tetranor-PGDM ELISA Kit

Item No. 501001

www.caymanchem.com

Customer Service 800.364.9897

Technical Support 888.526.5351

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

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	96 wells Quantity/Size	480 wells Quantity/Size
401002	tetranor-PGDM ELISA Antiserum	1 vial/100 dtn	1 vial/500 dtn
401000	tetranor-PGDM AChE Tracer	1 vial/100 dtn	1 vial/500 dtn
401004	tetranor-PGDM 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
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 tetranor-PGDM 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).

INTRODUCTION

Background

Prostaglandin D synthases (PGDS) catalyze the isomerization of PGH_2 to produce PGD_2. PGD_2 induces sleep, regulates nociception, inhibits platelet aggregation, and acts as an allergic mediator. Two distinct isoforms of PGD synthase have been identified, the lipocalin-type enzyme and the hematopoietic enzymes.[1-3] Lipocalin-type PGDS (L-PGDS) is localized in the central nervous system and male genital organs of various mammals, as well as in the human heart. This enzyme was originally identified as β-trace, a major protein in human cerebrospinal fluid.[1] Hematopoietic PGDS (H-PGDS) is widely distributed in peripheral tissues and is localized in antigen-presenting cells, mast cells, and megakaryocytes. PGD_2 is produced by H-PGDS in large quantities by allergen-stimulated mast cells and acts as a pro-inflammatory mediator in allergic reactions. The biological effects of PGD_2 are transduced by at least two 7-transmembrane G protein-coupled receptors, designated DP_1 and $CRTH_2/DP_2$.[4,5]

tetranor-PGDM is a major metabolite of PGD_2 found in human and mouse urine.[6] In human urine, tetranor-PGDM is significantly more abundant than the PGD_2 metabolites 11β-$PGF_{2\alpha}$ and 2,3-dinor-11β-$PGF_{2\alpha}$ and is the only endogenous PGD_2 metabolite detectable in mouse urine by LC-MS. Normal levels of tetranor-PGDM in human and mouse urine are 1.5 ng/mg creatinine and 8.1 ng/mg creatinine respectively.[6]

About This Assay

Cayman's tetranor-PGDM ELISA Kit is a competitive assay that can be used for quantification of tetranor-PGDM in urine. This assay converts tetranor-PGDM to a stable derivative, tetranor-PGJM, that can be easily quantified. The assay has a range from 6.4-4,000 pg/ml and a sensitivity (80% B/B_0) of approximately 40 pg/ml.

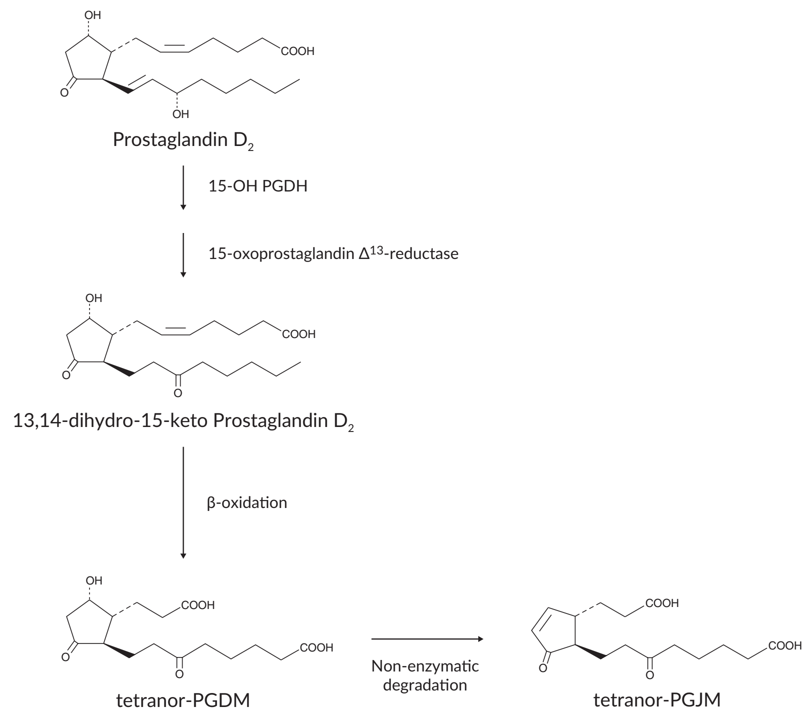


Figure 1. Metabolism of PGD_2

Description of AChE Competitive ELISAs

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

Absorbance $\propto$ [Bound tetranor-PGDM Tracer] $\propto$ 1/[tetranor-PGDM]

A schematic of this process is shown in Figure 2, on page 9.

