



Prostaglandin E₂ Express ELISA Kit

Item No. 500141

www.caymanchem.com

Customer Service 800.364.9897

Technical Support 888.526.5351

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

TABLE OF CONTENTS

GENERAL INFORMATION	3	Materials Supplied
	4	Safety Data
	4	Precautions
	5	If You Have Problems
	5	Storage and Stability
	5	Materials Needed but Not Supplied
INTRODUCTION	6	Background
	6	About This Assay
	7	Principle of This Assay
	8	Definition of Key Terms
PRE-ASSAY PREPARATION	10	Buffer Preparation
	11	Sample Preparation
	16	Sample Matrix Properties
ASSAY PROTOCOL	18	Preparation of Assay-Specific Reagents
	21	Plate Set Up
	22	Performing the Assay
ANALYSIS	26	Calculations
	28	Performance Characteristics
RESOURCES	31	Troubleshooting
	32	References
	33	Plate Template
	34	Notes
	35	Warranty and Limitation of Remedy

GENERAL INFORMATION

Materials Supplied

Item Number	Item	96 wells Quantity/Size	480 wells Quantity/Size
400142	Prostaglandin E ₂ Express ELISA Monoclonal Antibody	1 vial/100 dtn	1 vial/500 dtn
400140	Prostaglandin E ₂ Express AChE Tracer	1 vial/100 dtn	1 vial/500 dtn
400144	Prostaglandin E ₂ Express 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
400008/400009	Goat Anti-Mouse IgG-Coated Plate	1 plate	5 plates
400012	96-Well Cover Sheet	1 cover	5 covers
400050	Ellman's Reagent	3 vials/100 dtn	6 vials/250 dtn
400040	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 E₂ Express 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. An orbital microplate shaker
3. Adjustable pipettes; multichannel or repeating pipettor recommended
4. 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).*
5. Materials used for **Sample Preparation** (see page 11)

Background

Prostaglandin E₂ (PGE₂) is an eicosanoid formed from arachidonic acid by COX enzymes and PGE₂ synthase (PGES) *via* PGG₂ and PGH₂ intermediates.¹ It can be produced *de novo* in all cells following cell activation and the release of arachidonic acid from plasma membrane phospholipids or when exogenous free arachidonate is available.² It acts in an autocrine or paracrine fashion to bind to its receptors, EP₁, EP₂, EP₃, and EP₄, which have differing tissue distribution and cellular localizations, to initiate signaling through various pathways, including the protein kinase A (PKA), β-catenin, EGF, MAPK, NF-κB, and PI3K/Akt pathways.¹⁻⁴ Due to this, PGE₂ is involved in a wide variety of biological processes, including inflammation, fertility and parturition, gastric mucosal integrity and motility, and immune modulation in and outside of cancer.^{1,2,4-8}

About This Assay

Cayman's PGE₂ Express ELISA is a competitive assay that can be used for quantification of PGE₂ in urine, plasma, serum, tissue homogenates, and culture media samples. The assay has a range of 15.6-2,000 pg/ml, a sensitivity (80% B/B₀) of approximately 40 pg/ml, and a lower limit of detection (LLOD) of 11 pg/ml.

Principle of This Assay

This assay is based on the competition between PGE₂ and a PGE₂-acetylcholinesterase (AChE) conjugate (PGE₂ Express AChE Tracer) for a limited amount of PGE₂ Express ELISA Monoclonal Antibody. Because the concentration of the PGE₂ Express AChE Tracer is held constant while the concentration of PGE₂ varies, the amount of PGE₂ Express AChE Tracer that is able to bind to the PGE₂ Express ELISA Monoclonal Antibody will be inversely proportional to the concentration of PGE₂ in the well. This antibody-PGE₂ complex binds to 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 PGE₂ Express AChE Tracer bound to the well, which is inversely proportional to the amount of free PGE₂ present in the well during the incubation, as described in the equation:

$$\text{Absorbance} \propto [\text{Bound PGE}_2 \text{ Express AChE Tracer}] \propto 1/[\text{PGE}_2]$$

A schematic of this process is shown in Figure 1, below.

