to 155 pg/mg creatinine for Celecoxib™) without affecting COX-1 derived TXA_2 formation in platelets.[10,11] Genetic disruption or inhibition of COX-2 in mice substantially reduces 2,3-dinor-6-keto $PGF_{1\alpha}$ without affecting TXA_2 synthesis, whereas, COX-1 knockdown or inhibition has the opposite effect.[12] It is apparent that quantification of 2,3-dinor-6-keto $PGF_{1\alpha}$ is required to gain a more complete understanding of systemic PGI_2 production, particularly as therapeutics targeting the arachidonic acid metabolic pathway, whether COX-2 or downstream enzymes such as mPGES-1, are further developed and evaluated.[12]

About This Assay

Cayman's 2,3-dinor-6-keto $PGF_{1\alpha}$ EIA Kit is a competitive assay that can be used for quantification of 2,3-dinor-6-keto $PGF_{1\alpha}$ in tissue culture supernatants and other sample matrices. The EIA typically displays an IC_{50} (50% B/B_0) of aproximately 400 pg/ml and a detection limit (80% B/B_0) of approximately 100 pg/ml.

Description of ACE™ Competitive EIAs[13,14]

This assay is based on the competition between 2,3-dinor-6-keto $PGF_{1\alpha}$ and a 2,3-dinor-6-keto $PGF_{1\alpha}$-acetylcholinesterase (AChE) conjugate (2,3-dinor-6-keto $PGF_{1\alpha}$ tracer) for a limited number of 2,3-dinor-6-keto $PGF_{1\alpha}$ monoclonal antibody binding sites. Because the concentration of the 2,3-dinor-6-keto $PGF_{1\alpha}$ tracer is held constant while the concentration of 2,3-dinor-6-keto $PGF_{1\alpha}$ varies, the amount of 2,3-dinor-6-keto $PGF_{1\alpha}$ tracer that is able to bind to the monoclonal antibody will be inversely proportional to the concentration of 2,3-dinor-6-keto $PGF_{1\alpha}$ in the well. This antibody-2,3-dinor-6-keto $PGF_{1\alpha}$ complex (either free or tracer) binds to the goat polyclonal anti-mouse 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 2,3-dinor-6-keto $PGF_{1\alpha}$ tracer bound to the well, which is inversely proportional to the amount of free 2,3-dinor-6-keto $PGF_{1\alpha}$ present in the well during the incubation; or

$$\text{Absorbance} \propto [\text{Bound 2,3-dinor-6-keto } PGF_{1\alpha} \text{ Tracer}] \propto 1/[\text{2,3-dinor-6-keto } PGF_{1\alpha}]$$

A schematic of this process is shown in Figure 1, on page 7.

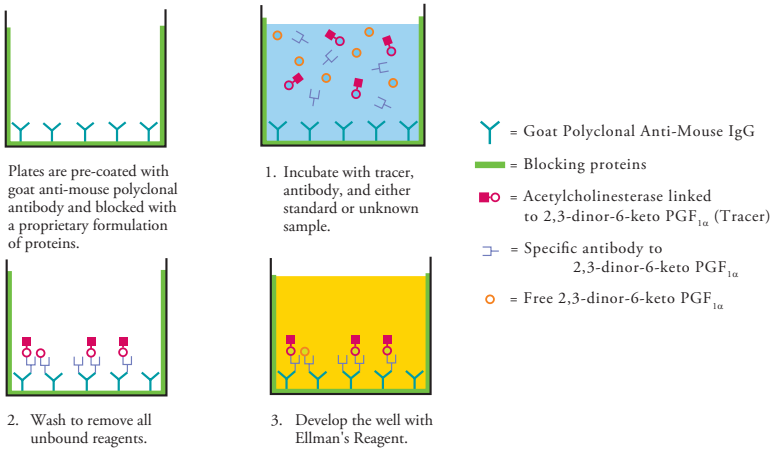


Figure 1. Schematic of the ACE™ EIA

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 2, 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.

