Prostaglandin D Synthase Inhibitor Screening Assay Kit

Item No. 10006595

Customer Service 800.364.9897 * **Technical Support** 888.526.5351
www.caymanchem.com

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Materials Supplied

Item No.	Item	96 wells Quantity/Size
412042	Prostaglandin D_2 Express EIA Monoclonal Antibody	1 vial/100 dtn
412040	Prostaglandin D_2 Express AChE Tracer	2 vials/100 dtn
412044	Prostaglandin D_2 Express EIA Standard	1 vial
400060	EIA Buffer Concentrate (10X)	2 vials/10 ml
400062	Wash Buffer Concentrate (400X)	1 vial/5 ml
400035	Polysorbate 20	1 vial/3 ml
400009	Goat Anti-Mouse IgG Coated Plate	1 plate
400012	96-Well Cover Sheet	1 cover
400050	Ellman's Reagent	3 vials/100 dtn
10007572	Reaction Buffer (10X)	1 vial
10007556	Prostaglandin D Synthase (hematopoietic-type)	1 vial
10007557	Prostaglandin D Synthase (lipocalin-type)	1 vial
10007558	Glutathione	1 vial
10007559	Dithiothreitol	1 vial
10007560	Prostaglandin H_2	1 vial
10007561	Hydrochloric Acid	1 vial
10007562	Iron (II) Chloride	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 laboratory research use only: not for administration to humans. Not for human or veterinary diagnostic or therapeutic use.

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 ACE™ EIA Kits. This kit may not perform as described if any reagent or procedure is replaced or modified.

For research use only. Not for human or diagnostic use.

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

Fax: 734-971-3641

E-Mail: techserv@caymanchem.com

Hours: M-F 8:00 AM to 5:30 PM EST

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 -80°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 EIA 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 EIA. *NOTE: UltraPure water is available for purchase from Cayman (Item No. 400000).*
4. Disposal glass test tubes (13 mm x 100 mm).

INTRODUCTION

Background

Prostaglandin D synthase (PGDS) catalyzes the isomerization of prostaglandin H_2 (PGH_2) to produce PGD_2. PGD_2 induces sleep, regulates nociception, inhibits platelet aggregation, and acts as an allergic mediator. Two distinct types of PGDS have been identified, the lipocalin and the hematopoietic-type enzymes.[1-4] The lipocalin-type PGDS (L-PGDS) is localized in the central nervous system, male genital organs of various mammals, and the human heart. This enzyme has been identified as β-trace, which is a major protein in human cerebrospinal fluid.[1] Hematopoietic-type PGDS (H-PGDS) is widely distributed in the peripheral tissues and is localized in the 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. H-PGDS requires glutathione for activity and belongs to the sigma-class of glutathione S-transferases.[2,5]

About This Assay

Cayman's PGDS Inhibitor Screening Assay Kit measures PGD_2 generated by PGDS. The prostanoid product is quantified *via* enzyme immunoassay (EIA) using a PGD_2 monoclonal antibody. The assay includes both human recombinant PGDS enzymes (lipocalin and hematopoietic) in order to screen isozyme-specific inhibitors.

Description of ACE™ Competitive EIAs[6,7]

This assay is based on the competition between PGD_2 and a PGD_2-acetylcholinesterase (AChE) conjugate (PGD_2 Tracer) for a limited number of PGD_2 monoclonal antibody binding sites. Because the concentration of the PGD_2 Tracer is held constant while the concentration of PGD_2 varies, the amount of PGD_2 Tracer that is able to bind to the PGD_2 Monoclonal Antibody will be inversely proportional to the concentration of PGD_2 in the well. This antibody-PGD_2 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 PGD_2 Tracer bound to the well, which is inversely proportional to the amount of free PGD_2 present in the well during the incubation; or

$$\text{Absorbance} \propto [\text{Bound } PGD_2 \text{ Tracer}] \propto 1/[PGD_2]$$

