Version 22-04/24

User's Manual

Prostaglandin D2 ELISA Kit

REF **DEIA5036**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

CD's PGD2 ELISA Kit is a competitive assay that can be used for quantification of PGD2 in cell culture lysates and enzymatic reactions. The assay has a range from 19.5-2,500 pg/ml and a sensitivity (80% B/B0) of approximately 55 pg/ml.

General Description

Prostaglandin D2 (PGD2) is biosynthesized in the brain by a soluble, 26 kDa glutathione-independent lipocalin-type PGD2 synthase.This PGD2 accumulates in the cerebrospinal fluid (CSF), where it induces physiologic sleep in rats and humans. PGD2 is also synthesized in mast cells and leukocytes by a cellular, myeloid-type, glutathione-dependent PGD synthase. This PGD2 which is formed in the intracellular and vascular compartments is rapidly metabolized to 11β-PGF2α.Thus, plasma measurements of PGD2 synthesis are most appropriately focused on the measurement of 11β-PGF2α.

Measurement of the parent eicosanoid PGD2 is appropriate in cell culture lysates and in CSF, where concentrations of several hundred pg/ml have been measured.

All studies of PGD2 biosynthesis should take into consideration the chemical instability of PGD2 and its rapid degradation in the presence of serum proteins such as albumin. PGD2 also readily degrades in both acidic and basic media to give a variety of decomposition products including PGJ2, Δ12-PGJ2, and 15-deoxy-Δ12,14-PGJ2. For these reasons, antibodies against PGD2 have generally been prepared against conjugates of a stable methoximine derivative (i.e., PGD2-MOX). Our PGD2-MOX and PGD2-MOX Express ELISA Kits are based on this chemistry and require the PGD2 in all samples to be converted to the stable methoximine derivative prior to performing the assay. These assays are well suited and recommended for the measurement of PGD2 in complex matrices. The antibody utilized in this PGD2 ELISA was generated in a unique way allowing the direct measure of PGD2 without prior conversion to the methoximine compound.

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. 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.

Principles of Testing

This assay is based on the competition between PGD2 and a PGD2- acetylcholinesterase (AChE) conjugate (PGD2 tracer) for a limited number of PGD2 monoclonal antibody binding sites. Because the concentration of the PGD2 tracer is held constant while the concentration of PGD2 varies, the amount of PGD2 tracer that is able to bind to the monoclonal antibody will be inversely proportional to the concentration of PGD2 in the well. This antibody-PGD2 complex (either free or tracer) binds to the goat polyclonal anti-mouse IgG that has been previously attached to the well. The plate is washed to remove any unbound reagents and then Ellman's Reagent (which contains the substrate to AChE) is added to the well. The product of this enzymatic reaction has a distinct yellow color and absorbs strongly at 412 nm. The intensity of this color, determined spectrophotometrically, is proportional to the amount of PGD2 tracer bound to the well, which is inversely proportional to the amount of free PGD2 present in the well during the incubation; or

Absorbance ∝ [Bound PGD2 Tracer] ∝ 1/[PGD2]

Reagents And Materials Provided

1. Prostaglandin D2 EIA Monoclonal Antibody: 1 vial/100 dtn
2. Prostaglandin D2 AChE Tracer: 1 vial/100 dtn
3. Prostaglandin D2 EIA Standard: 1 vial
4. EIA Buffer Concentrate (10×): 2 vials/10 mL
5. Wash Buffer Concentrate (400×): 1 vial/5 mL
6. Polysorbate 20: 1 vial/3 mL
7. Goat Anti-Mouse IgG Coated Plate: 1 plate
8. 96-Well Cover Sheet: 1 cover
9. Ellman's Reagent: 3 vials/100 dtn
10. EIA Tracer Dye: 1 vial
11. EIA Antiserum Dye: 1 vial

Materials Required 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.
4. Materials used for Sample Preparation.

Storage

Store at 4°C for frequent use, at -20°C for infrequent use. Avoid multiple freeze-thaw cycles. For more detailed information, please download the following document on our website.

Specimen Collection And Preparation

This assay has been validated for use with cell lysates and purified enzyme preparations. Proper sample storage and preparation are essential for consistent and accurate results.

General Precautions:

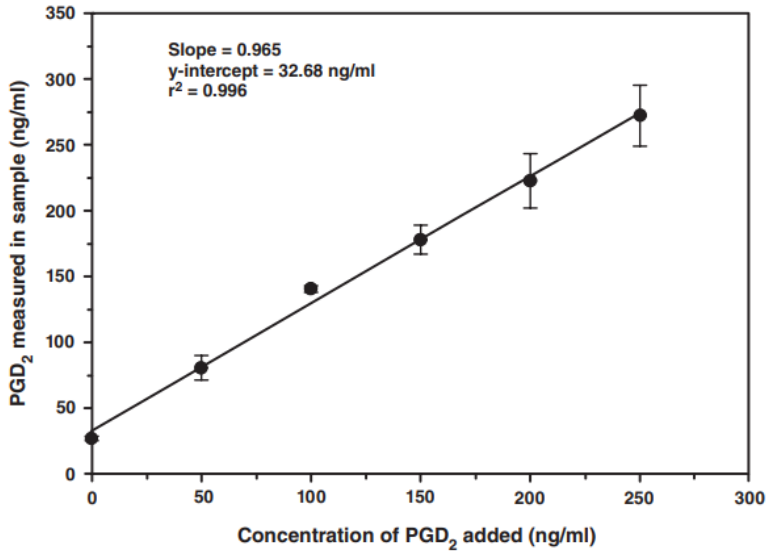
1. All samples must be free of organic solvents prior to assay.
2. Samples should be assayed immediately after collection; samples that cannot be assayed immediately should be stored at -80°C.
3. Samples of mouse and rat origin may contain antibodies which interfere with the assay by binding to the goat anti-mouse plate. We recommend that all murine samples be purified prior to use in this assay.