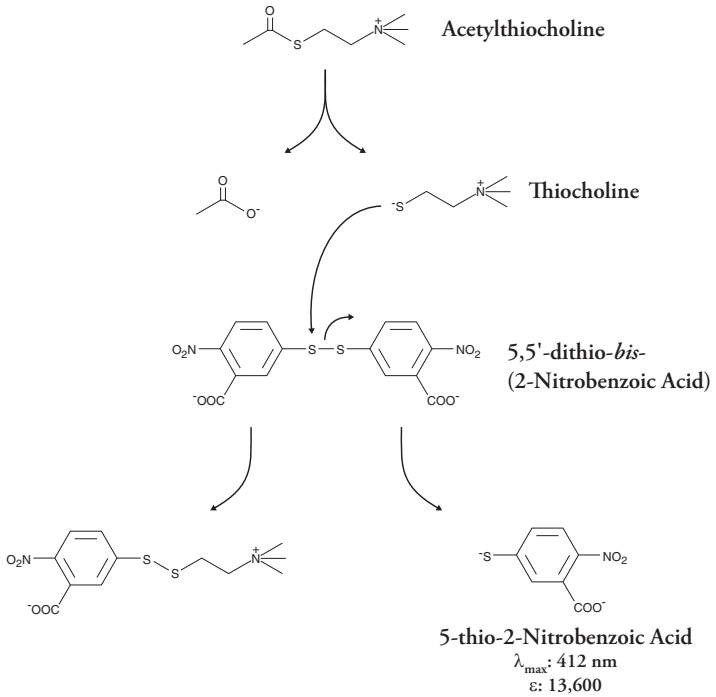


Figure 2. Reaction catalyzed by acetylcholinesterase

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 (Catalog No. 400000).

Buffer Preparation

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

1. 2,3-dinor-6-keto Prostaglandin $F_{1\alpha}$ Assay Buffer Preparation

Dilute the contents of one vial of 2,3-dinor-6-keto $PGF_{1\alpha}$ Assay Buffer Concentrate (Catalog No. 10008829) 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; Catalog No. 400062): Dilute to a total volume of 2 liters with UltraPure water and add 1 ml of Tween 20 (Catalog No. 400035).

OR

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

Urine and plasma are the primary matrices applicable for measurement of 2,3-dinor-6-keto-$PGF_{1\alpha}$. Cell culture samples are not likely to contain significant levels of 2,3-dinor-6-keto $PGF_{1\alpha}$. The applicable indicator of PGI_2 formed in cell culture samples is 6-keto $PGF_{1\alpha}$. Due to the specialized buffering system employed in this assay, we have found that purification of both types of samples is essential to obtain reproducible results in the EIA. A simple liquid-liquid extraction protocol for sample purification is provided below. Other sample types have not been validated for use in this EIA.

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.
- Samples of mouse origin may contain antibodies which interfere with the assay by binding to the goat anti-mouse plate. We recommend that all mouse samples be purified prior to use in this assay.

Determination of Recovery

Determination of percent recovery is recommended when any sample purification is performed. Described below is a method that can be employed to monitor 2,3-dinor-6-keto $PGF_{1\alpha}$ recovery. The sample must be split prior to purification and an appropriate amount of 2,3-dinor-6-keto $PGF_{1\alpha}$ added to one aliquot. The spiked sample is then assayed *via* EIA alongside the unspiked sample. Calculations for recovery are found in the **Analysis** section on page 21.

Purification Protocol

NOTE: We do not recommend the use of plastic vials or caps for this procedure. The ethyl acetate may extract interfering compounds from the plastic.

