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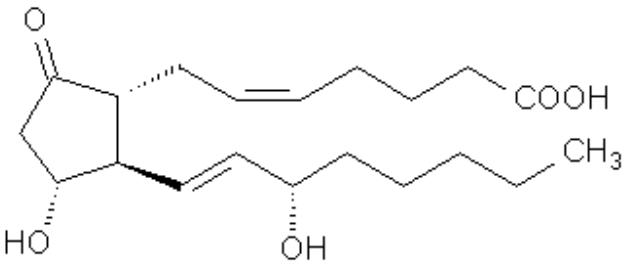
ProductInformation

Prostaglandin E_2 EIA

Product Number **CS0200**
Storage Temperature –20 °C

Technical Bulletin

Product Description



PGE_2

Prostaglandin E_2 EIA is a four-hour competitive enzyme immunoassay for the quantitative determination of Prostaglandin E_2 (PGE_2) concentrations in cell culture supernatants, saliva, urine, serum and plasma. PGE_2 present in the samples or standards competes with the fixed amount of PGE_2 conjugated to alkaline phosphatase for the limited number of binding sites on the monoclonal anti-PGE_2 antibody. During the first incubation, the antigen-antibody complex binds to the anti-mouse IgG antibody-coated multiwell plate. The excess conjugate and unbound sample are washed away and a substrate is added. During the second incubation the bound enzyme reacts with the substrate. The enzyme reaction is stopped and the absorbance read at 405 nm. The intensity of the yellow color is inversely proportional to the concentration of PGE_2 in the standards or the samples. The concentration is calculated on the basis of optical reading of standard dilutions.

The cyclooxygenase and lipoxygenase pathways are two major synthetic pathways relevant to human disease. The initial synthetic step for both pathways involves the cleavage of arachidonic acid. Arachidonic acid is stored esterified in phospholipids of cell membranes.[1] It is released from the cell membrane upon demand via phospholipase A2.[2] The free arachidonic acid is then oxygenated by either the cyclooxygenase or lipoxygenase pathway. The end products of these pathways are called eicosanoids. Prostaglandins and thromboxane are products of the cyclooxygenase pathway and leukotrienes are products of the lipoxygenase pathway. Eicosanoids are synthesized in response to immediate need and are not stored in significant amounts for later release.

Prostaglandin E_2 (PGE_2) is formed in a variety of cells from prostaglandin H_2, which is synthesized from arachidonic acid by the enzyme prostaglandin synthetase. PGE_2 and thromboxane A_2 are structurally related and their receptors are also homologous. PGE_2 produces a broad range of biologic actions in diverse tissues, including vasodilation, both anti- and proinflammatory action, modulation of sleep/wake cycles, and facilitation of the replication of human immunodeficiency virus. It elevates cAMP levels, stimulates bone resorption, and has thermoregulatory effects. It has been shown to be a regulator of sodium excretion and renal hemodynamics. COX2, EP_2, and EP_4 expression and PGE_2 synthesis are up regulated in cervical cancer tissue and PGE_2 may regulate neoplastic cell function in cervical carcinoma in an autocrine/paracrine manner via the EP_2/EP_4 receptors. Besides its effects on reproduction, PGE_2 regulates regional blood flow in various vascular beds.[3-7]

Reagents

- **Prostaglandin E_2 (PGE_2) Standard, 1 vial, Product No. P 1871** – 0.5 mL (50,000 pg/mL) in a buffer with preservative.
- **Anti-Mouse IgG-coated 96 well plate, 1 plate, Product No. I 6158**
- **Assay Buffer, 30 mL, Product No. A 4228 –** contains sodium azide. Ready to use.
- **Monoclonal Anti-PGE_2, 6 mL, Product No. P 1621 –** from mouse, contains sodium azide, yellow dye. Ready to use.
- **Prostaglandin E_2 (PGE_2)-Alkaline Phosphatase Conjugate, 6 mL, Product No. P 1746 -** contains sodium azide, blue dye. Ready to use.

