



---

## Fluprostenol ELISA Kit

---

Item No. 516761

[www.caymanchem.com](http://www.caymanchem.com)

Customer Service 800.364.9897

Technical Support 888.526.5351

1180 E. Ellsworth Rd • Ann Arbor, MI • USA

## TABLE OF CONTENTS

<b>GENERAL INFORMATION</b>	3	Materials Supplied
	4	Safety Data
	4	Precautions
	5	If You Have Problems
	5	Storage and Stability
	5	Materials Needed but Not Supplied
<b>INTRODUCTION</b>	6	Background
	7	About This Assay
	8	Description of AChE Competitive ELISAs
	10	Biochemistry of Acetylcholinesterase
	12	Definition of Key Terms
<b>PRE-ASSAY PREPARATION</b>	13	Buffer Preparation
	14	Sample Preparation
	15	Sample Purification
<b>ASSAY PROTOCOL</b>	17	Preparation of Assay-Specific Reagents
	19	Plate Set Up
	20	Performing the Assay
<b>ANALYSIS</b>	23	Calculations
	25	Performance Characteristics
<b>RESOURCES</b>	29	Troubleshooting
	30	References
	31	Plate Template
	32	Notes
	32	Warranty and Limitation of Remedy

## GENERAL INFORMATION

### Materials Supplied

Item Number	Item Name	96 wells Quantity/Size	480 wells Quantity/Size
416762	Fluprostenol ELISA Antiserum	1 vial/100 dtn	1 vial/500 dtn
416760	Fluprostenol AChE Tracer	1 vial/100 dtn	1 vial/500 dtn
416764	Fluprostenol ELISA Standard	1 vial	1 vial
400060	ELISA 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	Polysorbate 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

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.



**WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.**

## Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

## 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 Fluprostenol ELISA Kit. This kit may not perform as described if any reagent or procedure is replaced or modified.

When compared to quantification by LC/MS or GC/MS, it is not uncommon for immunoassays to report higher analyte concentrations. While LC/MS or GC/MS analyses typically measure only a single compound, antibodies used in immunoassays sometimes recognize not only the target molecule, but also structurally related molecules, including biologically relevant metabolites. In many cases, measurement of both the parent molecule and metabolites is more representative of the overall biological response than is the measurement of a short-lived parent molecule. It is the responsibility of the researcher to understand the limits of both assay systems and to interpret their data accordingly.

## If You Have Problems

### Technical Service Contact Information

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

Email: techserv@caymanchem.com

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 repeating pipettor.
3. A source of 'UltraPure' water. Water used to prepare all ELISA 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 ELISA. *NOTE: UltraPure water is available for purchase from Cayman (Item No. 400000).*
4. Materials used for Sample Preparation (see page 14).

## Background

Fluprostenol is 16-*m*-trifluoromethylphenoxy tetranor prostaglandin F<sub>2α</sub> (PGF<sub>2α</sub>), an F-series PG analog which has been approved for use as an ocular hypotensive drug for the treatment of glaucoma.<sup>1</sup> In clinical trials, (+)-fluprostenol at a dose of 0.004% once daily decreased intraocular pressure by 28.1-30.7%.<sup>2</sup> A typical treatment regime consists of topical optical administration of the prodrug, fluprostenol isopropyl ester, which crosses the cornea and is hydrolyzed to the biologically active form, (+)-fluprostenol. Fluprostenol is thought to exert its pharmacological activity by binding directly to the FP receptor.<sup>1,2</sup>

Fluprostenol is used in the veterinary field for induction of estrus in mares. It is also a potent luteolytic agent. When given to hamsters on the fourth, fifth, or sixth day of pregnancy, fluprostenol is 100 times more potent than PGF<sub>2α</sub> in causing termination of pregnancy.<sup>3,4</sup>

## About This Assay

Cayman's Fluprostenol ELISA is a competitive immunoassay that can be used to measure the free acid form of fluprostenol. It also detects the isopropyl ester form of fluprostenol, but with less sensitivity. The assay is most appropriate for use when only one of the two forms is present. Samples containing a mixture of both the isopropyl ester and free acid should be purified and the two compounds separated prior to ELISA analysis. The assay has a range from 7.8-1,000 pg/ml and a sensitivity (80% B/B<sub>0</sub>) of approximately 16 pg/ml.