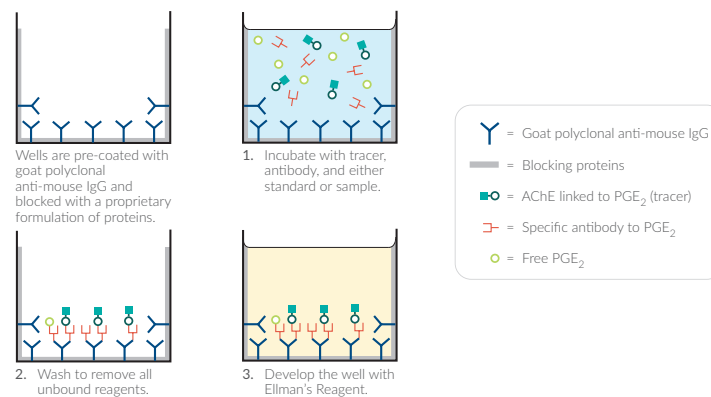


Figure 1. Schematic of the ELISA

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 the non-specific binding (NSB) wells.

TA (Total Activity): total enzymatic activity of the AChE-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.

B₀ (Maximum Binding): maximum amount of the tracer that the antibody can bind in the absence of free analyte.

%B/B₀ (%Bound/Maximum Bound): ratio of the absorbance of a sample or standard well to the average absorbance of the maximum binding (B₀) well.

Standard Curve: a plot of the %B/B₀ 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.

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 mid-point (50% B/B₀) value of the tested molecule to the mid-point (50% B/B₀) value of the primary analyte when each is measured in assay buffer using the following formula:

$$\% \text{ Cross Reactivity} = \left[\frac{50\% \text{ B/B}_0 \text{ value for the primary analyte}}{50\% \text{ B/B}_0 \text{ value for the potential cross reactant}} \right] \times 100\%$$

LLOD (Lower Limit of Detection): the smallest measure that can be detected with reasonable certainty for a given analytical procedure. The LLOD is defined as a concentration two standard deviations higher than the mean zero value.

Buffer Preparation

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

NOTE: It is normal for the concentrated buffer to contain crystalline salts after thawing. These will completely dissolve upon dilution with ultrapure water.

1. ELISA Buffer (1X) 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.

2. Wash Buffer (1X) Preparation

5 ml vial Wash Buffer Concentrate (400X) (96-well kit; Item No. 400062): Dilute to a total volume of 2 L 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 L with ultrapure water and add 2.5 ml of Polysorbate 20 (Item No. 400035).

Smaller volumes of Wash Buffer (1X) can be prepared by diluting the Wash Buffer concentrate (400X) 1:400 and adding 0.5 ml of Polysorbate 20 per 1 L of Wash Buffer (1X).

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

This assay has been demonstrated to work with a wide range of samples including urine, plasma, serum, tissue homogenates, and culture media samples. Proper sample storage and preparation are essential for consistent and accurate results. Please read this section thoroughly 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 mouse and rat origin may contain antibodies that interfere with the assay by binding to the goat anti-mouse plate. We recommend that all mouse and rat samples be purified prior to use in this assay.

Testing for Interference

Samples often contain contaminants, which can interfere in immunoassays. 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 within the linear portion of the standard curve (*i.e.*, between 20-80% B/B₀). If the two different dilutions of the sample show good correlation (differ by 20% or less) in the final calculated PGE₂ concentration, purification is not required. If you do not see good correlation of the different dilutions, purification is advised.

Urine

Since interference in urine is infrequent, dilutions of 1:2 and greater show a direct linear correlation between PGE₂ immunoreactivity and PGE₂ concentration. However, the amount of PGE₂ in normal urine is very low in comparison with other potentially immunoreactive metabolites.² A more accurate index of PGE₂ biosynthesis and excretion can be obtained using our Prostaglandin E Metabolite ELISA Kit (Item No. 514531).