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

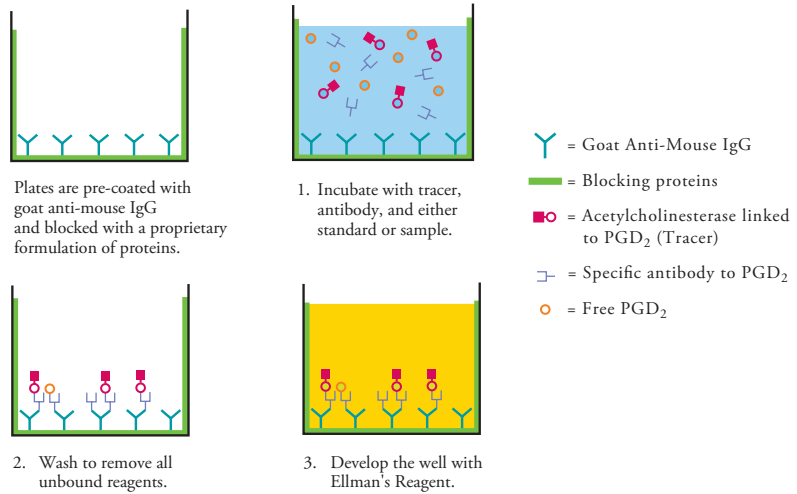


Figure 1. Schematic of the ACE™ EIA

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 ACE™ 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 9). 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.

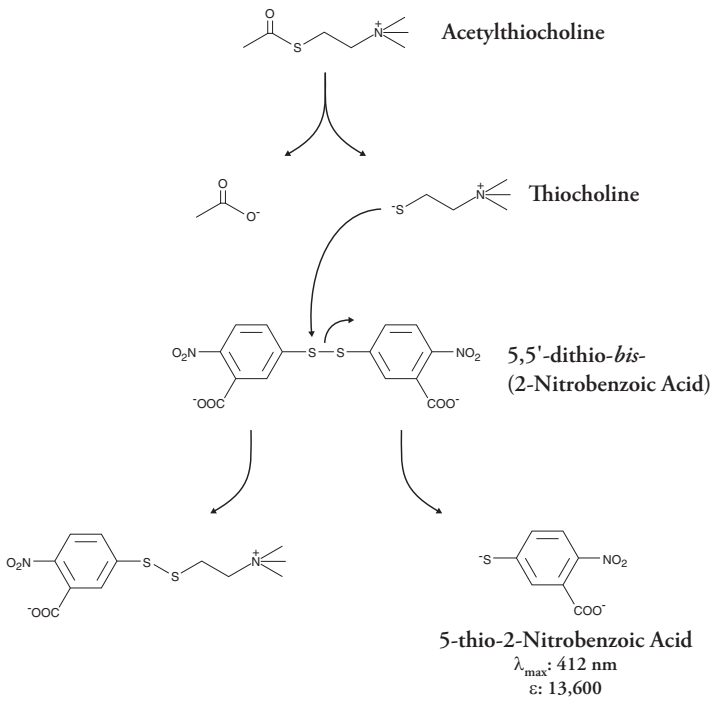


Figure 2. Reaction catalyzed by acetylcholinesterase

PGDS REACTION PROCEDURE

The use of both enzymes is not a requirement of the assay; one or both enzymes may be used depending on the nature of the study. The EIA plate will allow for 36 PGDS reactions (in duplicate) at one dilution or 18 PGDS reactions (in duplicate) at two dilutions.

IMPORTANT: Please read both PGDS Reaction Procedure and EIA Procedure sections carefully before initiating your experiments!

PGDS Reagent Preparation

1. **Reaction Buffer (10X) - (Item No. 10007572)**

   Dilute 5 ml of Reaction Buffer concentrate with 45 ml of UltraPure water. This final Reaction Buffer (0.1 M Tris-HCl, pH 8.0) is used in the PGDS reactions. When stored at room temperature, this diluted Reaction Buffer is stable for at least one month.

2. **PGDS (hematopoietic-type) - (Item No. 10007556)**

   This vial contains a solution of human recombinant PGDS (hematopoietic-type). Store the thawed enzyme on ice. Prior to use, dilute 40 µl of enzyme with 160 µl of diluted Reaction Buffer. This is sufficient enzyme for 20 reactions. If not performing 20 reactions, adjust the amount of diluted enzyme accordingly by diluting the enzyme 1:5 with reaction buffer. The diluted enzyme is stable for two hours on ice.

3. **PGDS (lipocalin-type) - (Item No. 10007557)**

   This vial contains a solution of human recombinant PGDS (lipocalin-type). Store the thawed enzyme on ice. Prior to use, dilute 100 µl of enzyme with 100 µl of diluted Reaction Buffer. This is sufficient enzyme for 20 reactions. If not performing 20 reactions, adjust the amount of diluted enzyme accordingly by diluting the enzyme 1:1 with reaction buffer. The diluted enzyme is stable for two hours on ice.

4. **Glutathione - (Item No. 10007558)**

   This vial contains a solution of reduced glutathione. It is ready to use in the PGDS (hematopoietic) reactions.

