Prostaglandin D Synthase (lipocalin-type; human) ELISA Kit

Item No. 10007684

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	96 wells Quantity/Size
10010915	Anti-L-PGDS (human) ELISA Strip Plate	1 plate
10010916	L-PGDS (human) ELISA Biotinylated Detection Antibody	1 vial/100 dtn
10010917	L-PGDS (human) ELISA Standard	2 vials/200 ng
400060	ELISA Buffer Concentrate (10X)	2 vials/10 ml
400062	Wash Buffer Concentrate (400X)	1 vial/5 ml
400035	Polysorbate 20	1 vial/3 ml
400012	96-Well Cover Sheet	1 cover
400064	L-PGDS (human) Streptavidin-HRP	2 vials/1.5 ml
400110	Non-specific Mouse Serum	1 vial/100 dtn
400074	TMB Substrate Solution	1 vial/12 ml
10011355	HRP Stop Solution	1 vial/12 ml

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's Prostaglandin D Synthase (lipocalin-type; human) ELISA Kit. This kit may not perform as described if any reagent or procedure is replaced or modified. The Stop Solution provided with this kit is an acid solution. Please wear appropriate personal protection equipment (*e.g.*, safety glasses, gloves, and lab-coat) when using this material.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Fax: 734-971-3640

E-Mail: 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 4°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 at 450 nm.
2. Adjustable pipettes and a repeating pipettor.
3. A source of ultrapure water, with a resistivity of 18.2 MΩ·cm and total organic carbon (TOC) levels of <10 ppb, is recommended. Pure water - glass-distilled or deionized - may not be acceptable. *NOTE: UltraPure Water is available for purchase from Cayman (Item No. 400000).*
4. Materials used for **Sample Preparation** (see page 11).

INTRODUCTION

Biochemistry of Prostaglandin D Synthase

Prostaglandin D synthase (PGDS) catalyzes the isomerization of PGH_2 to produce PGD_2. Lipocalin-type PGDS (L-PGDS, a.k.a. β-trace) is a homodimer with each subunit ranging in size from 20-31 kDa, depending on glycosylation. L-PGDS has two functions: it catalyzes the conversion of PGH_2 to PGD_2 and acts as a carrier protein for lipid-like molecules (*i.e.*, retinoids and thyroid hormones).[1] PGD_2 is involved in sleep regulation,[2] nociception,[3] platelet aggregation,[4] and allergic and inflammatory responses. There is also some evidence to indicate the involvement of PGD_2 and its J-series metabolites in pregnancy and parturition.[5]

About This Assay

Cayman's L-PGDS ELISA Kit is an immunometric (*i.e.*, sandwich) ELISA. The standard curve ranges from 1.56-100 ng/ml, with a limit of detection of 1.56 ng/ml. Inter- and intra-assay CVs of less than 15% may be achieved at most concentrations. L-PGDS is present in a variety of body fluids including cerebrospinal fluid, seminal fluid, and plasma. This assay has been validated using cerebrospinal fluid which contains approximately 12-30 µg/ml of L-PGDS.

Description of Immunometric ELISAs

Each well of the microwell plate supplied in the kit has been coated with Mouse anti-L-PGDS monoclonal antibody. L-PGDS, if present in the biological fluid sample, will bind to the immobilized anti-L-PGDS antibody. A biotin-conjugated L-PGDS detection antibody reagent is then added to the well and is bound by L-PGDS. An HRP-conjugated streptavidin reagent is then added and binds to the biotin, allowing quantitation of the L-PGDS. Addition of the HRP Substrate 3,3',5,5'-tetramethylbenzidine (TMB), followed by Stop Solution produces a yellow colored product which can be measured spectrophotometrically. The intensity of the color is directly proportional to the amount of bound streptavidin-HRP, which is proportional to the concentration of the biotinylated L-PGDS antibody, which is proportional to the L-PGDS.

$$\text{Absorbance} \propto [\text{Biotinylated L-PGDS antibody}] \propto [\text{L-PGDS}]$$

A schematic of this process is shown in Figure 1, on page 9.