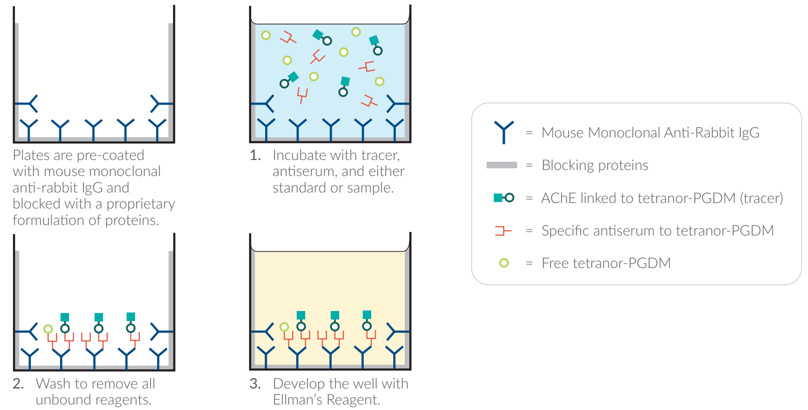


Figure 2. Schematic of the AChE ELISA

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 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 (ε = 13,600).

AChE has several advantages over other enzymes commonly used for enzyme immunoassays. Unlike horseradish peroxidase, AChE does not self-inactivate during turnover. This property of AChE also allows 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.

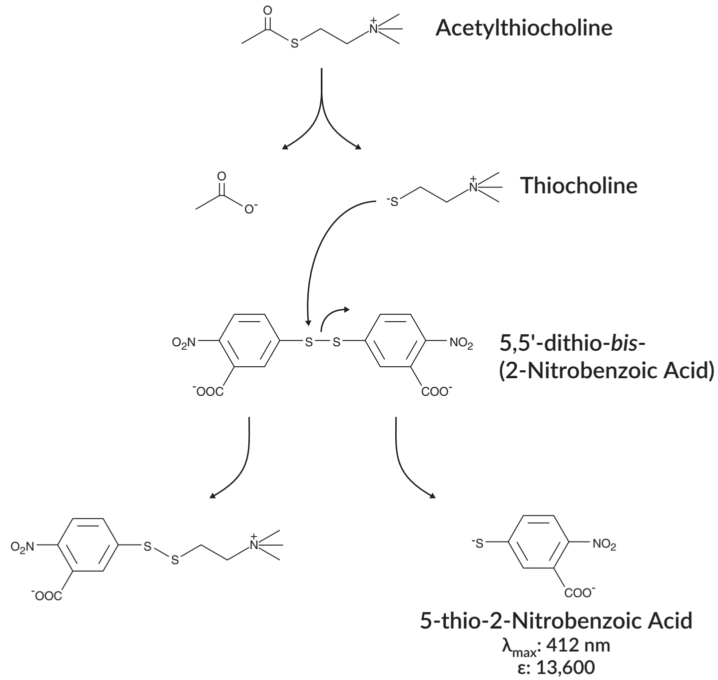


Figure 3. Reaction catalyzed by acetylcholinesterase

Definition of Key Terms

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

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

NSB (Non-Specific Binding): non-immunological binding of the tracer to the well. Even in the absence of specific antibody a very small amount of tracer still binds to the well; the NSB is a measure of this low binding. Do not forget to subtract the Blank absorbance values.

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

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

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

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

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/B_0) value of the tested molecule to the mid-point (50% B/B_0) 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

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

This assay has been validated for urine samples (diluted at least 1:2). 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 rabbit origin may contain antibodies which interfere with the assay by binding to the mouse anti-rabbit IgG plate. We recommend that all rabbit samples be purified prior to use in this assay.

Urine

Samples that cannot be assayed immediately should be stored at -80°C until ready to assay. Urinary concentrations of tetranor-PGDM vary considerably. It is recommended that the values obtained from urine samples be standardized to creatinine levels using Cayman's Creatinine ELISA Kit (Item No. 502330), Creatinine (urinary) Colorimetric Assay Kit (Item No. 500701), or a similar assay.