1. Aliquot an equal amount of each sample into two clean glass test tubes (500 µl 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 10, a 10 ml aliquot will be concentrated by a factor of 20, etc.). Label the first tube "sample # " and the second "sample # + spike".
2. Add a spike of 2,3-dinor-6-keto $PGF_{1\alpha}$ (1,000 pg/ml is recommended) to the "sample + spike" tube. Perform steps 3-7 below for both the unspiked and spiked tubes.
3. Acidify with an equal volume 1 M citrate buffer pH 4.0.
4. Add four volumes of ethyl acetate and vortex 2 x 10 seconds. Remove the top fraction and place into a clean test tube. Repeat two more times and combine the extracts into one tube.*
5. Evaporate to dryness either by 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.
6. Resuspend in 500 µl of assay buffer and vortex. Use this for EIA analysis.

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

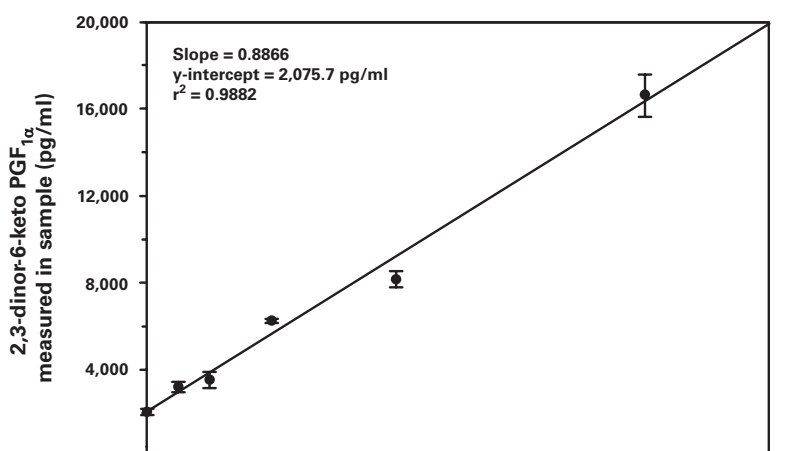


Figure 3. Recovery of 2,3-dinor-6-keto $PGF_{1\alpha}$ from urine

Urine samples were spiked with 2,3-dinor-6-keto $PGF_{1\alpha}$, extracted as described in the **Sample Preparation** section, on page 12, and analyzed using the 2,3-dinor-6-keto $PGF_{1\alpha}$ EIA Kit. The y-intercept corresponds to the amount of 2,3-dinor-6-keto $PGF_{1\alpha}$ in unspiked urine. Error bars represent standard deviations obtained from multiple dilutions of each sample.

ASSAY PROTOCOL

Preparation of Assay-Specific Reagents

2,3-dinor-6-keto Prostaglandin $F_{1\alpha}$ 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 2,3-dinor-6-keto $PGF_{1\alpha}$ EIA Standard (Catalog No. 10008828) 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.

To prepare the standard for use in EIA: Obtain eight clean test tubes and number them #1 through #8. Aliquot 900 µl assay buffer to tube #1 and 500 µl assay buffer to tubes #2-8. Transfer 100 µl of the bulk standard (50 ng/ml) to tube #1 and mix thoroughly. The concentration of this standard, the first point on the standard curve, will be 5 ng/ml (5,000 pg/ml). Serially dilute the standard by removing 500 µl from tube #1 and placing it in tube #2; mix thoroughly. Next, remove 500 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.

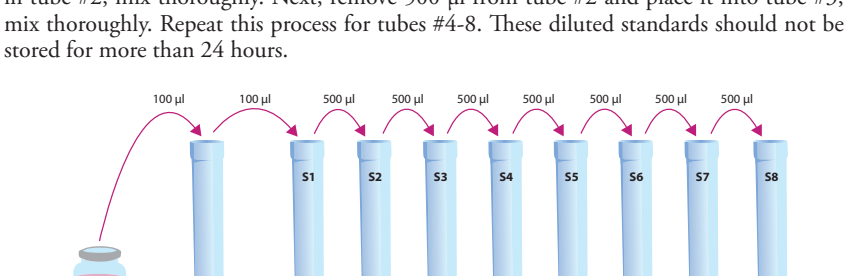


Figure 4. Preparation of the 2,3-dinor-6-keto $PGF_{1\alpha}$ standards

2,3-dinor-6-keto Prostaglandin $F_{1\alpha}$ AChE Tracer

Reconstitute the 2,3-dinor-6-keto $PGF_{1\alpha}$ AChE Tracer as follows:

100 dtn 2,3-dinor-6-keto $PGF_{1\alpha}$ AChE Tracer (96-well kit; Catalog No. 10008830): Reconstitute with 6 ml 2,3-dinor-6-keto $PGF_{1\alpha}$ Assay Buffer.