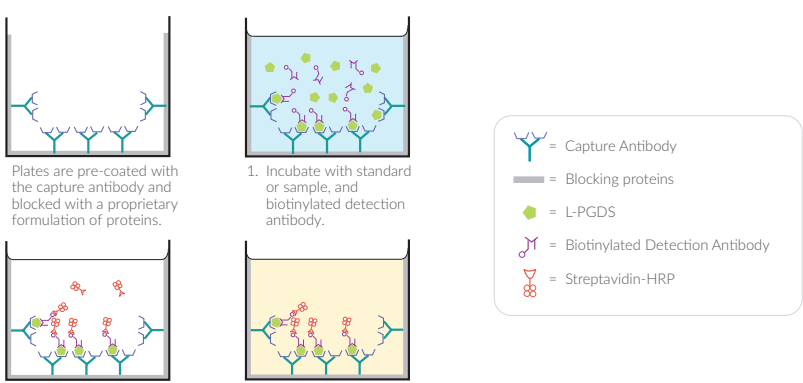

Figure 1. Schematic of the AChE ELISA

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 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.

2. **Wash Buffer Preparation**

   5 ml vial Wash Buffer Concentrate (400X) (Item No. 400062): Dilute to a total volume of 2 liters with deionized water and add 1 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. It is normal for the concentrated buffer to contain crystalline salts after thawing. These will completely dissolve upon dilution with water.

Sample Preparation

In general, samples can be assayed with no prior purification. If human cerebrospinal fluid (CSF) or plasma is to be tested, one must add non-specific mouse serum (Item No. 400110) to each sample and each point of the standard curve (see below). This will compensate for the effects of human anti-mouse IgG which may be present in the samples.

ASSAY PROTOCOL

Preparation of Assay-Specific Reagents

L-PGDS (human) ELISA Standard

Reconstitute one vial of the L-PGDS ELISA Standard (Item No. 10010917) with 2 ml of ELISA Buffer. Mix gently, but do not vortex. The concentration of this standard, the first point on the standard curve, will be 100 ng/ml. This reconstituted standard is very unstable; therefore each vial should be discarded within 24 hours of being reconstituted.

NOTE: If assaying culture medium samples that have not been diluted with ELISA Buffer, culture medium should be used in place of ELISA Buffer for dilution of the standard curve.

To prepare the standard for use in ELISA: Obtain seven clean test tubes and number them #2 through #8. Aliquot 500 µl ELISA Buffer into tubes #2-8. Serially dilute the standard by removing 500 µl from the stock standard vial #1 and placing in tube #2; mix gently but do not vortex. Next, remove 500 µl from tube #2 and place it into tube #3; mix gently but do not vortex. Repeat this process for tubes #4-7. Do not add any L-PGDS standard to tube #8. This tube is the zero-point vial, the lowest point on the standard curve. The diluted standards may be stored at 4°C for no more than 24 hours.

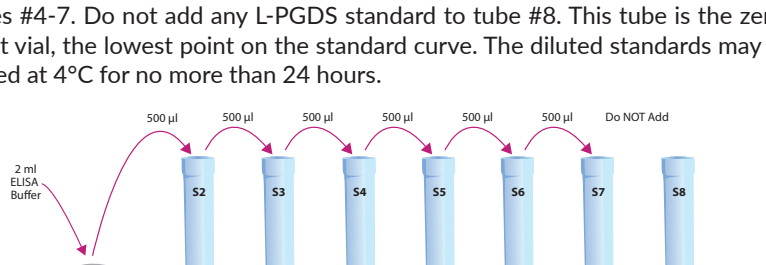

Figure 2. Preparation of the L-PGDS standards

L-PGDS (human) ELISA Biotinylated Detection Antibody

Reconstitute the L-PGDS (human) ELISA Biotinylated Detection Antibody as follows:

L-PGDS (human) ELISA Biotinylated Detection Antibody (Item No. 10010916): Reconstitute with 12 ml ELISA Buffer.

