Prostaglandin E Metabolite ELISA Kit

Item No. 514531

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	96 wells Quantity/Size	480 wells Quantity/Size
414532	Prostaglandin E Metabolite ELISA Antiserum	1 vial/100 dtn	1 vial/500 dtn
414530	Prostaglandin E Metabolite AChE Tracer	1 vial/100 dtn	1 vial/500 dtn
414534	Prostaglandin E Metabolite 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
400005/400007	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
400032	Phosphate Buffer	1 vial/100 dtn	2 vials/250 dtn
400027	Carbonate Buffer	1 vial/100 dtn	1 vial/500 dtn
400040	ELISA Tracer Dye	1 vial	1 vial
400042	ELISA 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) 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 Prostaglandin E Metabolite 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, with a resistivity of 18.2 MΩ·cm and total organic carbon (TOC) levels of <10 ppb, is recommended. Pure water - glass-distilled or deionized - may not be acceptable. *NOTE: UltraPure Water is available for purchase from Cayman (Item No. 400000).*
4. Materials used for **Sample Preparation** (see page 11).

INTRODUCTION

Background

Prostaglandin E_2 (PGE_2) is an eicosanoid formed from arachidonic acid by COX enzymes and PGE_2 synthase (PGES) *via* PGG_2 and PGH_2 intermediates.[1] It can be produced *de novo* in all cells following cell activation and the subsequent release of arachidonic acid from plasma membrane phospholipids or when exogenous free arachidonate is available.[1] It acts in an autocrine or paracrine fashion to bind to its receptors, EP_1, EP_2, EP_3, and EP_4, to initiate signaling through various pathways. PGE_2 is involved in a wide variety of biological processes, including inflammation, fertility, gastric mucosal integrity and motility, and immune modulation.[1-6] *In vivo*, PGE_2 is rapidly metabolized to the unstable metabolite 13,14-dihydro-15-keto-PGE_2, which undergoes further metabolism and non-enzymatic degradation.[7-8] For this reason, mammalian samples often contain very little PGE_2, and measurement of its metabolites is necessary to provide a reliable estimate of PGE_2 production.

About This Assay

Cayman's Prostaglandin E Metabolite (PGEM) assay converts downstream metabolites of PGE_2, such as 13,14-dihydro-15-keto PGE_2 and 13,14-dihydro-15-keto PGA_2, to stable derivatives that can be easily quantified in a competitive ELISA format. This assay is the method of choice for analysis of samples, such as plasma, serum and urine, in which PGE_2 has undergone extensive metabolism prior to collection. The assay has a range from 0.39-50 pg/ml and an average sensitivity (80% B/B_0) of 2 pg/ml.