5. **Dithiothreitol - (Item No. 10007559)**

   This vial contains a solution of dithiothreitol (DTT). It is ready to use in the PGDS (lipocalin) reactions.

6. **Prostaglandin H_2 - (Item No. 10007560)**

   This vial contains a 2.5 mM solution of PGH_2 in ethanol. PGH_2 is temperature sensitive; store on ice. The reagent is ready to use as supplied. The final concentration of PGH_2 in the assay is 50 µM. Store PGH_2 at -80°C when not in use.

7. **Hydrochloric Acid - (Item No. 10007561)**

   This vial contains 1 M HCl. Dilute 500 µl with 4.5 ml of UltraPure water to yield a concentration of 0.1 M. This diluted HCl is used to prepare the Iron (II) chloride solution. Both HCl solutions are stable for at least one month at room temperature.

8. **Iron (II) Chloride - (Item No. 10007562)**

   This vial contains crystalline Iron (II) chloride. Add 2 ml of 0.1 M HCl to the vial and vortex. The solution is stable for eight hours. If not performing all of the reactions in one day, weigh 4 mg of Iron (II) chloride into another vial and add 1 ml of 0.1 M HCl. You will need 10 µl of the solution for each reaction. *NOTE: Iron (II) chloride is used to reduce residual PGH_2 to 12(S)-HHTrE which is not detected by the EIA.*

Performing PGDS Reactions

Pipetting Hints

- It is recommended that a repeating pipettor be used to deliver PGH_2 to the test tubes. This saves time and helps to maintain more precise incubation times.
- Use different tips to pipette the buffer, enzyme, GSH, DTT, inhibitor, and PGH_2.
- 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 test tube.

- *The final volume of the reaction is 500 µl in all of the test tubes.*
- *You do not have to use both enzymes. You can use either PGDS (hematopoietic or lipocalcin) for the study. The EIA plate will allow for 36 PGDS reactions (in duplicate) at one dilution or 18 PGDS reactions (in duplicate) at two dilutions.*
- *Use the Reaction Buffer (dilute) in the reactions.*
- *It is recommended that you perform no more than ten reactions at one time.*

1. **Background tubes** - Add the following reagents to two test tubes: 460 µl of reaction buffer and 20 µl of either GSH or DTT.
2. **PGDS (hematopoietic) 100% Initial Activity tubes** - Add 450 µl of reaction buffer, 20 µl of GSH, and 10 µl of PGDS (hematopoietic) to two test tubes.
3. **PGDS (hematopoietic) Inhibitor tubes** - Add 450 µl of reaction buffer, 20 µl of GSH, and 10 µl of PGDS (hematopoietic) to the necessary number of test tubes.
4. **PGDS (lipocalin) 100% Initial Activity tubes** - Add 450 µl of reaction buffer, 20 µl of DTT, and 10 µl of PGDS (lipocalin) to two test tubes.
5. **PGDS (lipocalin) Inhibitor tubes** - Add 450 µl of reaction buffer, 20 µl of DTT, and 10 µl of PGDS (lipocalin) to the necessary number of test tubes.
6. Add 10 µl of inhibitor* to the Inhibitor tubes and 10 µl of reaction buffer or solvent (which ever your inhibitor was dissolved in), to the 100% Initial Activity and background tubes and vortex.
7. Initiate the reaction by adding 10 µl of PGH_2 to all the test tubes. Vortex and incubate for 1 minute at room temperature.
8. Add 50 µl of 1 M HCl to each test tube to stop enzyme catalysis. Add 10 µl of Iron (II) chloride solution to each test tube and vortex.
9. Repeat steps 2-8 if performing more reactions.
10. PGD_2 is quantified by EIA. Proceed to the EIA Procedure (page 14). The reactions are stable for 3 days at 0-4°C if tightly capped.

*Inhibitors can be dissolved in reaction buffer, methanol, DMSO, or ethanol. If the inhibitor is not soluble in the reaction buffer then the inhibitor can be added to the reaction in solvent. The inhibitor volume should not exceed 10 µl. We recommend that several concentrations of the inhibitor be used in cases where effective inhibition is unknown.

EIA PROCEDURE

NOTE: Water used to prepare all EIA 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 EIA. UltraPure water may be purchased from Cayman (Item No. 400000).