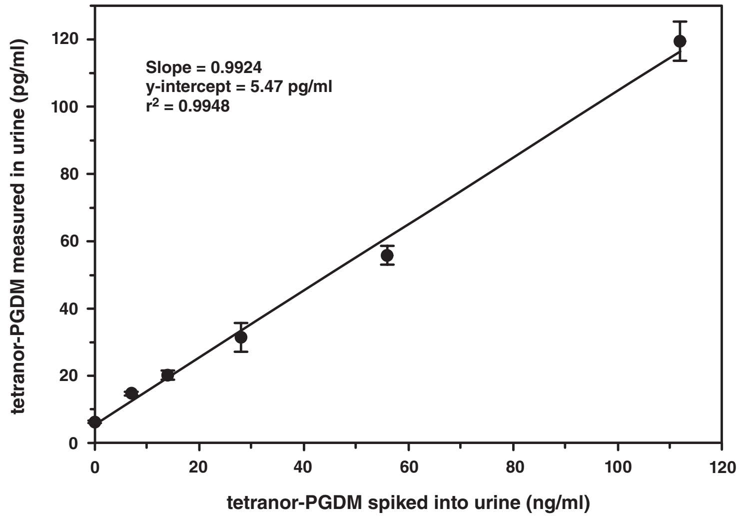


Figure 4. Recovery of tetranor-PGDM from urine
Urine samples were spiked with tetranor-PGDM, diluted, and analyzed using the tetranor-PGDM ELISA Kit. The y-intercept corresponds to the amount of tetranor-PGDM in unspiked urine. Error bars represent standard deviations obtained from multiple dilutions of each sample.

ASSAY PROTOCOL

Derivatization of Standards and Samples to tetranor-PGJM

Prior to use, the standards and samples must be converted from tetranor-PGDM to tetranor-PGJM by a simple derivitization process described below. The derivitization process (dilution and prolonged heating) may result in a loss of 2-5% of the initial sample volume. Thus, to reduce errors due to minor sample volume loss, it is recommended that the standard and the test samples be derivitized concurrently. The tetranor-PGDM AChE Tracer does not need to be derivatized.

Derivatization of the tetranor-PGDM 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 tetranor-PGDM ELISA Standard (Item No. 401004) into a clean microcentrifuge tube containing 900 µl UltraPure water. The concentration of this solution (the bulk standard) will be 100 ng/ml. Cap or seal the tube and incubate at 60°C for 18 hours (overnight). Invert the tube gently to mix, and cool to room temperature prior to use in the assay. The derivatized standard is stable for two weeks at 4°C.

Derivatization of the Samples

Aliquot 1 ml of each urine sample into a clean microcentrifuge tube. Cap or seal the tube and incubate at 60°C for 18 hours (overnight). Centrifuge the tube at 10,000 rpm for three minutes to remove any contaminating precipitates. Cool to room temperature prior to use in the assay.

Preparing the Standard Curve

To prepare the standard for use in ELISA: Obtain eight clean test tubes and number them #1 through #8. Aliquot 960 µl ELISA Buffer to tube #1 and 600 µl ELISA Buffer to tubes #2-8. Transfer 40 µl of the derivatized standard (100 ng/ml) to tube #1 and mix thoroughly. Serially dilute the standard by removing 400 µl from tube #1 and placing in tube #2; mix thoroughly. Next, remove 400 µl from tube #2 and place it into tube #3; mix thoroughly. Repeat this process for tubes #4-8. These diluted standards should not be stored for more than 24 hours.

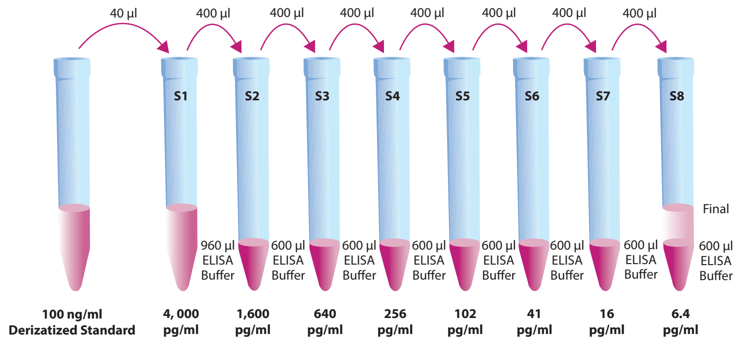


Figure 5. Preparation of the tetranor-PGDM standards

Preparation of Assay-Specific Reagents

tetranor-PGDM AChE Tracer

Reconstitute the tetranor-PGDM AChE Tracer as follows:

100 dtn tetranor-PGDM AChE Tracer (96-well kit; Item No. 401000): Reconstitute with 6 ml ELISA Buffer.

OR

500 dtn tetranor-PGDM AChE Tracer (480-well kit; Item No. 401000): Reconstitute with 30 ml ELISA Buffer.