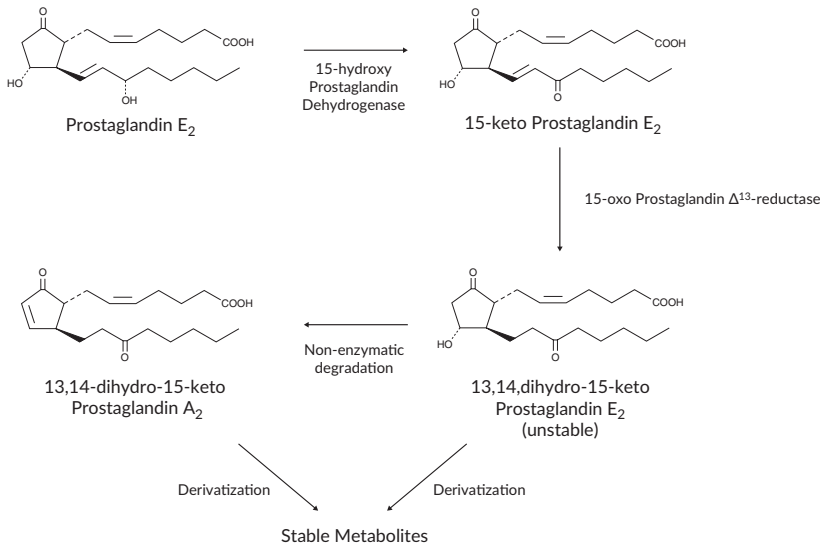


Figure 1. Metabolism of PGE_2

Principle Of This Assay

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

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

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

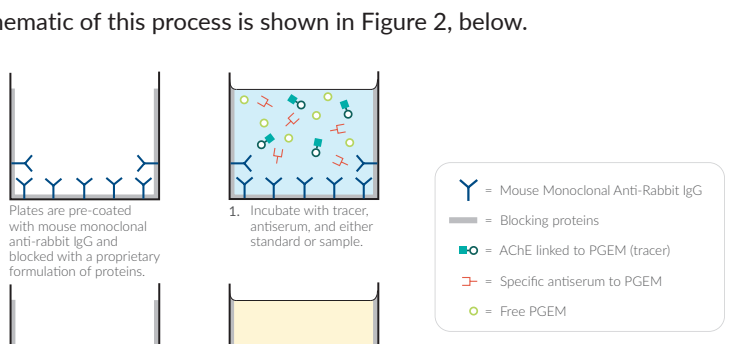


Figure 2. Schematic of the AChE ELISA

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 analyte enzyme abbreviation-linked 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 sample or standard well to the average absorbance of the maximum binding (B_0) wells.

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): one dtn is the amount of reagent used per well.

Cross Reactivity: numerical representation of the relative reactivity of this assay towards structurally related molecules as compared to the primary analyte of interest. Biomolecules that possess similar epitopes to the analyte can compete with the assay tracer for binding to the primary antibody. Substances that are superior to the analyte in displacing the tracer result in a cross reactivity that is greater than 100%. Substances that are inferior to the primary analyte in displacing the tracer result in a cross reactivity that is less than 100%. Cross reactivity is calculated by comparing the midpoint (50% B/B0) value of the tested molecule to the midpoint (50% B/B0) value of the primary analyte when each is measured in assay buffer using the following formula:

$$\%\ \text{Cross Reactivity} = \left[\frac{50\%\ B/B_0 \text{ value for the primary analyte}}{50\%\ B/B_0 \text{ value for the potential cross reactant}} \right] \times 100\%$$

PRE-ASSAY PREPARATION

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.

3. **Phosphate Buffer**

   Prepare a 1 M Phosphate Buffer solution by dissolving the contents of the 100 dtn vial of Phosphate Buffer (Item No. 400032) in 30 ml ultrapure water, or dissolve the contents of one of the 250 dtn vials of Phosphate Buffer (Item No. 400032) in 75 ml ultrapure water.

4. **Carbonate Buffer**

   Prepare a 1 M Carbonate Buffer solution by dissolving the contents of the 100 dtn vial of Carbonate Buffer (Item No. 400027) in 25 ml ultrapure water, or dissolve the contents of the 500 dtn vial of Carbonate Buffer (Item No. 400027) in 125 ml ultrapure water.

5. **PGEM Assay Buffer**

   Prepare 20 ml of PGEM Assay Buffer by combining 13 ml ELISA Buffer, 3 ml Carbonate Buffer, and 4 ml Phosphate Buffer. This quantity of buffer should be more than sufficient to complete one 96-well plate.

Sample Preparation

This assay has been validated for a wide range of samples including urine, and plasma. Proper sample storage and preparation are essential for consistent and accurate results. Please read this section carefully before beginning the assay.

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.
- AEBSF (Pefabloc SC®) and PMSF inhibit AChE. Samples containing these protease inhibitors should not be used in this assay.

Testing for Interference

In general, urine samples may be derivatized, diluted with PGE Metabolite Buffer and added directly to the assay well. Plasma, serum, and 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, derivatize one or two test samples, then dilute with PGE Metabolite Buffer to obtain at least two different dilutions within the linear portion of the standard curve. If the two different dilutions of the sample show good correlation (differ by 20% or less) in the final calculated PGEM concentrations, purification is not required. If you do not see good correlation of the different dilutions, purification is advised. The protocol described on page 14 is one such method.

Plasma and Serum

Analysis of plasma and serum samples without purification may lead to inconsistent results. We recommend using the purification protocol described below for all plasma samples.

Plasma samples should be collected in vacutainers containing sodium citrate, heparin, or EDTA. Serum samples should be collected in vacutainers without a coagulant. Vacutainers can also be supplemented with indomethacin to give a final concentration of at least 10 µM. Indomethacin will prevent *ex vivo* formation of prostaglandins, which have the potential to interfere with this assay (although most prostaglandins do not appear to exhibit any cross reactivity (see page 30)). Samples that cannot be assayed immediately should be stored at -80°C.