## Description of AChE Competitive ELISAs<sup>5,6</sup>

This assay is based on the competition between fluprostenol and a fluprostenol-acetylcholinesterase (AChE) conjugate (fluprostenol tracer) for a limited number of fluprostenol-specific rabbit antiserum binding sites. Because the concentration of the fluprostenol tracer is held constant while the concentration of fluprostenol varies, the amount of fluprostenol tracer that is able to bind to the rabbit antiserum will be inversely proportional to the concentration of fluprostenol in the well. This rabbit antiserum-fluprostenol (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 fluprostenol tracer bound to the well, which is inversely proportional to the amount of free fluprostenol present in the well during the incubation; or

$$\text{Absorbance} \propto [\text{Bound Fluprostenol Tracer}] \propto 1/[\text{Fluprostenol}]$$

A schematic of this process is shown in Figure 1, on page 8.

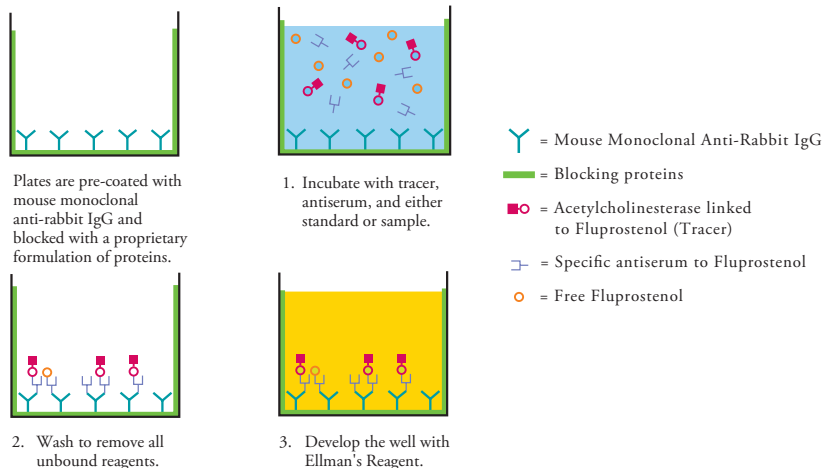


Figure 1. Schematic of the AChE ELISA

## Biochemistry of Acetylcholinesterase

The electric organ of the electric eel, *E. electricus*, contains an avid 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 ( $64,000 \text{ s}^{-1}$ ) for the hydrolysis of acetylthiocholine.

A molecule of the analyte covalently attached to a molecule of AChE serves as the tracer in AChE 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 11). 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 ( $\epsilon = 13,600$ ).

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 redevelopment 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.

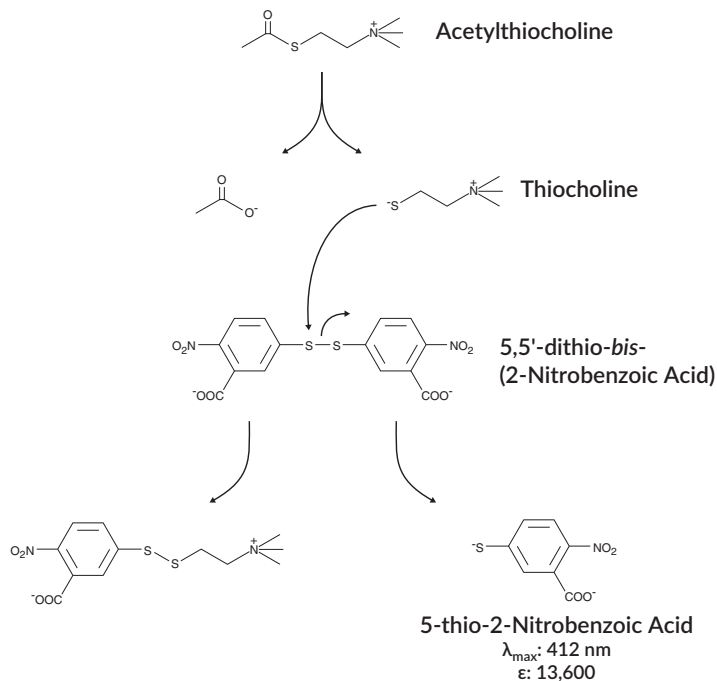


Figure 2. Reaction catalyzed by acetylcholinesterase

## Definition of Key Terms

**Blk (Blank):** background absorbance caused by Ellman's Reagent. The blank absorbance should be subtracted from the absorbance readings of all the other wells, including NSB wells.