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

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

tetranor-PGDM ELISA Antiserum

Reconstitute the tetranor-PGDM ELISA Antiserum as follows:

100 dtn tetranor-PGDM ELISA Antiserum (96-well kit; Item No. 401002): Reconstitute with 6 ml ELISA Buffer.

OR

500 dtn tetranor-PGDM ELISA Antiserum (480-well kit; Item No. 401002): Reconstitute with 30 ml ELISA Buffer.

Store the reconstituted tetranor-PGDM 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). *NOTE: Do not store antiserum with dye for more than 24 hours.*

Plate Set Up

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

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

A suggested plate format is shown in Figure 6, below. The user may vary the location and type of wells present as necessary for each particular experiment. The plate format provided below has been designed to allow for easy data analysis using a convenient spreadsheet offered by Cayman (see page 24, for more details). We suggest you record the contents of each well on the template sheet provided (see page 34).

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

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

Figure 6. Sample plate format

Performing the Assay

Pipetting Hints

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

Addition of the Reagents

1. **ELISA Buffer**

   Add 100 µl ELISA Buffer to NSB wells. Add 50 µl ELISA Buffer to B_0 wells.

2. **tetranor-PGDM 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 derivatized sample per well. Each sample should be assayed at a minimum of two dilutions. Each dilution should be assayed in duplicate (triplicate recommended).

4. **tetranor-PGDM AChE Tracer**

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

5. **tetranor-PGDM ELISA Antiserum**

   Add 50 µl 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_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 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_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 are in the range of 0.3-1.0 A.U. (blank subtracted). If the absorbance of the wells exceeds 1.5, wash the plate, add fresh Ellman's Reagent and let it develop again.

ANALYSIS

Many plate readers come with data reduction software that plot data automatically. Alternatively a spreadsheet program can be used. The data should be plotted as either %B/B_0 *versus* log concentration using a four-parameter logistic fit or as logit B/B_0 *versus* log concentration using a linear fit. *NOTE: Cayman has a computer spreadsheet available for data 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; the corrected B_0 divided by the actual TA (10X measured absorbance) will give the %Bound. This value should closely approximate the %Bound that can be calculated from the ***Sample Data*** *(see page 27). 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 32 for* ***Troubleshooting****).*

Plot the Standard Curve

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

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

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

Performance Characteristics

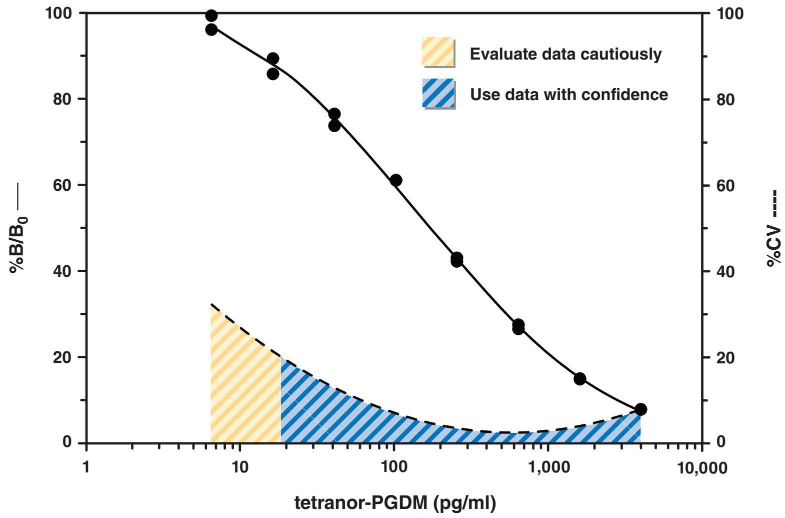
Sample Data

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	0.504	0.497	0.501	0.495
NSB	0.007	0.005	0.006	0.006
B_0	1.242	1.351		
	1.311	1.276	1.295	1.289

Dose (pg/ml)	Raw Data		Corrected		$\%B/B_0$	
4,000	0.108	0.107	0.102	0.101	7.94	7.87
1,600	0.200	0.197	0.194	0.191	15.05	14.83
640	0.361	0.348	0.355	0.342	27.52	26.57
256	0.561	0.551	0.555	0.545	43.07	42.27
102	0.795	0.795	0.789	0.789	61.21	61.19
41	0.992	0.957	0.986	0.951	76.48	73.79
16	1.157	1.113	1.151	1.107	89.32	85.86
6.4	1.245	1.286	1.239	1.280	96.11	99.34

Table 2. Typical results



Assay Range = 6.4-4,000 pg/ml
Sensitivity (defined as 80% B/B_0) = 40 pg/ml
Mid-point (defined as 50% B/B_0) = 125-250 pg/ml

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

Figure 6. Typical standard curve

Precision:

The intra-assay CVs have been determined at multiple points on the standard curve. These data are summarized in the graph on page 28 and in the table below.