Sample Purification

Acetone Precipitation

Acetone precipitation is recommended for removal of proteins from heterogeneous samples. For some samples, acetone precipitation may be the only purification that is required.

1. Aliquot samples into clean tubes.
2. Add four volumes of ice-cold acetone.
3. Incubate at -20°C for a minimum of thirty minutes.
4. Pellet proteins by centrifugation in a tabletop centrifuge at a minimum of 400 x g for five minutes.
5. Transfer supernatants to clean tubes.
6. Dry under a gentle stream of nitrogen.
7. Resuspend samples in the original volume of ELISA Buffer.
8. The samples are now ready for derivatization.

ASSAY PROTOCOL

Derivatization of Standards and Samples to PGEM

Derivatization Hints

- Allow the derivatization to proceed overnight to ensure that all the PGE_2 metabolites derivatize completely.
- Derivatize all standards and samples for the same amount of time.

Derivatization of the PGEM 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 µl of the PGEM ELISA Standard (Item No. 414534) into a clean test tube, then dilute with 900 µl ultrapure water. The concentration of this solution (the bulk standard) will be 40 ng/ml.

Aliquot 50 µl of this solution into a clean tube and dilute to a total volume of 1 ml with ELISA Buffer (*i.e.*, add 950 µl). Add 300 µl of Carbonate Buffer and incubate at 37°C overnight. Then add 400 µl Phosphate Buffer and 300 µl ELISA Buffer. This solution is 1,000 pg/ml.

Derivatization of the Samples

Aliquot 500 µl of each sample into a clean test tube. Add 150 µl of Carbonate Buffer and incubate overnight at 37°C. Then add 200 µl of Phosphate Buffer and 150 µl of ELISA Buffer. Depending on your sample type, the samples may now be ready to use directly in the assay.

NOTE: Because of the high salt concentration in the PGEM Standards, it is crucial that PGEM Assay Buffer is used rather than ELISA Buffer to dilute samples if further dilution is necessary.

For plasma samples, the following ethyl acetate extraction protocol is suggested after derivatization.

Acidification and Ethyl Acetate Extraction

We suggest the following procedure for samples requiring additional purification following derivatization:

1. Acidify the sample to ~pH 4 by the addition of 1M acetate buffer. (To avoid having to measure the pH of each individual sample, adjust the pH of an equivalent volume of sample matrix to pH 4.0 using the 1M acetate buffer. *NOTE: For samples of different volumes, the amount of acid should be adjusted to maintain this ratio of acid to sample.*)
2. Add two volumes of ethyl acetate to each derivatized sample.
3. Vortex thoroughly to mix.
4. Centrifuge samples at a minimum of 200 x g for five minutes.
5. Transfer the ethyl acetate (upper layer) to a clean glass tube.
6. Repeat this extraction three more times, combining all of the ethyl acetate layers from each individual sample.
7. Dry samples under a gentle stream of nitrogen.
8. Resuspend samples in PGEM Assay Buffer, in the original sample volume.
9. The samples are now ready to use in the assay

NOTE: Because of the high salt concentration in the PGEM Standards, it is crucial that PGEM Assay Buffer is used rather than ELISA Buffer to dilute samples if further dilution is necessary.

Preparing the Standard Curve

NOTE: Because of the high salt concentration in the 1,000 pg/ml solution, all the points of the standard curve must contain the same salt concentration. Thus, when performing the serial dilution, use the PGEM Assay Buffer.

To prepare the standard for use in ELISA: Obtain eight clean test tubes and number them #1 through #8. Aliquot 950 µl PGEM Assay Buffer to tube #1 and 500 µl PGEM Assay Buffer to tubes #2-8. Transfer 50 µl of the derivatized standard (1,000 pg/ml) to tube #1 and mix thoroughly. 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.