**TA (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. Do not forget to subtract the Blank absorbance values.

**B<sub>0</sub> (Maximum Binding):** maximum amount of the tracer that the antibody can bind in the absence of free analyte.

**%B/B<sub>0</sub> (%Bound/Maximum Bound):** ratio of the absorbance of a particular sample or standard well to that of the maximum binding (B<sub>0</sub>) well.

**Standard Curve:** a plot of the %B/B<sub>0</sub> 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 ELISA 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 ELISA. 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. ELISA Buffer Preparation

Dilute the contents of one vial of ELISA 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 Polysorbate 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 Polysorbate 20 (Item No. 400035).

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

*NOTE: Polysorbate 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

In general, urine and tissue culture supernatant samples may be diluted with ELISA Buffer and added directly to the assay well. Aqueous humor, plasma, serum, 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 20 and 750 pg/ml (*i.e.*, between 20-80% B/B<sub>0</sub>). If the two different dilutions of the sample show good correlation (differ by 20% or less) in the final calculated fluprostenol concentration, purification is not required. If you do not see good correlation of the different dilutions, purification is advised. The **Sample Purification**, on pages 15-16, is one such method.

### 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 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.

## Sample Purification

1. Aliquot a known amount of each sample into a clean test tube (500  $\mu$ l is recommended). If your samples need to be concentrated, a larger volume should be used (e.g., a 5 ml sample will be concentrated by a factor of 10, a 10 ml sample will be concentrated by a factor of 20, etc.).
2. *Precipitation of proteins using ethanol is optional and may not be needed if samples are clean enough to flow through the SPE Cartridge (C-18). Body fluids such as plasma and urine can typically be applied directly to the SPE Cartridge (C-18) after the acidification step (step 4) below.* To precipitate proteins, add ethanol (approximately four times the sample volume) to each tube. Vortex to mix thoroughly. Incubate samples at 4°C for five minutes, then centrifuge a 3,000 x g for 10 minutes to remove precipitated proteins. Transfer the supernatant to a clean test tube. Evaporate the ethanol under nitrogen.
3. Adjust the pH of the sample to ~4.0 using 1.0 M acetate buffer (or citrate buffer). (Standardize the pH adjustment using the sample matrix prior to proceeding with a large number of samples; approximately 1-2 equivalents of buffer is required for most biological samples.) If the samples are cloudy or contain precipitate, either filter or centrifuge to remove the precipitate. Particulate matter in the sample may clog the SPE Cartridge (C-18), resulting in loss of the sample.

4. Activate an SPE Cartridge (C-18) (6 ml) (Item No. 400020) by rinsing with 5 ml methanol followed by 5 ml UltraPure water. Do not allow the SPE Cartridge (C-18) to dry.
5. Pass the sample through the SPE Cartridge (C-18). Wash the cartridge with 5 ml UltraPure water and discard the wash. Elute the fluprostenol with 5 ml ethyl acetate containing 1% methanol. Higher recovery and better reproducibility may be obtained if the sample is applied and eluted by gravity. The wash steps may be performed under vacuum or pressure.\*
6. Evaporate the ethyl acetate to dryness 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 ELISA.
7. Add 500  $\mu$ l of ELISA Buffer and vortex. Use this for ELISA analysis. It is common for an insoluble precipitate to remain after the addition of ELISA Buffer; this will not affect the assay.

\*If it is necessary to stop during this purification, samples may be stored in the ethyl acetate/methanol solution at -20°C.  
obtained from multiple dilutions of each sample.