Dose (pg/ml)	%CV* Intra-assay variation
4,000	6.0
1,600	4.3
640	5.6
256	4.8
102	6.8
41	9.2
16	19.9
6.4	34.5

Table 3. Intra-assay variation
*%CV represents the variation in concentration (not absorbance) as determined using a reference standard curve.

Level	%CV Intra-assay variation	Average (ng/ml)	%CV Inter-assay variation	Average (ng/ml)
High	7.5	25.2	15.1	28.1
Medium	7.8	11.5	16.2	14.0
Low	8.5	0.792	10.4	0.824

Table 4. Urine sample validation

Urine samples containing a high, medium, or low level of tetranor-PGDM were measured 60 times each using a single set of reagents. The calculated %CV is reported as intra-assay variance. A separate series of urine samples containing a high, medium, or low level of tetranor-PGDM were measured four times each using eight independent sets of reagents. The calculated %CV is reported as inter-assay variance.

Cross Reactivity:

Compound	Cross Reactivity
tetranor-PGDM	100%
tetranor-PGJM	100%
tetranor-PGAM	2.08%
tetranor-PGEM	0.03%
tetranor-PGFM	<0.01%
Prostaglandin A_2	<0.01%
Prostaglandin D_2	<0.01%
13,14-dihydro-15-keto Prostaglandin D_2	<0.01%
Prostaglandin E_2	<0.01%
13,14-dihydro-15-keto Prostaglandin E_2	<0.01%
Prostaglandin $F_{2\alpha}$	<0.01%
13,14-dihydro-15-keto Prostaglandin $F_{2\alpha}$	<0.01%
Prostaglandin J_2	<0.01%

Table 5. Cross Reactivity of the tetranor-PGDM 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.e.*, more than 20% difference)	Interfering substances are present	Purify sample prior to analysis by ELISA[7]
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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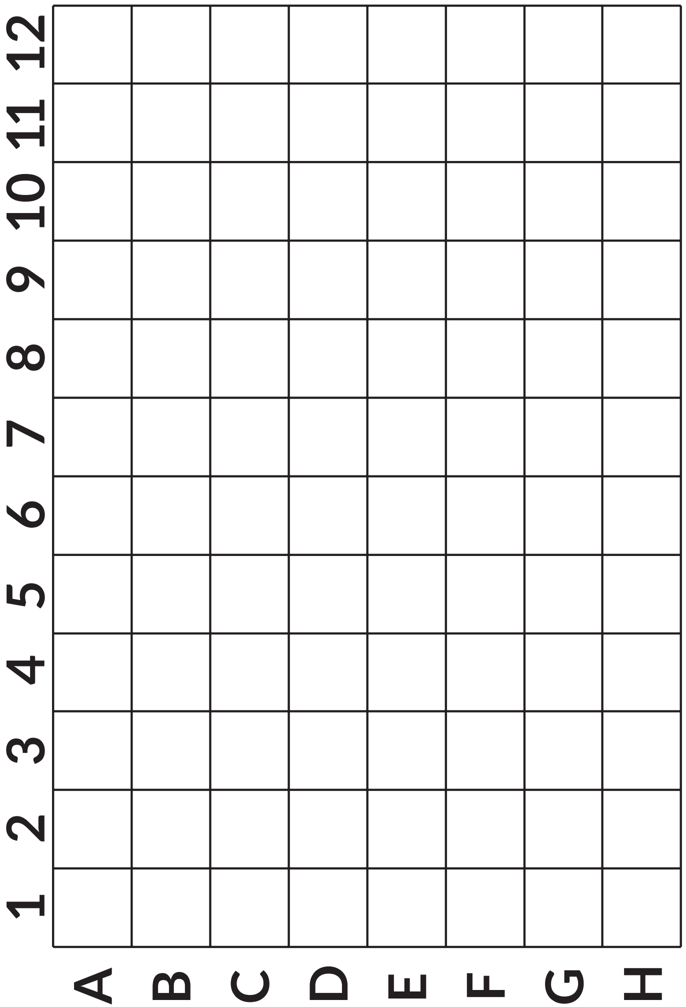

NOTES

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