Store the reconstituted L-PGDS (human) ELISA Biotinylated Detection Antibody at 4°C. It will be stable for at least two weeks. A 20% surplus of antibody has been included to account for any incidental losses.

L-PGDS (human) Streptavidin-HRP

This reagent is supplied as a concentrated (10X) stock solution of Streptavidin conjugated to HRP. On the day of the assay, prepare a Working Solution by adding 1.2 ml of the Streptavidin-HRP (Item No. 400064) to 10.8 ml ELISA Buffer (12 ml total). This Working Solution is stable for 24 hours at 4°C, protected from light. In the event that two or more experiments are performed with this kit more than 24 hours apart, two vials of stock solution has been provided to produce additional 12 ml of the Working Solution.

Non-specific Mouse Serum

The Non-specific Mouse Serum supplied with this kit is to be used when analyzing plasma, serum, cerebrospinal fluid or any other sample that contains heterophilic antibodies. Reconstitute the Non-specific Mouse Serum as follows:

Non-specific Mouse Serum (Item No. 400110): Reconstitute with 2.5 ml UltraPure Water.

Store the reconstituted Non-specific Mouse Serum at 4°C and use within four weeks. 25 µl of Mouse Serum should be added to each 500 µl aliquot of sample or standard prior to addition to the well. Remember, you must also add Mouse Serum to each point of the standard curve (25 µl of Mouse Serum per 500 µl of standard).

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) 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 3, 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 **Analysis**, page 18, for more details). We suggest you record the contents of each well on the template sheet provided (see page 26).

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	1	1	1	9	9	9	17	17	17	25
B	S2	S2	2	2	2	10	10	10	18	18	18	25
C	S3	S3	3	3	3	11	11	11	19	19	19	25
D	S4	S4	4	4	4	12	12	12	20	20	20	26
E	S5	S5	5	5	5	13	13	13	21	21	21	26
F	S6	S6	6	6	6	14	14	14	22	22	22	26
G	S7	S7	7	7	7	15	15	15	23	23	23	Blk
H	S8	S8	8	8	8	16	16	16	24	24	24	Blk

Blk - Blank
S1-S8 - Standards 1-8
1-26 - Samples

Figure 3. Sample plate format

Performing the Assay

NOTE: This assay is performed in two steps prior to the addition of the substrate.

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 First Set of Reagents

1. **L-PGDS (human) ELISA Standard**

   Add 100 µl from tube #8 to both of the lowest standard wells (S8). Add 100 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.

2. **Samples**

   Add 100 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).

3. **L-PGDS (human) ELISA Biotinylated Detection Antibody**

   Add 100 µl to each well *except* the Blank (Blk) wells.

Well	Standard	Samples	Antibody
Blk	-	-	-
Standards	100 µl	-	100 µl
Samples	-	100 µl	100 µl

Table 1. Pipetting summary

Incubation of the Plate

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

Addition of the Second Set of Reagents

1. Empty the wells and rinse four times with Wash Buffer. Each well should be completely filled with Wash Buffer during each wash. Invert the plate between wash steps to empty the fluid from the wells. After the last wash, gently tap the inverted plate on absorbent paper to remove the residual Wash Buffer.
2. Add 100 µl of L-PGDS ELISA Streptavidin-HRP to each well except the Blank (Blk) wells.

Incubation of the Plate

Cover the plate with plastic film and incubate for one hour at room temperature on an orbital shaker.