- **p-Nitrophenylphosphate (pNPP) Substrate, 20 mL, Product No. N 6911 -** Ready to use.
- **Wash Buffer Concentrate, 20X, 30 mL, Product No. W 3139 –** TRIS buffered saline containing 0.2% Hydorol M as preservative.
- **Stop Solution, 5 mL, Product No. S 9443 –** a solution of trisodium phosphate. Ready to use.
- **Plate sealer, 1 each, Product No. P 1496**

Reagents and Equipment required but not provided

- Multiwell plate reader capable of readings at 405 nm, preferably with corrections between 570 and 590 nm.
- Horizontal orbital multiwell plate shaker capable of maintaining a speed of 500 +/- 50 rpm.
- Calibrated adjustable precision pipettes for volumes between 5 µL and 1,000 µL.
- If the sample requires extraction, cell extraction materials are needed (see recommended extraction procedure).
- Deionized or distilled water.
- Plate washer (optional), use squirt bottle, manifold dispenser, etc.
- Glass or plastic 1.0 – 1.5 mL tubes for diluting and aliquoting standard.
- Absorbent paper towels to blot the plate.
- Calibrated beakers and graduated cylinders in various sizes.
- Vortex mixer.
- Graph paper: linear, log-log, or semi-log, as desired.
- Prostaglandin synthetase inhibitor, such as indomethacin (Product No. I 8280), or meclofenamic acid sodium salt (Product No. M 4531)

Precautions and Disclaimer

The kit is for R&D use only, not for drug, household or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Sample Preparation

- Samples containing mouse IgG may interfere with this assay.
- The assay is validated for cell culture samples, saliva, urine, plasma and serum.
- Samples may be assayed directly or after extraction.
- If samples are not assayed immediately, a prostaglandin synthetase inhibitor, such as indomethacin should be added to all samples at approximately 10 µg/mL before storage.
- Cell culture supernatants should be separated from the cells and frozen, if not analyzed shortly after collection. Avoid multiple freeze/thaw cycles.
- Cell culture samples may be assayed undiluted or diluted in cell culture media.
- Use the same cell culture medium for blanks, controls and standard dilutions.
- Samples, which normally have low levels of PGE_2, (below assay sensitivity) require extraction.
- Sera and plasma require a 20-fold dilution in Assay Buffer.
- Urine samples require a 50-fold dilution. A suggested 50-fold dilution is 10 µL sample + 490 µL Assay Buffer.
- All saliva samples require a 10-fold dilution. A suggested 10-fold dilution is 50 µL sample + 450 µL Assay Buffer.
- To ensure accurate results, always dilute the standards and blanks in the same diluent as the samples.

<u>Materials Required for Extraction</u>

- 2 N HCl
- Ethanol
- Hexane
- Ethyl acetate
- 200 mg C_{18} Reverse Phase Column
- High specific activity tritiated PGE_2 (for determination of extraction efficiency).

<u>Protocol for Sample Extraction</u>

1. Acidify sample to pH 3.5 with 2N HCl. (~ 50 µL HCl per 1 mL of plasma).
2. Equilibrate at 2-8 °C for 15 minutes.
3. Centrifuge samples in a microcentrifuge for 2 minutes to remove any precipitate.
4. Prepare the C_{18} reverse phase column by washing with 10 mL of ethanol followed by 10 mL of deionized water.
5. Apply the sample under a slight positive pressure to obtain a flow rate of about 0.5 mL/minute.
6. Wash the column with 10 mL of water, followed by 10 mL of 15% ethanol, and finally 10 mL hexane.
7. Elute the sample from the column by addition of 10 mL ethyl acetate.
8. If analysis is to be carried out immediately, evaporate samples under a stream of nitrogen.

9. Add 50 µL ethanol to the dried samples and reconstitute sample with at least 200 µL of Assay Buffer or cell culture medium.
10. If analysis is to be delayed, store samples as the eluted ethyl acetate solutions at –70 °C until the time of assay.
11. Repeat steps 8,9.

Note: Refer to citations 8-9 for details of extraction protocols.