OR

500 dtn 2,3-dinor-6-keto $PGF_{1\alpha}$ AChE Tracer (480-well kit; Catalog No. 10008830): Reconstitute with 30 ml 2,3-dinor-6-keto $PGF_{1\alpha}$ Assay Buffer.

Store the reconstituted 2,3-dinor-6-keto $PGF_{1\alpha}$ 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.*

2,3-dinor-6-keto Prostaglandin $F_{1\alpha}$ EIA Monoclonal Antibody

Reconstitute the 2,3-dinor-6-keto $PGF_{1\alpha}$ EIA Monoclonal Antibody as follows:

100 dtn 2,3-dinor-6-keto $PGF_{1\alpha}$ EIA Monoclonal Antibody (96-well kit; Catalog No. 10008831): Reconstitute with 6 ml 2,3-dinor-6-keto $PGF_{1\alpha}$ Assay Buffer.

OR

500 dtn 2,3-dinor-6-keto $PGF_{1\alpha}$ EIA Monoclonal Antibody (480-well kit; Catalog No. 10008831): Reconstitute with 30 ml 2,3-dinor-6-keto $PGF_{1\alpha}$ Assay Buffer.

Store the reconstituted 2,3-dinor-6-keto $PGF_{1\alpha}$ EIA Monoclonal Antibody at 4°C. It will be stable for at least four weeks. A 20% surplus of antibody has been included to account for any incidental losses.

Antiserum Dye Instructions (optional)

This dye may be added to the antibody, if desired, to aid in visualization of antibody-containing wells. Add the dye to the reconstituted antibody at a final dilution of 1:100 (add 60 µl of dye to 6 ml antibody or add 300 µl of dye to 30 ml of antibody). *NOTE: Do not store antibody 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 5, 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 5. 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. **2,3-dinor-6-keto Prostaglandin $F_{1\alpha}$ Assay Buffer**

   Add 150 µl assay buffer to Non-Specific Binding (NSB) wells. Add 100 µl assay buffer to Maximum Binding (B_0) wells.

2. **2,3-dinor-6-keto Prostaglandin $F_{1\alpha}$ EIA Standard**

   Add 100 µl from tube #8 to both of the lowest standard wells (S8). Add 100 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 100 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. **2,3-dinor-6-keto Prostaglandin $F_{1\alpha}$ AChE Tracer**

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

5. **2,3-dinor-6-keto Prostaglandin $F_{1\alpha}$ EIA Monoclonal Antibody**

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

Well	2,3-dinor-6-keto $PGF_{1\alpha}$ Assay Buffer	Standard/ Sample	Tracer	Antibody
Blk	-	-	-	-
TA	-	-	5 µl (at devl. step)	-
NSB	150 µl	-	50 µl	-
B_0	100 µl	-	50 µl	50 µl
Std/Sample	-	100 µl	50 µl	50 µl

Table 1. Pipetting summary

Incubation of the Plate

Cover each plate with plastic film (Catalog 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; Catalog No. 400050): Reconstitute with 20 ml of UltraPure water.

   OR

   250 dtn vial Ellman's Reagent (480-well kit; Catalog 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 $\%B/B_0$ *versus* log concentration using either a 4-parameter logistic or log-logit curve 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). Multiply by 100 to obtain $\%B/B_0$. Repeat for S2-S8 and all sample wells.