## Preparation of Assay-Specific Reagents

### Fluprostenol ELISA 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  $\mu$ l of the Fluprostenol ELISA Standard (Item No. 416764) into a clean test tube, then dilute with 900  $\mu$ l UltraPure water. The concentration of this solution (the bulk standard) will be 10 ng/ml. Extra standard has been provided in the event additional standard curves are desired.

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

To prepare the standard for use in ELISA: Obtain eight clean test tubes and number them #1 through #8. Aliquot 900  $\mu$ l ELISA Buffer to tube #1 and 500  $\mu$ l ELISA Buffer to tubes #2-8. Transfer 100  $\mu$ l of the bulk standard (10 ng/ml) to tube #1 and mix thoroughly. Serially dilute the standard by removing 500  $\mu$ l from tube #1 and placing in tube #2; mix thoroughly. Next, remove 500  $\mu$ l 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.

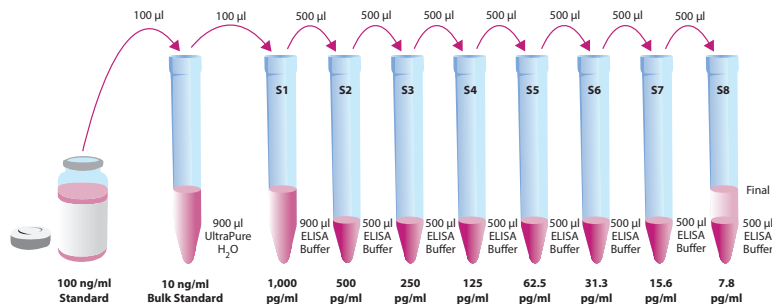


Figure 3. Preparation of the Fluprostenol standards

### Fluprostenol AChE Tracer

Reconstitute the Fluprostenol AChE Tracer as follows:

**100 dtn Fluprostenol AChE Tracer (96-well kit; Item No. 416760):**  
Reconstitute with 6 ml ELISA Buffer.

OR

**500 dtn Fluprostenol AChE Tracer (480-well kit; Item No. 416760):**  
Reconstitute with 30 ml ELISA Buffer.

Store the reconstituted Fluprostenol AChE Tracer at 4°C (*do not freeze!*) and use within two weeks. A 20% surplus of tracer has been included to account for any incidental losses.

### Fluprostenol ELISA Antiserum

Reconstitute the Fluprostenol ELISA Antiserum as follows:

**100 dtn Fluprostenol ELISA Antiserum (96-well kit; Item No. 416762):**  
Reconstitute with 6 ml ELISA Buffer.

OR

**500 dtn Fluprostenol ELISA Antiserum (480-well kit; Item No. 416762):**  
Reconstitute with 30 ml ELISA Buffer.

Store the reconstituted Fluprostenol ELISA 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.

## 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 4, 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 23, 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 4. 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. ELISA Buffer

Add 100  $\mu$ l ELISA Buffer to NSB wells. Add 50  $\mu$ l ELISA Buffer to  $B_0$  wells. If culture medium was used to dilute the standard curve, substitute 50  $\mu$ l of culture medium for ELISA Buffer in the NSB and  $B_0$  wells (*i.e.*, add 50  $\mu$ l culture medium to NSB and  $B_0$  wells and 50  $\mu$ l ELISA Buffer to NSB wells).

#### 2. Fluprostenol ELISA Standard

Add 50  $\mu$ l from tube #8 to both of the lowest standard wells (S8). Add 50  $\mu$ l 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  $\mu$ l 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. Fluprostenol AChE Tracer

Add 50  $\mu$ l to each well *except* the TA and the Blk wells.

#### 5. Fluprostenol ELISA Antiserum

Add 50  $\mu$ l to each well *except* the TA, the NSB, and the Blk wells.