Development of the Plate

1. Empty the wells and rinse four times with Wash Buffer.
2. Add 100 µl of TMB Substrate Solution (Item No. 400074) to each well of the plate.
3. Cover the plate with a 96-Well Cover Sheet and incubate for 10-15 minutes at room temperature in the dark. Development of the blue color can be monitored at 650 nm. When the maximum standard (S1) O.D. value reaches 0.5-0.6, Stop Solution should be added to the entire plate.
4. DO NOT WASH THE PLATE. Add 100 µl of HRP Stop Solution (Item No. 10011355) to each well of the plate. Blue wells should turn yellow and colorless wells should remain colorless. *NOTE: The Stop Solution in this kit contains an acid. Wear appropriate protection and use caution when handling this solution.*

Reading the Plate

1. Wipe the bottom of the plate with a clean tissue to remove fingerprints, dirt, etc.
2. Read the plate at a wavelength of 450 nm.

ANALYSIS

Many plate readers come with data reduction software that plots data automatically. Alternatively a spreadsheet program can be used. *NOTE: Cayman has a computer spreadsheet available for data analysis. Please contact Technical Service or visit our website (www.caymanchem.com/analysis/immuno) to obtain a free copy of this convenient data analysis tool.*

Calculations

Plotting the Standard Curve and Determining the Sample Concentration

Using computer reduction software, plot absorbance (linear y-axis) *versus* concentration (linear x-axis) for standards (S1-S8) and fit the data with a quadratic equation. Using the equation of the line, calculate the concentration L-PGDS in each sample.

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. Development of the plate for 15 minutes typically results in an absorbance of >1.0 O.D. units for the 100 ng/ml standard.

L-PGDS (ng/ml)	Absorbance	
100	1.556	1.625
50	0.929	0.878
25	0.469	0.454
12.5	0.252	0.309
6.25	0.143	0.169
3.13	0.093	0.090
1.56	0.068	0.068
0	0.051	0.036

Table 2. Typical results

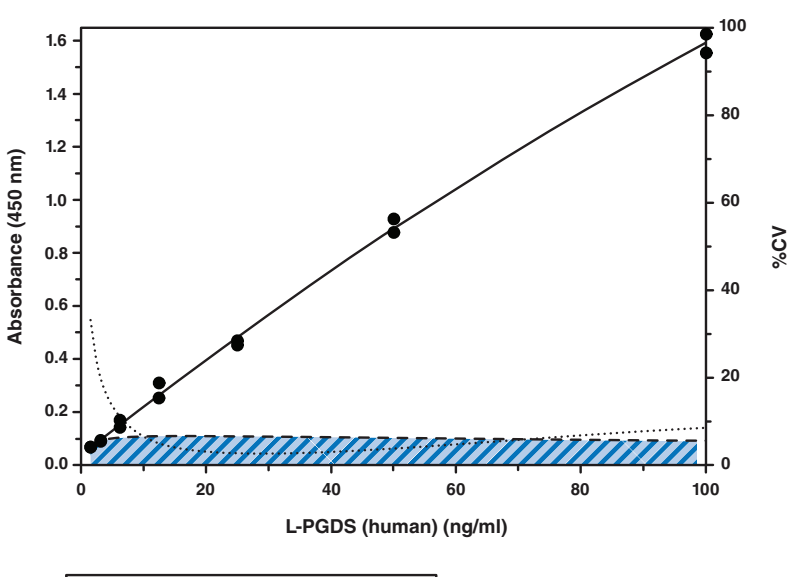

Figure 4. Typical standard curve
The minimum detectable concentration is 1.56 ng/ml.

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 20.

L-PGDS (ng/ml)	%CV* Intra-assay variation	%CV* Inter-assay variation
100	5.3	6.8
50	7.2	5.3
25	5.7	4.4
12.5	7.0	6.5
6.25	6.0	10.5
3.13	5.9	13.9
1.56	4.4	36.9
0	†	†

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.

LPGDS (ng/ml)	Mean of O.D.	Standard Deviation (S.D.)	O.D. - (1.64 x S.D.)
100	1.537	0.041	1.469
50	1.163	0.132	0.947
25	0.600	0.070	0.485
12.5	0.313	0.021	0.280
6.25	0.169	0.010	0.153
3.13	0.102	0.006	0.093
1.56	0.072	0.003	0.067
0	0.051	0.008	0.064*

*O.D. + (1.64 x S.D.)