Reagent Preparation

Prostaglandin E_2 Standard

1. Standards can be made either in glass or plastic tubes.
2. Pre-rinse pipette tips and change the tips before each dilution.
3. Equilibrate standard and all reagents to room temperature.
4. Prepare serial standard dilutions as follows:

Tube #	Assay Buffer or cell culture medium µL	Standard from tube (#): µL	Final Standard Concentration pg/mL
0	Standard vial 50,000 pg/mL		
1	950 µL	50 µL (0)	2,500
2	500 µL	500 µL (1)	1,250
3	500 µL	500 µL (2)	625
4	500 µL	500 µL (3)	313
5	500 µL	500 µL (4)	156
6	500 µL	500 µL (5)	78.1
7	500 µL	500 µL (6)	39.1

5. Diluted standards should be used within 60 minutes of preparation.

PGE_2 -Alkaline phosphatase conjugate

1. The activity of the PGE_2 conjugate is dependent on the presence of Mg^{2+} and Zn^{2+} ions.
2. The activity of PGE_2 conjugate is affected by high concentrations of chelators, such as EDTA and EGTA. Samples that contain <10 mM EDTA or EGTA can be assayed without interference. Samples containing higher concentrations of chelators must be diluted prior to assay.
3. Equilibrate conjugate to room temperature before use.
4. After use, store the remaining conjugate at –20 °C for the shelf life of the kit.
5. For Total Activity (TA) wells: dilute 50 µL conjugate in 450 µL Assay Buffer – add 5 µL to TA wells.

Wash Buffer

1. Use only Wash Buffer provided in the kit.
2. Inorganic phosphate is a strong competitive inhibitor of alkaline phosphatase; **avoid the use of PBS-based wash buffers** and other sources of inorganic phosphate contamination.
3. Warm buffer to room temperature.
4. Dilute 5 mL Wash Buffer Concentrate with 95 mL deionized water.
5. Label as **Working Wash buffer**
6. Diluted buffer may be stored at room temperature for up to 3 months.

Storage/Stability

- The kit is shipped on dry ice.
- Store unopened kit at –20 °C until use. Do not store past kit shelf life.
- After opening:
  - Conjugate must remain frozen at –20 °C.
  - The rest of the components may be stored at 2-8 °C.

Refer to the Certificate of Analysis for kit shelf life. To obtain C of A go to www sigma-aldrich.com

Procedure

Precautions

- Allow all reagents to equilibrate to room temperature (15-30 °C) for at least 30 minutes before opening the kit.
- Use only the pre-coated 96 multiwell capture plate provided with the kit.
- Multiwell strips should be equilibrated to room temperature in the sealed foil bag.
- Remove desired number of strips, reseal the bag and refrigerate unused wells desiccated at 2-8 °C to maintain plate integrity.
- When not in use all kit components should be stored as described in **Storage/Stability.**
- Do not use reagents after the kit expiration date.
- Assay all standards, controls and samples in duplicate.
- If particulate matter is present, centrifuge or filter prior to analysis.
- A standard curve must be run with each assay
- Maintain a consistent order of component and reagent addition from well to well. This ensures equal incubation times for all wells.
- Run in-house controls with every assay. If control values fall outside pre-established ranges, the accuracy of the assay may be suspect.

- All reagents are lot-specific. Do not mix reagents from different kit lots.
- Minimize contamination by endogenous alkaline phosphatase, present especially in the substrate solution. Avoid touching pipette tips and other items with bare hands.
- Alkaline phosphatase is a temperature sensitive enzyme. Optical Density (OD) units may vary with temperature changes.
- Pre-rinse the pipette tip with the reagent and use fresh pipette tips for each sample, standard or reagent.
- Pipette standards and samples to the bottom of the wells.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Read absorbances within 2 hours of assay completion.

Washing directions

1. The purpose of washing is to remove unbound proteins and other non-specific parts of lysate.
2. Incomplete washing will adversely affect the assay and render false results.
3. Avoid the use of phosphate-based buffers to prevent contamination with endogenous phosphate.
4. Washing may be performed using automated washer, manifold pipette or squirt bottle.
5. Wash cycle three times, blotting as dry as possible after the 3rd wash.
6. When washing manually, fill wells with Wash Buffer, aspirate thoroughly and tap dry on absorbent tissue.
7. It is recommended to use laboratory tape to hold plate strips to the plate frame while performing the plate washing and drying procedure to avoid strips coming free of the frame.

Assay Procedure

Refer to the diagram on page 7 to view the assay layout.