Well	ELISA Buffer	Standard/ Sample	Tracer	Antiserum
Blk	-	-	-	-
TA	-	-	5 µl (at devl. step)	-
NSB	100 µl	-	50 µl	-
B <sub>0</sub>	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 overnight 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 TA 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<sub>0</sub> 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<sub>0</sub> wells have reached a minimum of 0.3 A.U. (blank subtracted). The plate should be read when the absorbance of the B<sub>0</sub> 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<sub>0</sub> versus log concentration using a four-parameter logistic fit or as logit B/B<sub>0</sub> versus log concentration using a linear fit. *NOTE: Cayman has a computer spreadsheet available for data analysis. Please contact Technical Service or visit our website ([www.caymanchem.com/analysis/elisa](http://www.caymanchem.com/analysis/elisa)) 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<sub>0</sub> wells.
3. Subtract the NSB average from the B<sub>0</sub> average. This is the corrected B<sub>0</sub> or corrected maximum binding.
4. Calculate the B/B<sub>0</sub> (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<sub>0</sub> (from Step 3). Repeat for S2-S8 and all sample wells. (To obtain %B/B<sub>0</sub> for a logistic four-parameter fit, multiply these values by 100.)

*NOTE: The TA values are not used in the standard curve calculations. Rather, they are used as a diagnostic tool; the corrected B<sub>0</sub> 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 25). 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 29 for **Troubleshooting**).*

#### Plot the Standard Curve

Plot %B/B<sub>0</sub> for standards S1-S8 versus Fluprostenol concentration using linear (y) and log (x) axes and perform a 4-parameter logistic fit.

Alternative Plot - The data can also be linearized using a logit transformation. The equation for this conversion is shown below. *NOTE: Do not use %B/B<sub>0</sub> in this calculation.*

$$\text{logit (B/B}_0\text{)} = \ln [\text{B/B}_0\text{}/(1 - \text{B/B}_0\text{)}]$$

Plot the data as logit (B/B<sub>0</sub>) versus log concentrations and perform a linear regression fit.

#### Determine the Sample Concentration

Calculate the B/B<sub>0</sub> (or %B/B<sub>0</sub>) 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<sub>0</sub> 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.*

## Performance Characteristics

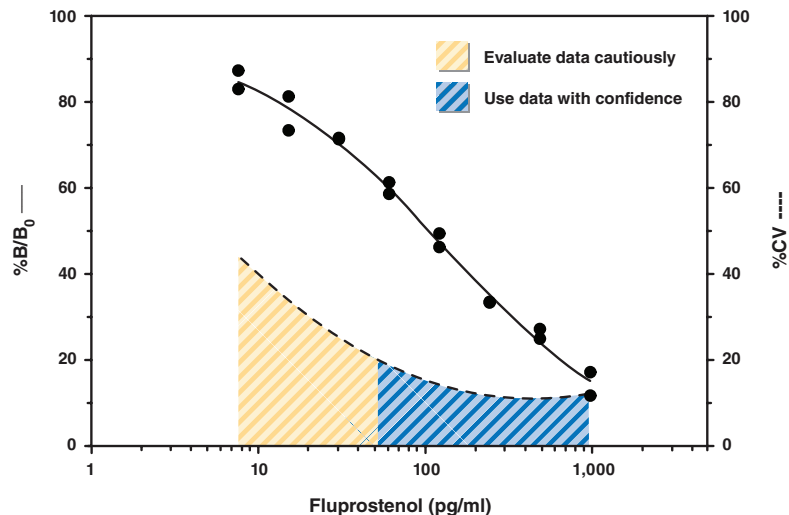
### Sample Data

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 value of your samples. Your results could differ substantially.

	Raw Data		Average	Corrected
Total Activity	0.785	0.828	0.806	
NSB	0	0	0	
$B_0$	0.427	0.470		
	0.466	0.427	0.448	0.448

Dose (pg/ml)	Raw Data		Corrected		%B/B <sub>0</sub>	
1,000	0.053	0.077	0.053	0.077	11.8	17.2
500	0.112	0.122	0.112	0.122	25.0	27.3
250	0.150	0.149	0.150	0.149	33.5	33.3
125	0.207	0.221	0.207	0.221	46.5	49.4
62.5	0.262	0.274	0.262	0.274	58.5	61.2
31.3	0.319	0.320	0.319	0.320	71.3	71.5
15.6	0.363	0.328	0.363	0.328	81.1	73.3
7.8	0.371	0.390	0.371	0.390	82.9	87.2

Table 2. Typical results



**Assay Range** = 7.8-1,000 pg/ml  
**Sensitivity** (defined as 80% B/B<sub>0</sub>) = ~16 pg/ml  
**Mid-point** (defined as 50% B/B<sub>0</sub>) = ~110 pg/ml

The sensitivity and mid-point were derived from the standard curve shown above. The standard was diluted with ELISA Buffer.