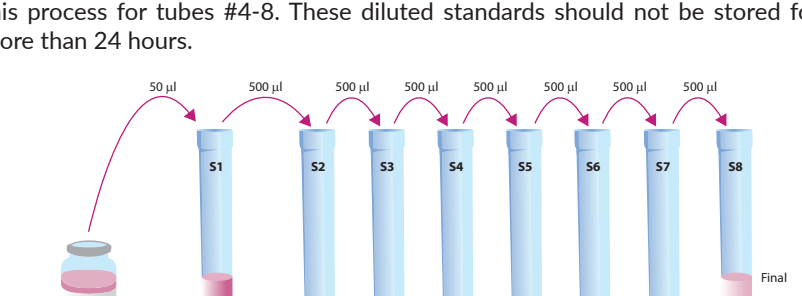


Figure 3. Preparation of the PGEM standards

Preparation of Assay-Specific Reagents

PGEM AChE Tracer

Reconstitute the PGEM AChE Tracer as follows:

100 dtn PGEM AChE Tracer (96-well kit; Item No. 414530): Reconstitute with 6 ml ELISA Buffer.

OR

500 dtn PGEM AChE Tracer (480-well kit; Item No. 414530): Reconstitute with 30 ml ELISA Buffer.

Store the reconstituted PGEM 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). Do not store tracer with dye.

PGEM ELISA Antiserum

Reconstitute the PGEM ELISA Antiserum as follows:

100 dtn PGEM ELISA Antiserum (96-well kit; Item No. 414532): Reconstitute with 6 ml ELISA Buffer.

OR

500 dtn PGEM ELISA Antiserum (480-well kit; Item No. 414532): Reconstitute with 30 ml ELISA Buffer.

Store the reconstituted PGEM 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.

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). Do not store antiserum with dye.

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), three 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 23, for more details). We suggest you record the contents of each well on the template sheet provided (see page 33).

	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. **PGEM Buffer**

   Add 50 µl ELISA Buffer and 50 µl of PGEM Buffer to NSB wells. Add 50 µl PGEM Buffer to B_0 wells.

2. **PGEM ELISA 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. **PGEM AChE Tracer**

   Add 50 µl to each well *except* the TA and the Blk wells.

5. **PGEM ELISA Antiserum**

   Add 50 µl to each well *except* the TA, the NSB, and the Blk wells.

Well	PGEM Buffer	ELISA Buffer	Standard/ Sample	Tracer	Antiserum
Blk	-	-	-	-	-
TA	-	-	-	5 µl (at devl. step)	-
NSB	50 µl	50 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 for 18 hours at room temperature.

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 be 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_0 wells ≥0.3 A.U. (blank subtracted)) in 60-90 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 in the range of 0.3-1.5 A.U. (blank subtracted). If the absorbance of the wells exceeds 2.0, wash the plate, add fresh Ellman's Reagent and let it develop again.

ANALYSIS

Many plate readers come with data reduction software that plots 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 analysis. Please contact Technical Service or visit our website (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_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 TA values are not used in the standard curve calculations. Rather, they are used as a diagnostic tool. Low or no absorbance from a TA well could indicate a dysfunction in the enzyme-substrate system.

Plot the Standard Curve

Plot %B/B_0 for standards S1-S8 *versus* PGEM 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.*

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

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 (or %B/B_0) value for each sample. Determine the concentration of each sample using the equation obtained from the standard curve. *NOTE: Remember to account for any concentration or dilution of the sample prior to the addition to the well and for urine samples, for a 1:2 sample dilution during derivatization.* 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.

Performance Characteristics

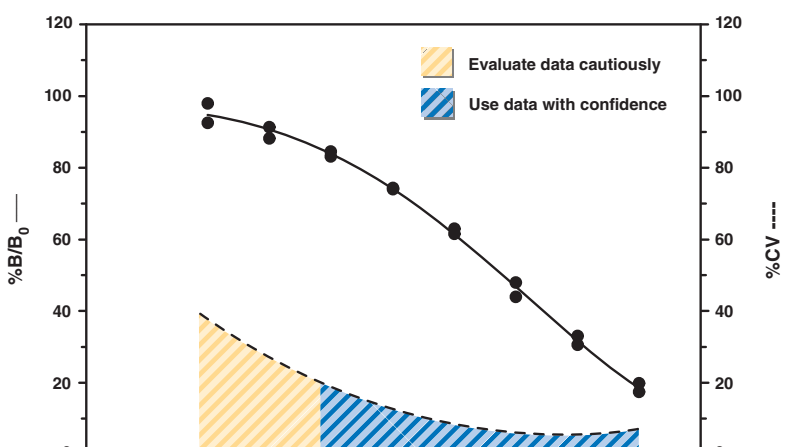
Sample Data

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	1.752	1.965	1.859	
NSB	-0.001	-0.003	-0.002	
B_0	0.674	0.726		
	0.684	0.719	0.701	0.703