Table 4. Determination of LLOQ

The lower limit of quantitation (LLOQ) is defined as the lowest standard concentration in which O.D. - (1.64 x S.D.) is higher than the blank value of O.D. + (1.64 x S.D.). The LLOQ is 1.56 ng/ml.

Cross Reactivity:

Compound	Cross Reactivity
Prostaglandin D Synthase (lipocalin-type; human)	100%
Prostaglandin D Synthase (hematopoietic-type; human)	<1%
FABP1 (human recombinant)	<1%
FABP2 (rat recombinant)	<1%
FABP3 (human recombinant)	<1%
FABP4 (human recombinant)	<1%
FABP5 (human recombinant)	<1%
FABP7 (human recombinant)	<1%
Prostaglandin F Synthase (human recombinant)	<1%
sRBP4 (human recombinant)	<1%
Prostaglandin D Synthase (lipocalin-type; murine)	cross reacts with capture antibody

Table 5. Cross Reactivity of the L-PGDS (human) ELISA.

RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates	A. Trace organic contaminants in the water source B. Contamination of water with organic solvents C. Poor pipetting/technique	A. Replace activated carbon filter or change source of UltraPure water
Poor development (low signal) of standard curve	A. Plate requires additional development time B. Standard was diluted incorrectly C. Standard is degraded	A. Return plate to shaker and re-read later
Analyses of two dilutions of a biological sample do not agree (*i.e.*, more than 20% difference)	Interfering substances are present	A. Add mouse serum and DTT to standards and samples B. Purify sample prior to analysis by ELISA[6]
Sample concentrations appear inconsistent with literature values	Matrix for samples and standards are different	A. Use sample matrix for all samples and standards B. Add mouse serum and DTT to standards and samples

References

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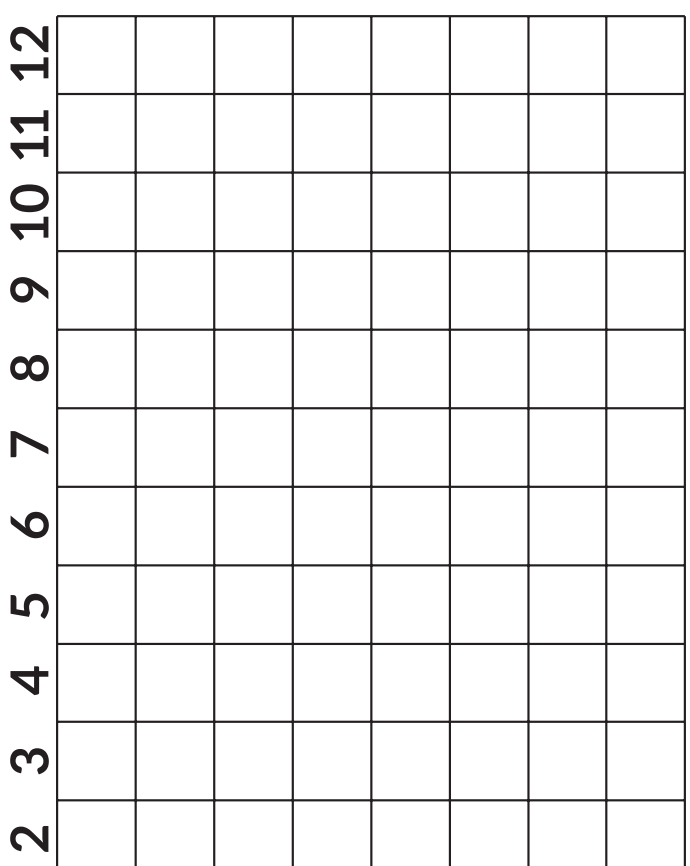

NOTES

Warranty and Limitation of Remedy

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