Figure 5. 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 26 and in the table below.

Dose (pg/ml)	%CV* Intra-assay variation	%CV* Inter-assay variation
1,000	12.3	3.8
500	13.4	15.4
250	8.7	11.9
125	16.5	16.1
62.5	13.2	17.8
31.3	†	†
15.6	†	†
7.8	†	†

**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
Fluprostenol	100%	tetranor-PGEM	<0.01%
2,3-dinor Fluprostenol	100%	tetranor-PGFM	<0.01%
Fluprostenol lactone diol	100%	Prostaglandin D <sub>2</sub>	<0.01%
9-keto Fluprostenol	83.0%	Prostaglandin E <sub>2</sub>	<0.01%
15(S)-Fluprostenol isopropyl ester	27.0%	Prostaglandin E <sub>2</sub> Ethanolamide	<0.01%
11-keto Fluprostenol	15.2%	6-keto Prostaglandin F <sub>1α</sub>	<0.01%
Fluprostenol isopropyl ester	10.0%	Prostaglandin F <sub>2α</sub>	<0.01%
9-keto Fluprostenol isopropyl ester	8.50%	Prostaglandin F <sub>2α</sub> Ethanolamide	<0.01%
15-keto Fluprostenol free acid	0.90%	5- <i>trans</i> Prostaglandin F <sub>2α</sub>	<0.01%
15-keto Fluprostenol isopropyl ester	0.02%	17-phenyl trinor Prostaglandin F <sub>2α</sub>	<0.01%
Latanoprost	<0.01%	17-phenyl trinor Prostaglandin F <sub>2α</sub> ethyl amide	<0.01%
Latanoprost (free acid)	<0.01%	Thromboxane B <sub>2</sub>	<0.01%
15- <i>epi</i> Latanoprost	<0.01%	Unoprostone	<0.01%
5,6- <i>trans</i> Latanoprost	<0.01%	Unoprostone isopropyl ester	<0.01%

**Table 4. Specificity of the Fluprostenol ELISA Antiserum**

## RESOURCES

### 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 (>10% of B <sub>0</sub> )	A. Poor washing B. Exposure of NSB wells to specific antibody	A. Rewash plate and redevelop
Very low B <sub>0</sub>	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 ELISA <sup>7</sup>
Only Total Activity (TA) wells develop	Trace organic contaminants in the water source	Replace activated carbon filter or change source of UltraPure water

### References

1. McCue, B.A., Cason, M.M., Curtis, M.A., *et al.* Determination of travoprost and travoprost free acid in human plasma by electrospray HPLC/MS/MS. *J. Pharm. Biomed. Analysis* **28**, 199-208 (2002).
2. Sorbera, L.A. and Castañer, J. Travoprost. *Drugs of the Future* **25**, 41-45 (2000).
3. Binder, D., Bowler, J., Brown, E.D., *et al.* 16-Aryloxyprostaglandins: A new class of potent luteolytic agent. *Prostaglandins* **6**, 87-90 (1974).
4. Dukes, M., Russell, W., and Walpole, A.L. Potent luteolytic agents related to prostaglandin F<sub>2α</sub>. *Nature* **250**, 330-331 (1974).
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 acetylcholinesterase as label: An alternative to radioimmunoassay. *Anal. Chem.* **57**, 1170-1173 (1985).
7. Maxey, K.M., Maddipati, K.R. and Birkmeier, J. Interference in enzyme immunoassays. *J. Clin. Immunoassay* **15**, 116-120 (1992).

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

## NOTES

### Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.

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.

©10/06/2022, Cayman Chemical Company, Ann Arbor, MI, All rights reserved. Printed in U.S.A.