1st Incubation

a. Remove the appropriate number of strips and return the unused strips to the pouch. Reseal pouch and refrigerate.
b. Add 100 µL of standard diluent (Assay Buffer or tissue culture medium) to the NSB wells and to the B_o (0 pg/mL) wells
c. Add 100 µL of standards #1-7 to the appropriate wells.
d. Add 100 µL of samples to the appropriate wells
e. Add 50 µL Assay Buffer to the NSB wells
f. Add 50 µL of PGE_2-alkaline phosphatase conjugate to all wells, except the Total Activity(TA) and Blank wells.
g. Add 50 µL of Monoclonal Anti-PGE_2 to all wells, except the Blank, TA and NSB wells.
h. All wells used should be green, except the NSB wells, which are blue. The Blank and TA wells are empty at this time and have no color.
i. Cover with plate cover and incubate 2 hours at room temperature on an orbital shaker at 500 rpm.
j. Wash wells for a total of 3 times following washing instructions.
k. After the final wash, blot dry on a lint free paper towel to remove any remaining wash buffer.

Substrate Incubation

a. Add 5 µL of PGE_2 conjugate (1:10 dilution) to the TA wells.
b. Add 200 µL of pNPP substrate to all wells. Cover.
c. Incubate 45 minutes at room temperature without shaking.

Stop Reaction

a. Add 50 µL of Stop Solution to each well.
b. Yellow color develops immediately and can be read in the multiwell plate reader at 405 nm with corrections at 570 or 590 nm.
c. Subtract the readings at 590 nm from the readings at 405 nm, to correct for optical imperfection of the plate.

Results

1. Average the duplicate readings for each standard and sample and subtract the average NSB optical density.
2. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit.
3. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on a linear y-axis against the concentration (pg/mL) on a logarithmic x-axis and draw the best fit curve through the points on the graph.
4. % B/B_o can be calculated by dividing the corrected OD for each standard or sample by the corrected B_o OD and multiplying by 100.
5. Calculate the concentration of PGE_2 corresponding to the mean absorbance or % B/B_o from the standard curve.

6. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

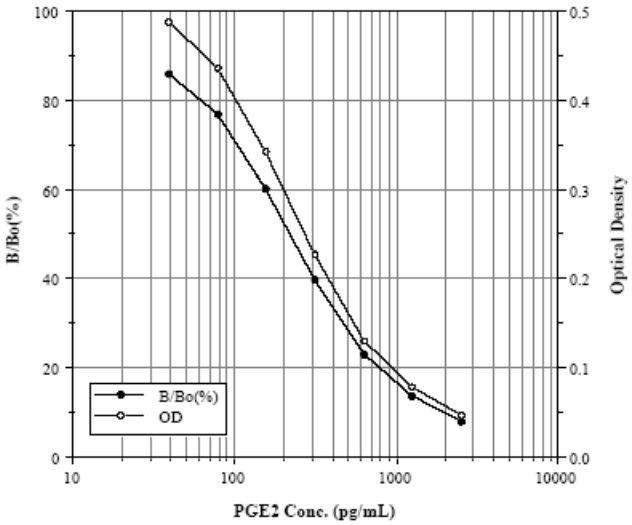
Product Profile

Typical Results

Well ID	Mean OD (–Blank) 405 nm	Average Net OD B	% B/B_o	PGE_2 pg/mL
Blank	(0.112)			
TA	0.362			
NSB	0.008	0.000	0.00%	
0 (B_o)	0.577	0.569	100%	**0**
S1	0.054	0.046	8.1%	**2,500**
S2	0.086	0.078	13.8%	**1,250**
S3	0.138	0.130	22.9 %	**626**
S4	0.235	0.227	39.9 %	**313**
S5	0.350	0.342	60.1%	**156**
S6	0.445	0.437	76.9%	**78.1**
S7	0.497	0.489	86.0%	**39.1**
Sample 1	0.168	0.160	29.1%	**489.8**
Sample 2	0.259	0.251	44.9%	**269.2**

Typical Standard Curve for PGE_2 EIA

This standard curve is provided for demonstration only. A standard curve should be generated for each assay.