EIA Buffer Preparation

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

1. EIA Buffer Preparation

Dilute the contents of one vial of EIA 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

Dilute the contents of the vial of Wash Buffer Concentrate (400X) (Item No. 400062) to a total volume of 2 liters with UltraPure water and add 1 ml of Polysorbate 20 (Item No. 400035). A smaller volume of Wash Buffer can be prepared by diluting the Wash Buffer Concentrate 1:400 and adding Polysorbate 20 (0.5 ml/L of Wash Buffer). The diluted buffer will be stable for two months at 4°C.

NOTE: Polysorbate 20 is a viscous liquid and cannot be measured by a pipette. A positive displacement device such as a syringe should be used to deliver small quantities accurately.

Preparation of Assay-Specific Reagents

Prostaglandin D_2 Express EIA Standard

Transfer 100 µl of the PGD_2 Express EIA Standard (Item No. 412044) into a clean test tube and dilute with 900 µl of UltraPure water. The concentration of this solution (the bulk standard) will be 150 ng/ml. This standard should not be stored for more than 24 hours.

To prepare the standard for use in EIA: Obtain eight clean test tubes and number them #1 through #8. Aliquot 900 µl EIA Buffer to tube #1 and 500 µl EIA Buffer to tubes #2-8. Transfer 100 µl of the bulk standard (150 ng/ml) to tube #1 and mix thoroughly. The concentration of this standard, the first point on the standard curve, will be 15 ng/ml (15,000 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.

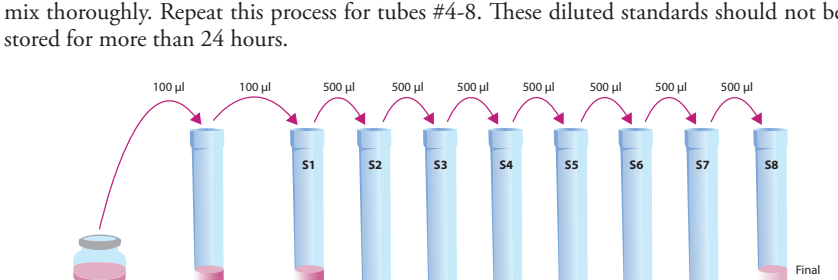


Figure 3. Preparation of the PGD_2 standards

PGD_2 Express AChE Tracer

Reconstitute one of the 100 dtn PGD_2 Express AChE Tracer vials (Item No. 412040) with 6 ml EIA Buffer. A 20% surplus of tracer has been included to account for any incidental losses. Store the reconstituted 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.

PGD_2 Express EIA Monoclonal Antibody

Reconstitute the 100 dtn PGD_2 Express EIA Monoclonal Antibody (Item No. 412042) with 6 ml EIA Buffer. Store the reconstituted 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.

PGDS Reaction Dilutions

Background Samples

Obtain three clean test tubes and label them BC1 through BC3. Aliquot 990 µl of EIA Buffer to tube BC1, add 10 µl of the background sample, and mix thoroughly. Aliquot 2.45 ml of EIA Buffer to BC2, add 50 µl of tube BC1 to tube BC2, and mix thoroughly. Tube BC2 contains a 1:5,000 dilution of the original sample. Aliquot 1 ml of EIA Buffer to tube BC3, add 1 ml of BC2 to tube BC3, and mix thoroughly. Tube BC3 contains a 1:10,000 dilution of the original sample. Tubes BC2 and BC3 will be run in the EIA. Do not use test tube BC1 in the EIA (this dilution is outside the usable range of the assay).

PGDS (hematopoietic) 100% Initial Activity Samples

Obtain three clean test tubes per sample and label them H1 through H3. Aliquot 990 µl of EIA Buffer to tube H1, add 10 µl of the sample, and mix thoroughly. Aliquot 2.45 ml of EIA Buffer to H2, add 50 µl of tube H1 to tube H2, and mix thoroughly. Tube H2 contains a 1:5,000 dilution of the original sample. Aliquot 1 ml of EIA Buffer to tube H3, add 1 ml of H2 to tube H3, and mix thoroughly. Tube H3 contains a 1:10,000 dilution of the original sample. Tubes H2 and H3 will be run in the EIA. Do not use test tube H1 in the EIA (this dilution is outside the usable range of the assay).

PGDS (hematopoietic) Inhibitor Samples

Obtain three clean test tubes per sample and label them IH1 through IH3. Aliquot 990 µl of EIA Buffer to tube IH1, add 10 µl of the sample, and mix thoroughly. Aliquot 2.45 ml of EIA Buffer to IH2, add 50 µl of tube IH1 to tube IH2, and mix thoroughly. Tube IH2 contains a 1:5,000 dilution of the original sample. Aliquot 1 ml of EIA Buffer to tube IH3, add 1 ml of IH2 to tube IH3, and mix thoroughly. Tube IH3 contains a 1:10,000 dilution of the original sample. Tubes IH2 and IH3 will be run in the EIA. Do not use test tube IH1 in the EIA (this dilution is outside the usable range of the assay).