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 23). 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 27 for* ***Troubleshooting****).*

Plot the Standard Curve

Plot %B/B_0 for standards S1-S8 *versus* 2,3-dinor-6-keto $PGF_{1\alpha}$ concentration using linear (y) and log (x) axes and fit the data to a 4-parameter logistic equation.

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 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.

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)

$$\text{Purification Recovery Factor} = \left[\frac{Y - V}{S}\right]$$

$$\text{2,3-dinor-6-keto } PGF_{1\alpha} \text{ (pg) in purified sample} = \left[\frac{V}{\text{Recovery Factor}}\right] \times 0.5 \text{ ml}$$

$$\text{2,3-dinor-6-keto } PGF_{1\alpha} \text{ in original sample (pg/ml)} = \frac{\text{2,3-dinor-6-keto } PGF_{1\alpha} \text{ (pg) in purified sample}}{\text{Volume of sample used for purification (ml)}}$$

Performance Characteristics

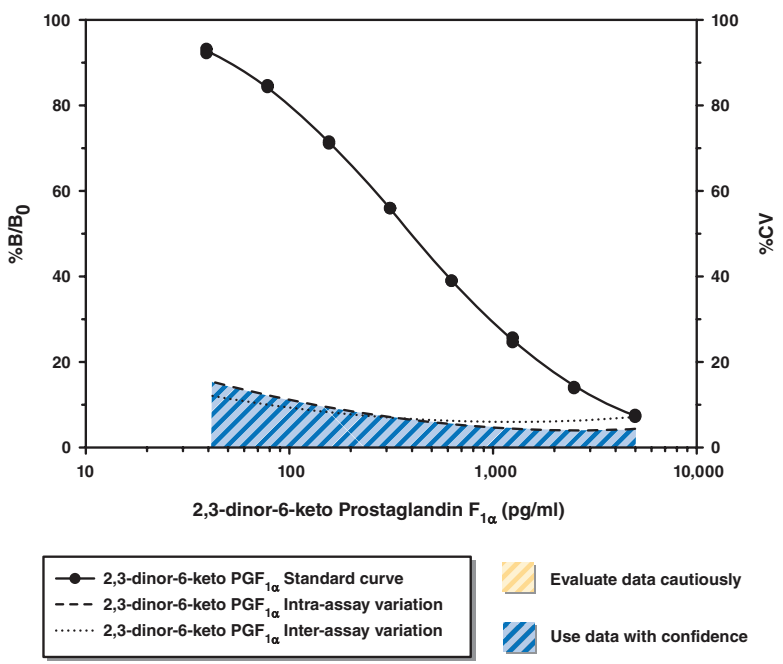
Sample Data

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	1.259	1.273	1.266	
NSB	-0.005	0.017	0.006	
B_0	1.091	1.149		
	1.188	1.247	1.169	1.163

Dose (pg/ml)	Raw Data		Corrected		%B/B_0	
5,000	0.096	0.091	0.090	0.085	7.7	7.3
2,500	0.170	0.172	0.164	0.166	14.1	14.2
1,250	0.294	0.306	0.288	0.300	24.8	25.8
625	0.461	0.461	0.455	0.455	39.2	39.1
313	0.649	0.658	0.643	0.652	55.3	56.1
156	0.839	0.835	0.833	0.829	71.7	71.3
78	0.988	0.986	0.982	0.980	84.5	84.3
39	1.081	1.092	1.075	1.086	92.4	93.4

Table 2. Typical results



50% B/B_0 - 400 pg/ml

Detection Limit (80% B/B_0) - 100 pg/ml

Figure 8. Typical standard curve

Precision:

The intra- and inter-assay CV's have been determined at multiple points on the standard curve. These data are summarized in the graph on page 24.

Dose (pg/ml)	%CV* Intra-assay variation	%CV* Inter-assay variation
5,000	4.3	6.9
2,500	4.7	5.4
1,250	4.5	7.1
625	5.5	7.6
313	7.2	9.7
156	9.0	5.9
78	12.7	5.5
39	15.8	16.0

Table 3. Intra- and inter-assay variation

*%CV represents the variation in concentration (not absorbance) as determined using a reference standard curve.