Plasma

Collect blood in vacutainers containing heparin or EDTA for plasma samples. It is recommended that the vacutainers also be supplemented with indomethacin to give a final concentration of at least 10 μ M. To obtain plasma, centrifuge blood at 1,000 x g for 15 minutes at 4°C. Pipette off the top plasma layer without disturbing the white buffy layer. Samples should be assayed immediately after collection; samples that cannot be assayed immediately should be stored at -80°C. Improper handling and storage of plasma samples may lead to inconsistent results. Purification of plasma samples prior to the assay is recommended (see **SPE (C18) Purification Protocol** on page 14).

Serum

Collect blood in vacutainers without a coagulant for serum samples. It is recommended that the vacutainers be supplemented with indomethacin to give a final concentration of at least 10 μ M. Allow samples to clot undisturbed for 30-60 minutes at room temperature. To obtain serum, centrifuge at 1,000-2,000 x g for 15-30 minutes at 4°C. Pipette off the top serum layer. Samples should be assayed immediately after collection; samples that cannot be assayed immediately should be stored at -80°C. Improper handling and storage of serum samples may lead to inconsistent results. Purification of serum samples prior to the assay is recommended (see **SPE (C18) Purification Protocol** on page 14).

Cell Culture Supernatants

In general, cell culture supernatants can be assayed without purification. Dilute samples with ELISA Buffer (1X) if necessary. If samples are tested undiluted or at low dilutions (samples with low concentrations of the analyte), prepare the standard curve in cell culture medium or assay buffer containing the same concentration of the cell culture medium as in the diluted sample. This will ensure that the matrix for the standards is comparable to the samples. We recommend that a test standard curve be run first to ensure that the assay will perform in a particular culture medium.

Tissue

Snap-freeze tissues in liquid nitrogen immediately upon collection and then store at -80°C. When assaying tissue, PGE₂ concentrations are usually normalized using either the wet weight of the tissue or the protein concentration of the lysate. We recommend that you weigh each sample prior to homogenization. If you wish to determine protein concentration of the tissue lysate, we recommend the use of Cayman's Protein Determination Kit (Item No. 704002).

1. Add 1 ml of cold homogenization buffer (10 mM phosphate, pH 7.4, and 10 μ M indomethacin) per 250 mg of tissue.
2. Homogenize samples either manually or using a Precellys® 24 Homogenizer. Recommendations for use of a Precellys® 24 Homogenizer are shown in the table below.

Organ	Speed (rpm)	Cycle Length (seconds)	Beads
Lung	5,200	20	CK28 Large Ceramic (Item No. 10011151)
Brain	5,500	20	CK28 Large Ceramic (Item No. 10011151)
Liver	5,200	15	CK28 Large Ceramic (Item No. 10011151)
Kidney	5,200	20	CK14 Small Ceramic (Item No. 10011152)
Heart	5,200	30	CK14 Small Ceramic (Item No. 10011152)

Table 1. Precellys® settings

3. Centrifuge homogenized samples at 8,000 x g for 10 minutes to pellet particulate matter.
4. Transfer supernatants to clean tubes.
5. If you wish to normalize your samples to protein concentration, reserve an aliquot of each supernatant for use in a protein assay.
6. Test tissue lysates for interference as described on page 11. If purification is necessary, we recommend using the procedure described below.

SPE (C18) Purification

The following protocol is a suggestion only. You may choose a different protocol based on your own requirements, sample type, and expertise.