PGDS (lipocalin) 100% Initial Activity Samples

Obtain three clean test tubes per sample and label them L1 through L3. Aliquot 990 µl of EIA Buffer to tube L1, add 10 µl of the sample, and mix thoroughly. Aliquot 1.8 ml of EIA Buffer to L2, add 200 µl of tube L1 to tube L2, and mix thoroughly. Tube L2 contains a 1:1,000 dilution of the original sample. Aliquot 1 ml of EIA Buffer to tube L3, add 1 ml of L2 to tube L3, and mix thoroughly. Tube L3 contains a 1:2,000 dilution of the original sample. Tubes L2 and L3 will be run in the EIA. Do not use test tube L1 in the EIA (this dilution is outside the usable range of the assay).

PGDS (lipocalin) Inhibitor Samples

Obtain three clean test tubes per sample and label them IL1 through IL3. Aliquot 990 µl of EIA Buffer to tube IL1, add 10 µl of the sample, and mix thoroughly. Aliquot 1.8 ml of EIA Buffer to IL2, add 200 µl of tube IL1 to tube IL2, and mix thoroughly. Tube IL2 contains a 1:1,000 dilution of the original sample. Aliquot 1 ml of EIA Buffer to tube IL3, add 1 ml of IL2 to tube IL3, and mix thoroughly. Tube IL3 contains a 1:2,000 dilution of the original sample. Tubes IL2 and IL3 will be run in the EIA. Do not use test tube IL1 in the EIA (this dilution is outside the usable range of the assay).

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.

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), one Total Activity well (TA), 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 PGDS reaction sample should be assayed at two dilutions and each dilution should be assayed in duplicate. A minimum of one 100% Initial Activity Sample should be assayed for both PGDSs.

A suggested plate format is shown in Figure 4, below. The user may vary the location and type of wells present as necessary for each particular experiment. The plate format provided below has been designed to allow for easy data analysis using a convenient spreadsheet offered by Cayman (see page 23, for more details). We suggest you record the contents of each well on the template sheet provided (see page 35).

Blk - Blank
TA - Total Activity
NSB - Non-Specific Binding
B_0 - Maximum Binding
S1-S8 - Standards 1-8
BC2&3 - Background Samples
‡ - 100% Initial Activity Samples (H2,H3, L2, & L3)
H - PGDS Inhibitor Samples (IH2, IH3, IL2, & IL3)

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blk	S1	S1	BC2	BC2	H	H	H	H	H	H	H
B	Blk	S2	S2	BC3	BC3	H	H	H	H	H	H	H
C	NSB	S3	S3	‡	‡	H	H	H	H	H	H	H
D	NSB	S4	S4	‡	‡	H	H	H	H	H	H	H
E	B_0	S5	S5	‡	‡	H	H	H	H	H	H	H
F	B_0	S6	S6	‡	‡	H	H	H	H	H	H	H
G	B_0	S7	S7	H	H	H	H	H	H	H	H	H
H	TA	S8	S8	H	H	H	H	H	H	H	H	H

Figure 4. Sample plate format

Performing the EIA

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. **EIA Buffer**

   Add 100 µl of EIA Buffer to NSB wells. Add 50 µl of EIA Buffer to B_0 wells.

2. **PGD_2 Express EIA 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. We recommend that you use the same pipette tip to aliquot all the standards. Before you pipette each standard, make sure to equilibrate the pipette tip in that standard.

3. **Background Samples (test tubes BC2 and BC3)**

   Add 50 µl of sample per well. Each sample should be assayed in duplicate.

4. **PGDS (hematopoietic) 100% Initial Activity Samples (test tubes H2 and H3)**

   Add 50 µl of sample per well. We recommend that you assay both H2 and H3. Each dilution should be assayed in duplicate.

5. **PGDS (hematopoietic) Inhibitor Samples (test tubes IH2 and IH3)**

   Add 50 µl of sample per well. We recommend that you assay both IH2 and IH3. Each dilution should be assayed in duplicate.

6. **PGDS (lipocalin) 100% Initial Activity Samples (test tubes L2 and L3)**

   Add 50 µl of sample per well. We recommend that you assay both L2 and L3. Each dilution should be assayed in duplicate.