Specificity:

Compound	Cross-reactivity
2,3-dinor-6-keto Prostaglandin $F_{1\alpha}$	100%
tetranor-PGFM	0.07%
Prostaglandin B_1	<0.01%
Prostaglandin B_2	<0.01%
Prostaglandin E_1	<0.01%
Prostaglandin E_2	<0.01%
Prostaglandin $F_{1\alpha}$	<0.01%
13,14-dihydro-Prostaglandin $F_{1\alpha}$	<0.01%
6,15-diketo-13,14-dihydro-Prostaglandin $F_{1\alpha}$	<0.01%
6-keto Prostaglandin $F_{1\alpha}$	<0.01%
Prostaglandin $F_{2\alpha}$	<0.01%
15-keto Prostaglandin $F_{2\alpha}$	<0.01%
Thromboxane B2	<0.01%

Table 4. Specificity of the 2,3-dinor-6-keto $PGF_{1\alpha}$ Monoclonal Antibody

RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates	A. Trace organic contaminants in the water source B. Contamination of water with organic solvents C. 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[15]
Only Total Activity (TA) wells develop	Trace organic contaminants in the water source	Replace activated carbon filter or change source of UltraPure water

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Related Products

2,3-dinor-6-keto Prostaglandin $F_{1\alpha}$ (sodium salt) - Cat. No. 15120
6-keto Prostaglandin $F_{1\alpha}$ - Cat. No. 515211
UltraPure Water - Cat. No. 400000

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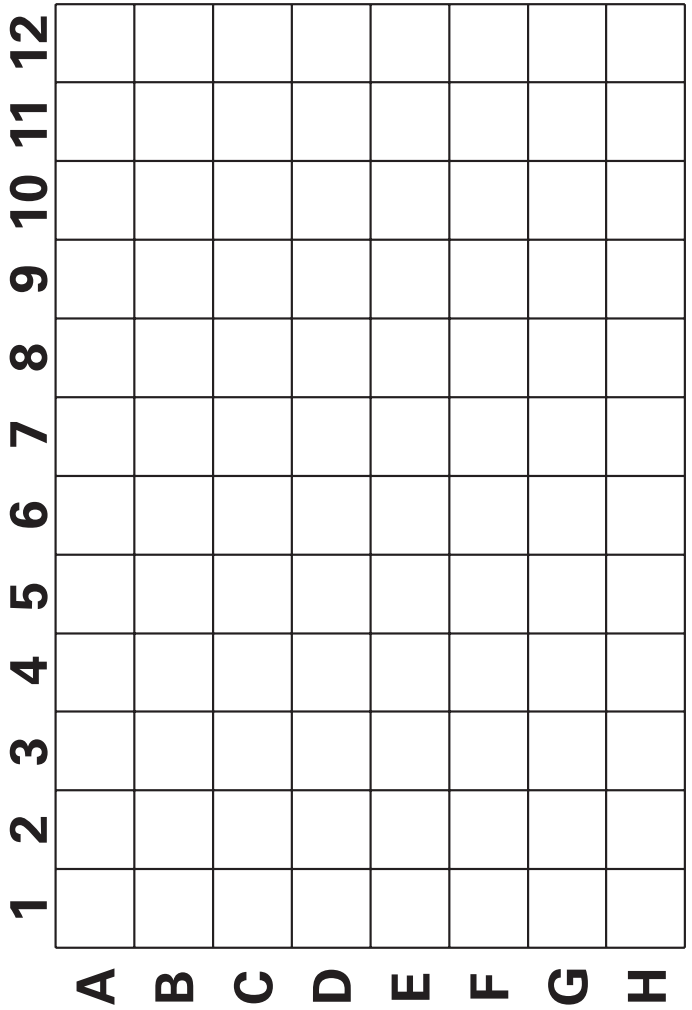
Said refund or replacement is conditioned on Buyer giving written notice to Cayman within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

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



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