Materials Needed

1. 1 M acetate buffer or 2N HCl, deionized water, ethanol, methanol, and ethyl acetate
2. 500 mg SPE cartridges (C18) (6 ml) (Item No. 400020)

SPE (C18) Purification Protocol

1. Aliquot a known amount of each sample into a clean test tube (500 µl 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 (C18). Body fluids such as urine, plasma, and serum can typically be applied directly to the SPE cartridge (C18) after the acidification step (step 3) 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 at 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. Acidify the sample to ~pH 4.0 by the addition of 1 M acetate buffer or by adding approximately 50 µl of 2N HCl to every 1 ml of sample if ethanol precipitation was not used. Standardize the pH adjustment using the sample matrix prior to proceeding with a large number of 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 (C18).
4. Prepare SPE (C18) columns by rinsing with 5 ml methanol followed by 5 ml deionized water. Do not allow the SPE cartridge (C18) to dry.
6. Apply the sample to the SPE cartridge (C18) and allow the sample to completely enter the packing material.
7. Wash the column with 5 ml deionized water. Discard the wash.
8. Elute the PGE₂ from the column with 5 ml methanol or 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.
9. Evaporate the ethyl acetate/methanol to dryness under a stream of nitrogen. It is very important that all of the organic solvent be removed as even small quantities will adversely affect the ELISA.
10. To resuspend the sample, add 500 µl ELISA Buffer (1X) and vortex. It is common for insoluble precipitate to remain in the sample after addition of ELISA Buffer (1X); this will not affect the assay. This sample is now ready for use in the ELISA.

Sample Matrix Properties

Spike and Recovery

Cell culture supernatant from RAW 264.7 cells grown in DMEM containing 10% FBS was spiked with different amounts of PGE₂, serially diluted with ELISA Buffer (1X), and analyzed using the PGE₂ Express ELISA Kit. The standard curve for this assay was prepared in ELISA Buffer (1X). The error bars represent standard deviations obtained from multiple dilutions of each sample.

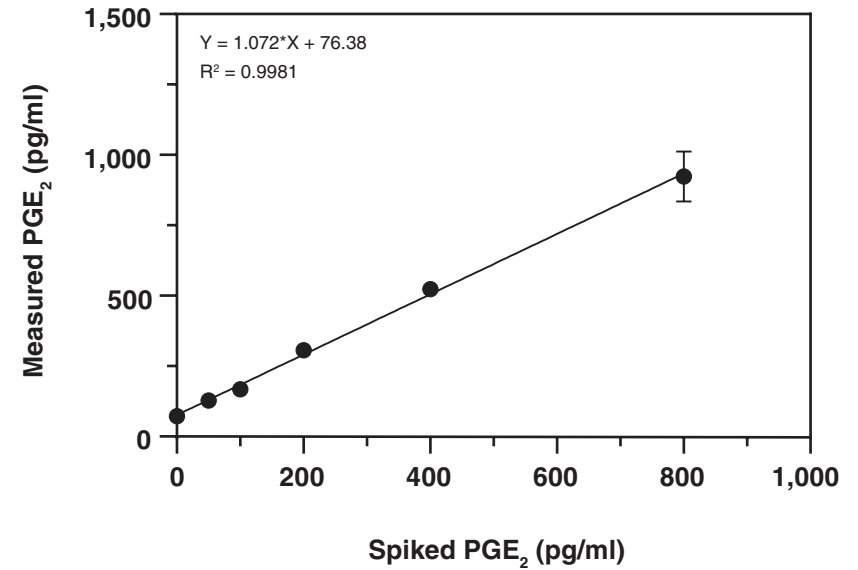


Figure 2. Spike and recovery of PGE₂ in RAW 264.7 cell culture supernatant

Preparation of Assay-Specific Reagents

PGE₂ Express ELISA Standard

Reconstitute the contents of the PGE₂ Express ELISA Standard (Item No. 400144) with 1.0 ml of ELISA Buffer (1X). The concentration of this solution (the bulk standard) will be 10 ng/ml. Store at 4°C; this standard will be stable for up to four weeks.

NOTE: If assaying culture medium samples undiluted or at low dilutions culture medium should be used in place of ELISA Buffer (1X) for dilution of the standard curve and samples.

To prepare the standard for use in ELISA: Obtain eight clean test tubes and number them #1 through #8. Aliquot 800 µl ELISA Buffer (1X) to tube #1 and 500 µl ELISA Buffer (1X) to tubes #2-8. Transfer 200 µl of the bulk standard (10 ng/ml) to tube #1 and mix thoroughly. The concentration of this standard, the first point on the standard curve, will be 2 ng/ml (2,000 pg/ml). Serially dilute the standard by removing 500 µl from tube #1 and placing 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.