7. **PGDS (lipocalin) Inhibitor Samples (test tubes IL2 and IL3)**

   Add 50 µl of sample per well. We recommend that you assay both IL2 and IL3. Each dilution should be assayed in duplicate.

8. **PGD_2 Express AChE Tracer**

   Add 50 µl of PGD_2 Express AChE Tracer to each well *except* the TA and the Blk wells.

9. **PGD_2 Express EIA Monoclonal Antibody**

   Add 50 µl of PGD_2 Express EIA Monoclonal Antibody to each well *except* the TA, the NSB, and the Blk wells

Well	EIA 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 1. Pipetting summary

Incubation of the Plate

Cover each plate with plastic film (Item No. 400012) and incubate two hours at room temperature on an orbital shaker.

Development of the Plate

1. When ready to develop the plate, reconstitute one 100 dtn vial of Ellman's Reagent (Item No. 400050) with 20 ml of UltraPure water. This reagent is sufficient to develop 100 wells.

   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 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 anaylsis. Please contact Technical Service or visit our website (www.caymanchem.com/analysis/eia) 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 26). 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 31 for* ***Troubleshooting****).*

Plot the Standard Curve

Plot $\%B/B_0$ for standards S1-S8 *versus* PGD_2 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.*

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

Plot the data as logit (B/B_0) *versus* log concentrations and perform a linear regression fit.

Determine the Sample Concentration

1. Calculate the $\%B/B_0$ value for each sample.
2. Determine the concentration of each sample by identifying the $\%B/B_0$ on the standard curve and reading the corresponding values on the x-axis. $\%B/B_0$ values greater than 80% and 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 would indicate interference in the EIA. Remember to multiply the PGDS samples by the appropriate dilution factor (BC2, H2, and IH2 = 5,000; BC3, H3, and IH3 = 10,000; L2 and IL2 = 1,000; L3 and IL3 = 2,000).
3. Subtract values obtained for the Background Samples from the 100% Initial Activity and Inhibitor samples.
4. Subtract each Inhibitor Sample from the 100% Initial Activity Sample, then divide by the 100% Initial Activity Sample, and multiply by 100 to give the percent inhibition.
5. Graph the percent inhibition or percent initial activity as a function of the inhibitor concentration to determine the IC_{50} value (concentration at which there was 50% inhibition). The inhibition of human recombinant PGDS (hematopoietic) by HQL-79 is shown in Figure 5 (on page 25) as an example.

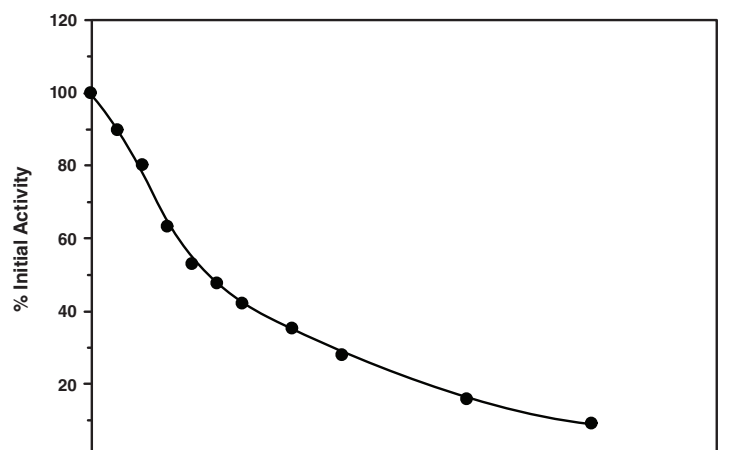


Figure 5. Inhibition of human recombinant PGDS (hematopoietic) by HQL-79 (IC_{50} = 45 µM).