Dose (pg/ml)	Raw Data		Corrected		%B/B_0	
50	0.138	0.120	0.140	0.122	19.88	17.44
25	0.230	0.213	0.232	0.215	33.08	30.60
12.5	0.335	0.307	0.337	0.309	47.96	44.01
6.25	0.441	0.431	0.443	0.433	63.00	61.63
3.13	0.518	0.521	0.520	0.523	74.00	74.37
1.56	0.592	0.582	0.594	0.584	84.54	83.16
0.78	0.640	0.618	0.642	0.620	91.35	88.20
0.39	0.649	0.687	0.651	0.689	92.58	97.98

Table 2. Typical results



Assay Range = 0.39-50 pg/ml
Sensitivity (defined as 80% B/B_0) = 2 pg/ml
Mid-point (defined as 50% B/B_0) = 8-14 pg/ml

The sensitivity was derived from the standard curve shown above. The standard was diluted with PGEM Buffer.

Figure 4. 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
50	8.1	18.2
25	5.4	7.2
12.5	5.9	8.3
6.25	5.5	11.2
3.13	12.8	8.0
1.56	25.1	13.4
0.78	23.7	39.3
0.39	N.D.	123

Table 3. Intra- and inter-assay Variation
*%CV represents the variation in concentration (not absorbance) of 40 repetitions of each point on the standard curve as determined using a reference standard curve.

Sample Matrix Properties

Spike and Recovery

Human urine sample was spiked with underivatized 13,14-dihydro-15-keto PGE_2, derivatized as described on page 14, diluted with ELISA Buffer (1X) and validated in the assay. Results are shown below. The error bars represent standard deviations obtained from multiple dilutions of each sample.

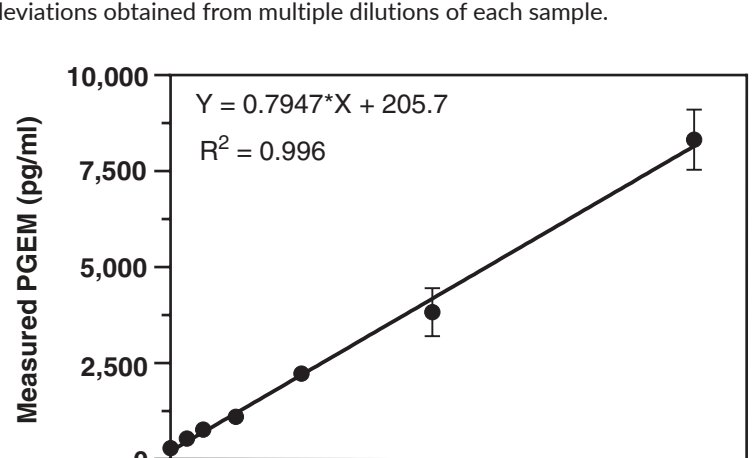


Figure 5. Spike and recovery in urine

Human plasma sample was spiked with underivatized 13,14-dihydro-15-keto PGE_2, purified by acetone precipitation described on page 13, derivatized as described on page 14, extracted with ethyl acetate as described on page 15, diluted with ELISA Buffer (1X) and validated in the assay. Results are shown below. The error bars represent standard deviations obtained from multiple dilutions of each sample.