Enzymatic Reactions

Reactions using purified enzyme can be diluted directly into ELISA Buffer and used in the assay without further purification. Suggestions for performing PGD2 synthase reactions may be obtained from our technical service staff by email (info@creativediagnostics.com).

Cell Lysates

Cells may be lysed by sonication in 0.1 M potassium phosphate, pH 7.4. Pellet cellular debris by centrifugation and transfer supernatants to clean tubes. These samples may then be diluted with ELISA Buffer and used in the assay without further purification.



Measurement of PGD2 in cell lysates. Cells were lysed as described in the Sample Preparation section. The lysates were then spiked with PGD2, diluted with ELISA Buffer, and analyzed using the PGD2 ELISA Kit. The y-intercept corresponds to the amount of PGD2 measured in unspiked lysates. Error bars represent standard deviations obtained from multiple dilutions of each sample.

Plate Preparation

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, 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 CD. We suggest you record the contents of each well on the template sheet provided.

Reagent 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 (10×) 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 (400×) (96-well kit): Dilute to a total volume of 2 liters with UltraPure water and add 1 ml of Polysorbate 20.

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. Prostaglandin D2 ELISA Standard

Transfer 100 µl of the PGD2 ELISA Standard into a clean test tube and dilute with 900 µl of UltraPure water. The concentration of this solution (the bulk standard) will be 25 ng/ml. This standard should not be stored for more than 24 hours.

NOTE: If assaying cell lysates or in vitro reactions that have not been diluted with ELISA Buffer, those matrices should be used in place of ELISA Buffer for dilution of the standard curve.

To prepare the standard for use in ELISA: Obtain eight clean test tubes and number them #1 through #8. Aliquot 900 µl ELISA Buffer to tube #1 and 500 µl ELISA Buffer to tubes #2-8. Transfer 100 µl of the bulk standard (25 ng/ml) to tube #1 and mix thoroughly. The concentration of this standard, the first point on the standard curve, will be 2.5 ng/ml (2,500 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.

4. Prostaglandin D2 AChE Tracer

Reconstitute the PGD2 AChE Tracer as follows:

100 dtn PGD2 AChE Tracer (96-well kit): Reconstitute with 6 ml ELISA Buffer.

Store the reconstituted PGD2 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.

5. 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).

6. Prostaglandin D2 ELISA Monoclonal Antibody

Reconstitute the PGD2 ELISA Monoclonal Antibody as follows:

100 dtn PGD2 ELISA Monoclonal Antibody (96-well kit):

Reconstitute with 6 ml ELISA Buffer.

Store the reconstituted PGD2 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.

7. 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).

Assay Procedure

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.

1. Pipetting Hints

a. Use different tips to pipette each reagent.

b. 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).

c. Do not expose the pipette tip to the reagent(s) already in the well.

2. Addition of the Reagents

a. ELISA Buffer

Add 100 µl ELISA Buffer to NSB wells. Add 50 µl ELISA Buffer to B0 wells. If another matrix was used to dilute the standard curve, substitute 50 µl of that matrix for ELISA Buffer in the NSB and B0 wells (i.e., add 50 µl sample matrix to NSB and B0 wells and 50 µl ELISA Buffer to NSB wells).

b. Prostaglandin D2 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.

c. 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).

d. Prostaglandin D2 AChE Tracer

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

e. Prostaglandin D2 ELISA Monoclonal Antibody

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

Well	ELISA Buffer	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: Pipetting summary Incubation of the Plate

Cover each plate with plastic film and incubate overnight at 4°C.

3. Development of the Plate

a. 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): Reconstitute with 20 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.

b. Empty the wells and rinse five times with Wash Buffer.

c. Add 200 µl of Ellman's Reagent to each well.

d. Add 5 µl of tracer to the TA wells.

e. 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., B0 wells ≥0.3 A.U. (blank subtracted)) in 90-120 minutes.

4. Reading the Plate

a. Wipe the bottom of the plate with a clean tissue to remove fingerprints, dirt, etc.

b. 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.

c. Read the plate at a wavelength between 405 and 420 nm. The absorbance may be checked periodically until the B0 wells have reached a minimum of 0.3 A.U. (blank subtracted). The plate should be read when the absorbance of the B0 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.

Calculation

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; the corrected B0 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. Erratic absorbance values and a low (or no) %Bound could indicate the presence of organic solvents in the buffer or other technical problems.

Plot the Standard Curve

Plot %B/B0 for standards S1-S8 versus PGD2 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/B0 in this calculation.

logit (B/B0) = ln [B/B0/(1 - B/B0)]

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

Determine the Sample Concentration

Calculate the B/B0 (%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.

Definition of Key Terms

1. 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.

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

3. 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.

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

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

6. Standard Curve: a plot of the %B/B0 values versus concentration of a series of wells containing various known amounts of analyte.

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

8. 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:

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

Typical Standard Curve

1. 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	0.404	0.413	0.409	0.407
NSB	0.003	0.001	0.002	
B_0	0.915	0.934		
	0.918	0.947	0.929	

2. Typical results

Precision

The intra- and inter-assay CVs have been determined at multiple points on the standard curve.

Intra- and inter-assay variation

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

+ Outside of the recommended usable range of the assay.

Detection Range

19.5-2,500 pg/ml

Sensitivity

Sensitivity (defined as 80% B/B0) = 55 pg/ml Mid-point (defined as 50% B/B0) = 170-310 pg/ml

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

Specificity

Precautions

Please read these instructions carefully before beginning this assay.

The reagents in this kit have been tested and formulated to work exclusively with CD's AChE ELISA Kits. 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.