EIA Performance Characteristics

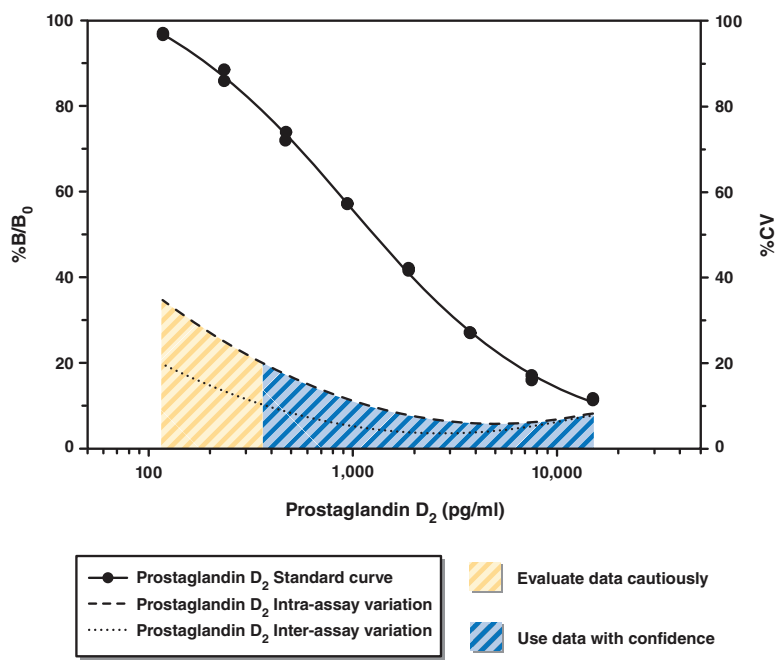
Sample Data

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	1.507	1.512	1.510	
NSB	0.000	0.000	0.000	
B_0	0.731	0.717		
	0.767	0.788	0.751	0.751

Dose (pg/ml)	Raw Data		Corrected		%B/B_0	
15,000	0.086	0.088	0.086	0.088	11.4	11.7
7,500	0.129	0.121	0.129	0.121	17.1	16.1
3,750	0.203	0.204	0.203	0.204	27.0	27.1
1,875	0.313	0.316	0.313	0.316	41.6	42.0
937.5	0.429	0.430	0.429	0.430	57.0	57.2
468.8	0.555	0.541	0.555	0.541	73.9	72.0
234.4	0.645	0.664	0.645	0.664	85.8	88.4
117.2	0.728	0.725	0.728	0.725	96.9	96.5

Table 2. Typical results



50% B/B_0 - 1,277 pg/ml
Detection Limit (80% B/B_0) - 343 pg/ml

Figure 6. 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 27 and in the table below.

Dose (pg/ml)	%CV* Intra-assay variation	%CV* Inter-assay variation
15,000	7.6	7.0
7,500	7.4	5.8
3,750	6.6	4.5
1,875	7.3	5.1
937.5	9.9	4.8
468.8	16.3	8.5
234.4	†	11.1
117.2	†	21.6

Table 3. Intra- and inter-assay variation
*%CV represents the variation in concentration (not absorbance) as determined using a reference standard curve.
†Outside of the recommended usable range of the assay.

Specificity:

Compound	Cross Reactivity	Compound	Cross Reactivity
Prostaglandin D_2	100%	6-keto Prostaglandin $F_{1\alpha}$	0.05%
Prostaglandin $F_{2\alpha}$	92.4%	13,14-dihydro-15-keto Prostaglandin D_2	0.02%
Prostaglandin J_2	21.6%	Arachidonic Acid	<0.01%
Prostaglandin E_2	2.86%	Leukotriene D_4	<0.01%
Thromboxane B_2	2.54%	tetranor-PGDM	<0.01%
11β-Prostaglandin $F_{2\alpha}$	1.99%	tetranor-PGEM	<0.01%
8-*iso* Prostaglandin $F_{2\alpha}$	1.90%	tetranor-PGFM	<0.01%
Prostaglandin A_2	0.72%	tetranor-PGJM	<0.01%
12(S)-HHTrE	0.16%		

Table 4. Specificity of the PGD_2 Express Monoclonal Antibody

Interference

It is possible that a PGDS inhibitor will interfere with the EIA and thus appear to exhibit no enzyme inhibition or exhibit a higher PGD_2 value than the 100% initial activity well. If the inhibitor exhibits no inhibition, you can repeat the PGDS reaction using a higher concentration of inhibitor and re-run the EIA. You can test for inhibitor interference by adding the inhibitor to a background sample as a control. Treat the control as a normal background sample. The sample should not yield anymore PGD_2 than a background sample without the inhibitor. If the inhibitor is detected by the antibody, the inhibitor is interfering with the EIA.