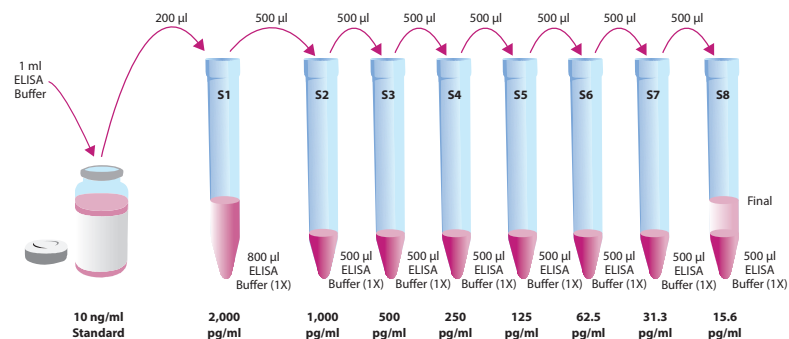


Figure 3. Preparation of the PGE₂ standards

Prostaglandin E₂ Express AChE Tracer

Reconstitute the PGE₂ Express AChE Tracer as follows:

100 dtn PGE₂ Express AChE Tracer (96-well kit; Item No. 400140):
Reconstitute with 6 ml ELISA Buffer (1X).

OR

500 dtn PGE₂ Express AChE Tracer (480-well kit; Item No. 400140):
Reconstitute with 30 ml ELISA Buffer (1X).

Store the reconstituted PGE₂ Express 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).

Prostaglandin E₂ Express ELISA Monoclonal Antibody

Reconstitute the PGE₂ Express Monoclonal Antibody as follows:

100 dtn PGE₂ Express ELISA Monoclonal Antibody (96-well kit; Item No. 400142): Reconstitute with 6 ml ELISA Buffer (1X).

OR

500 dtn PGE₂ Express ELISA Monoclonal Antibody (480-well kit; Item No. 400142): Reconstitute with 30 ml ELISA Buffer (1X).

Store the reconstituted PGE₂ Express ELISA 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).

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 Blk, two NSB, and two B₀ wells, 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 26, 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
B₀ - 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 μl ELISA Buffer (1X) to NSB wells. Add 50 μl ELISA Buffer (1X) to B₀ wells. If culture medium was used to dilute the standard curve, substitute 50 μl of culture medium for ELISA Buffer (1X) in the NSB and B₀ wells (*i.e.*, add 50 μl culture medium to NSB and B₀ wells and 50 μl ELISA Buffer (1X) to NSB wells).

2. Prostaglandin E₂ Express 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. Prostaglandin E₂ Express AChE Tracer

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

5. Prostaglandin E₂ Express ELISA Monoclonal Antibody

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

Well	ELISA Buffer (1X)	Standard/ Sample	Tracer	Antibody
Blk	-	-	-	-
TA	-	-	5 μl (at devl. step)	-
NSB	100 μl	-	50 μl	-
B ₀	50 μl	-	50 μl	50 μl
Std/Sample	-	50 μl	50 μl	50 μl

Table 2. Pipetting summary

Incubation of the Plate

Cover each plate with a 96-Well Cover Sheet (Item No. 400012) and incubate 60 minutes at room temperature on an orbital shaker.

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 (1X).
3. Add 200 μ l of Ellman's Reagent to each well.
4. Add 5 μ l of the reconstituted tracer to the TA wells.
5. Cover the plate with a 96-Well Cover Sheet. 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 (1X) 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 0.3-2.0 A.U. (blank subtracted). If the absorbance of the wells exceeds 2, 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/B₀ versus log concentration using a four-parameter logistic fit or as logit B/B₀ 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 B₀ wells.
3. Subtract the NSB average from the B₀ average. This is the corrected B₀ or corrected maximum binding.
4. Calculate the B/B₀ (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 B₀ (from Step 3). Repeat for S2-S8 and all sample wells. (To obtain %B/B₀ 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/B₀ for standards S1-S8 versus PGE₂ concentration using linear (y) and log (x) axes and perform a four-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/B₀ in this calculation.*

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

Plot the data as logit (B/B₀) versus log concentrations and perform a linear regression fit.