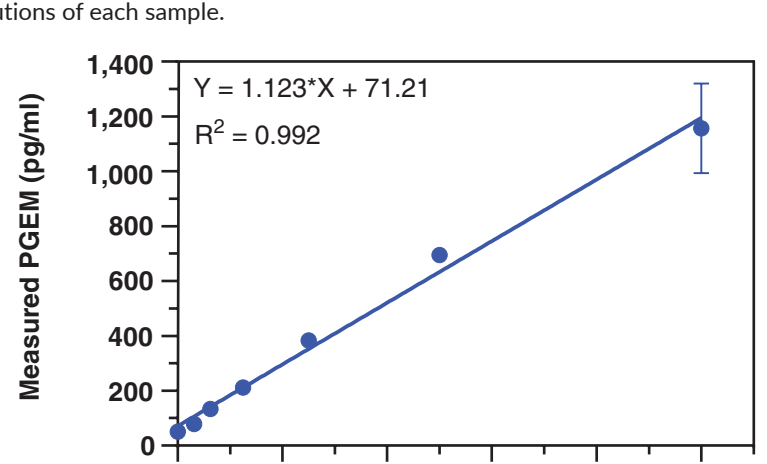


Figure 6. Spike and recovery in plasma

Cross Reactivity:

Compound	Cross Reactivity
13,14-dihydro-15-keto PGE_1*	100%
13,14-dihydro-15-keto PGE_2*	100%
Bicyclo Prostaglandin E_1	38%
13,14-dihydro-15-keto PGD_2*	0.08%
13,14-dihydro-15-keto $PGF_{2\alpha}$*	0.02%
Arachidonic Acid	<0.01%
Leukotriene B_4	<0.01%
tetranor-PGEM	<0.01%
tetranor-PGFM	<0.01%
Prostaglandin D_2	<0.01%
Prostaglandin E_1	<0.01%
6-keto Prostaglandin E_1	<0.01%
Prostaglandin E_2	<0.01%
Prostaglandin $F_{1\alpha}$	<0.01%
6-keto Prostaglandin $F_{1\alpha}$	<0.01%
Prostaglandin $F_{2\alpha}$	<0.01%
Thromboxane B_2	<0.01%

Table 4. Cross Reactivity of the PGEM ELISA
*Derivatized per Assay Protocol

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 (>0.100)	A. Poor washing B. Exposure of NSB wells to specific antibody	A. Re-wash 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 ELISA[9]
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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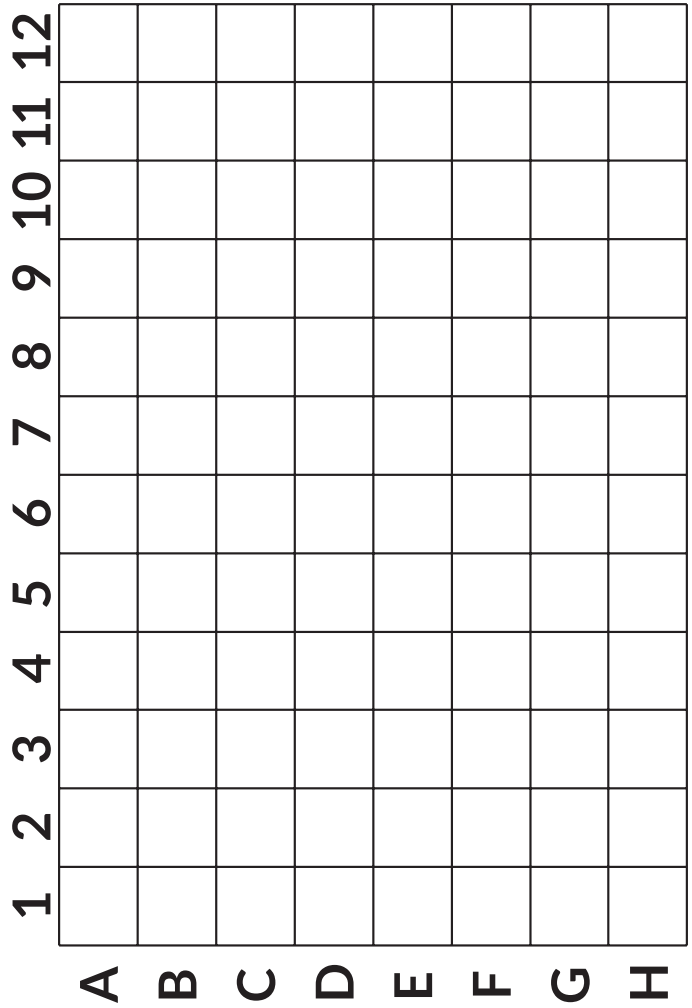

NOTES

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