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates	A. Trace organic contaminants in the water source B. Poor pipetting/technique	Replace activated carbon filter or change source of UltraPure water
High NSB (>0.035)	A. Poor washing B. Exposure of NSB wells to specific antibody	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 do not agree (*i.e.*, more than 20% difference)	Interfering substances are present	See **Interference** section on page 30
Only Total Activity (TA) wells develop	Trace organic contaminants in the water source	Replace activated carbon filter or change source of UltraPure water
No inhibition seen with compound	A. The concentration of the compound is not high enough B. The compound is not an inhibitor of the enzyme	Increase the compound concentration and re-assay

References

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6. Maclouf, J., Grassi, J., and Pradelles, P. Development of enzyme-immunoassay techniques for the measurement of eicosanoids, Chapter 5, *in* Prostaglandin and Lipid Metabolism in Radiation Injury. Walden, T.L., Jr. and Hughes, H.N., editors, Plenum Press, Rockville, 355-364 (1987).
7. Pradelles, P., Grassi, J., and Maclouf, J.A. Enzyme immunoassays of eicosanoids using acetylcholinesterase as label: An alternative to radioimmunoassay. *Anal. Chem.* **57**, 1170-1173 (1985).

Related Products

HQL-79 - Item No. 10134
tetranor-PGDM EIA Kit - Item No. 501001
Prostaglandin D_2 - Item No. 12010
Prostaglandin D_2 EIA Kit - Item No. 512031
Prostaglandin D_2 Express EIA Kit - Item No. 512041
Prostaglandin D_2 FPIA Kit - Red - Item No. 512051
Prostaglandin D_2-MOX EIA Kit - Item No. 512011
Prostaglandin D_2-MOX Express EIA Kit - Item No. 500151
UltraPure Water - Item No. 400000

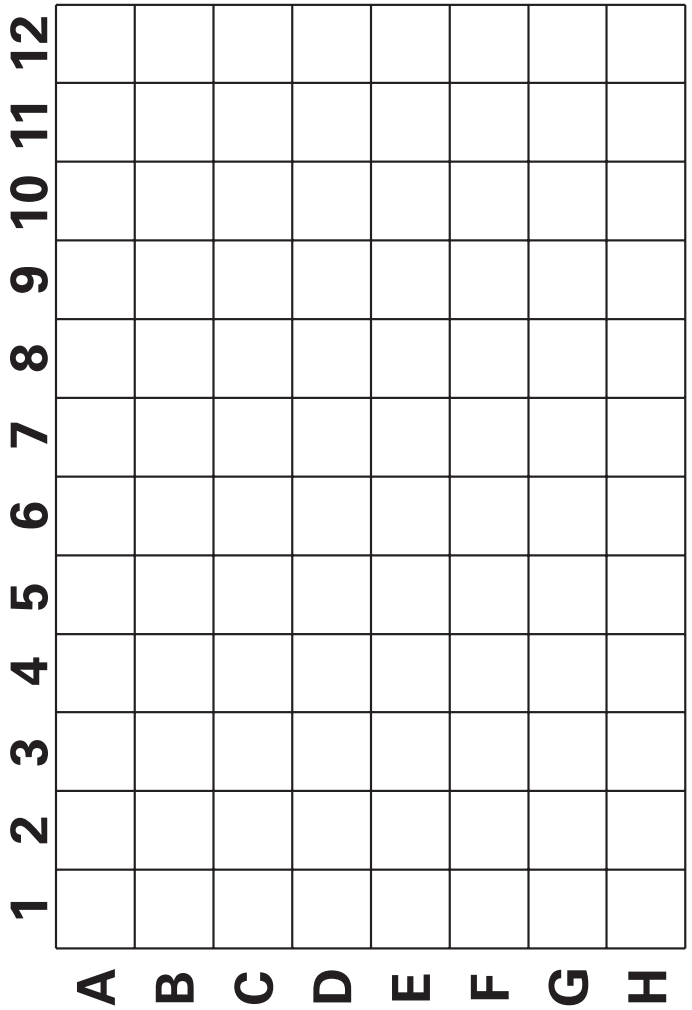
Warranty and Limitation of Remedy

Cayman Chemical Company makes **no warranty or guarantee** of any kind, whether written or oral, expressed or implied, including without limitation, any warranty of fitness for a particular purpose, suitability and merchantability, which extends beyond the description of the chemicals hereof. Cayman **warrants only** to the original customer that the material will meet our specifications at the time of delivery. Cayman will carry out its delivery obligations with due care and skill. Thus, in no event will Cayman have **any obligation or liability**, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if Cayman is informed about their possible existence. This limitation of liability does not apply in the case of intentional acts or negligence of Cayman, its directors or its employees.

Buyer's **exclusive remedy** and Cayman's sole liability hereunder shall be limited to a refund of the purchase price, or at Cayman's option, the replacement, at no cost to Buyer, of all material that does not meet our specifications.

Said refund or replacement is conditioned on Buyer giving written notice to Cayman within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

For further details, please refer to our Warranty and Limitation of Remedy located on our website and in our catalog.



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