Determine the Sample Concentration

Calculate the B/B₀ (or %B/B₀) 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/B₀ 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

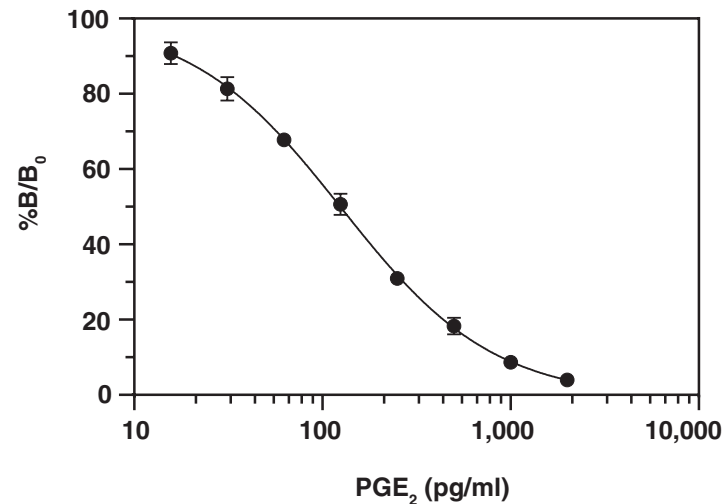
The standard curve presented here is an example of the data typically produced with this kit; however, your results will not be identical to these. You **must** run a new standard curve. Do not use the data below to determine the values of your samples. Your results could differ substantially.

PGE ₂ Standards (pg/ml) and Controls	Absorbance	NSB Corrected Absorbance	%B/B ₀	%CV* Intra-Assay Precision	%CV* Inter-Assay Precision
NSB	0.004	--	--	--	--
B ₀	1.284	--	--	--	--
2,000	0.052	0.048	4.0	10.3	3.6
1,000	0.111	0.107	8.6	6.5	2.0
500	0.235	0.231	18.3	3.8	3.1
250	0.397	0.393	30.9	3.9	2.2
125	0.651	0.647	50.7	4.4	2.8
62.5	0.871	0.867	67.8	9.5	3.9
31.25	1.045	1.041	81.3	18.2	5.9
15.63	1.166	1.162	90.8	43.0**	11.5

Table 3. Typical results

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

** Evaluate data in this range with caution



Assay Range = 15.625-2,000 pg/ml
Sensitivity (defined as 80% B/B₀) = 34.7 pg/ml
Mid-point (defined as 50% B/B₀) = 124.5 pg/ml
Lower Limit of Detection (LLOD) = 10.7 pg/ml
 The sensitivity and mid-point were derived from the standard curve shown above. The standard was diluted in ELISA Buffer (1X).

Figure 5. Typical standard curve

Cross Reactivity:

Compound	Cross Reactivity	Compound	Cross Reactivity
Prostaglandin E ₂	100%	tetranor-PGEM	<0.01%
Prostaglandin E ₂ Ethanolamide	100%	tetranor-PGFM	<0.01%
Prostaglandin E ₂ -1-glyceryl ester	100%	Prostaglandin A ₁	<0.01%
Prostaglandin E ₃	43%	8-iso Prostaglandin A ₂	<0.01%
Prostaglandin E ₁	18.7%	Prostaglandin A ₃	<0.01%
8-iso Prostaglandin E ₂	2.5%	Prostaglandin B ₁	<0.01%
Sulprostone	1.25%	Prostaglandin B ₂	<0.01%
6-keto Prostaglandin F _{1α}	1%	Prostaglandin D ₂	<0.01%
8-iso Prostaglandin F _{2α}	0.25%	11-deoxy Prostaglandin E ₂	<0.01%
Prostaglandin A ₂	0.04%	16,16-dimethyl Prostaglandin E ₂	<0.01%
13,14-dihydro-15-keto Prostaglandin E ₂	0.02%	19(R)-hydroxy Prostaglandin E ₂	<0.01%
Arachidonic Acid	<0.01%	20-hydroxy Prostaglandin E ₂	<0.01%
Arachidonoyl Ethanolamide	<0.01%	15-keto Prostaglandin E ₂	<0.01%
O-Arachidonoyl Ethanolamide	<0.01%	Prostaglandin F _{1α}	<0.01%
Butaprost	<0.01%	Prostaglandin F _{2α}	<0.01%
Conjugated Linoleic Acid (10E,12Z)	<0.01%	13,14-dihydro-15-keto Prostaglandin F _{2α}	<0.01%
8(S),15(S)-DiHETE	<0.01%	Prostaglandin F _{3α}	<0.01%
Leukotriene B ₄	<0.01%	Prostaglandin J ₂	<0.01%
Misoprostol	<0.01%	15-deoxy-Δ ^{12,14} -Prostaglandin J ₂	<0.01%
Misoprostol (free acid)	<0.01%	Thromboxane B ₂	<0.01%

Table 4. Cross reactivity of the PGE₂ Express ELISA

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 ₀)	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>i.e.</i> , more than 20% difference)	Interfering substances are present	Purify sample prior to analysis by ELISA?
Only TA wells develop	Trace organic contaminants in the water source	Replace activated carbon filter or change source of ultrapure water

References

1. Wang, B., Wu, L., Chen, J., *et al.* Metabolism pathways of arachidonic acids: Mechanisms and potential therapeutic targets. *Signal Transduct. Target. Ther.* **6(1)**, 94 (2021).
2. Legler, D.F., Bruckner, M., Allmen, E.U., *et al.* Prostaglandin E₂ at new glance: Novel insights in functional diversity offer therapeutic chances. *Int. J. Biochem. Cell Biol.* **42(2)**, 198-201 (2010).
3. Sugimoto, Y. and Narumiya, S. Prostaglandin E receptors. *J. Biol. Chem.* **282(16)**, 11613-11617 (2007).
4. Jin, K., Qian, C., Lin, J., *et al.* Cyclooxygenase-2-prostaglandin E₂ pathway: A key player in tumor-associated immune cells. *Front. Oncol.* **13**, 1099811 (2023).
5. Willis, A.L. and Cornelsen, M. Repeated injection of prostaglandin E₂ in rat paws induces chronic swelling and a marked decrease in pain threshold. *Prostaglandins* **3(3)**, 353-357 (1973).
6. Jackson, G.M., Sharp, H.T., and Varner, M.W. Cervical ripening before induction of labor: A randomized trial of prostaglandin E₂ gel versus low-dose oxytocin. *Am. J. Obstet. Gynecol.* **171(4)**, 1092-1096 (1994).
7. Robert, A., Schultz, J.R., Nezamis, J.E., *et al.* Gastric antisecretory and antiulcer properties of PGE₂, 15-methyl PGE₂, and 16,16-dimethyl PGE₂. Intravenous, oral and intrajejunal administration. *Gastroenterology* **70(3)**, 359-370 (1976).
8. Arvind, P., Papavassiliou, E.D., Tsioulis, G.J., *et al.* Prostaglandin E₂ down-regulates the expression of HLA-DR antigen in human colon adenocarcinoma cell lines. *Biochemistry* **34(16)**, 5604-5609 (1995).
9. Maxey, K.M., Maddipati, K.R. and Birkmeier, J. Interference in enzyme immunoassays. *J. Clin. Immunoassay* **15**, 116-120 (1992).

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

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.

©01/22/2024, Cayman Chemical Company, Ann Arbor, MI, All rights reserved. Printed in U.S.A.