Quality Control

Each laboratory should establish a quality control program to monitor the performance of the PGE_2 immunoassay. As a part of this program, TA, NSB, B_0, and Substrate Blank wells should be run in each assay. The average readings are calculated over the time. Any time the assay readings exceed the average, the assay may need to be re-run.

Typical Quality Control Parameters

Total Activity Added (TAA) = TA x 10 = 3.62
% NSB (NSB/TAA x 100) = 0.20%
% B_o (B_o/TAA x 100) = 15.7%
Quality of Fit = 1.000

Performance Characteristics

Sensitivity

The sensitivity of the PGE_2 assay is typically less than 13.4 pg/mL; it was calculated by dividing 2 times the standard deviation of the B_o optical densities by the difference between the average optical density for sixteen (16) wells run as B_o and the average optical density for sixteen (16) wells run with Standard # 7, then multiplying by 39.1 pg/mL

0.022/0.064 x 39.1 pg/mL = 13.4 pg/mL.

Linearity

A sample containing 50,000 pg/mL PGE_2 was diluted 6 times 1:2 in the kit Assay Buffer and measured in the assay. The data was plotted graphically as actual PGE_2 concentration versus measured PGE_2 concentration. The line obtained had a slope of 1.161 and a correlation coefficient of 1.000.

Precision

Intra-assay precision was determined by taking samples containing low, medium and high concentrations of PGE_2 and running these samples 24 times in the same assay.

Inter-assay precision was determined by measuring three samples with low, medium and high concentrations of PGE_2 in 8 different assays.

	PGE_2 pg/mL	Intra Assay %CV	Inter Assay %CV
Low	116	8.9	
Medium	492	5.8	
High	2,416	17.5	
Low	111		3.0
Medium	419		5.1
High	1,902		3.9

Recovery

The recovery of PGE_2 spiked into samples in various matrices was evaluated.

Sample	**% Recovery**	**Dilution**
Tissue Cultures	104.4	Undiluted
Human Saliva	123.3	1:10
Human Urine	108.9	1:10
Human Male Serum	126.1	1:10
Human Female Serum	113.7	1:10
Human Whole Blood	101.2	1:10

Cross Reactivity

The cross reactivity for a number of related compounds was determined by dissolving the cross reactant (purity checked by N.M.R. and other analytical methods) in Assay Buffer at concentrations from 39 to 500,000 pg/mL. These samples were measured in the PGE_2 assay and the PGE_2 concentration at 50% B/B_o calculated. The % cross reactivity was calculated by comparison with the actual concentration of cross reactant in the sample and expressed as a percentage.

Compound	**% Cross Reactivity**
PGE_2	100%
PGE_1	70%
PGE_3	16.3%
$PGF_{1\alpha}$	1.4%
$PGF_{2\alpha}$	0.7%
6-keto-$PGF_{1\alpha}$	0.6%
PGA_2	0.1%
PGB_1	0.1%
13,14-dihydro-15-keto-$PGF_{2\alpha}$	<0.1%
6,15-keto-13, 14-dihydro-$PGF_{1\alpha}$	<0.1%
Thromboxane B_2	<0.1%
2-Arachidonoylglycerol	<0.1%
Anandamide	<0.1%
PGD_2	<0.1%
Arachadonic acid	<0.1%

References

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PGE_2 Assay Flow Chart (CS0200)

	Blank	TA	NSB	B_0	Standards	Samples
Well ID	**A1, B1**	**C1, D1**	**E1, F1**	**G1, H1**	**A2-F3**	**G3-H12**
Standard Diluent			100 µL	100 µL		
Assay Buffer			50 µL			
Standard and/or Sample					100 µL	100 µL
Conjugate			50 µL	50 µL	50 µL	50 µL
Anti-PGE_2 Antibody				50 µL	50 µL	50 µL

Incubate 2 hours at Room Temperature with shaking
Wash 3X, blot dry

Conjugate 1:10 dilution		5 µL*				
Substrate	200 µL	200 µL	200 µL	200 µL	200 µL	200 µL

Incubate 45 minutes at Room Temperature without shaking

Stop Solution	50 µL	50 µL	50 µL	50 µL	50 µL	50 µL

Read at 405 nm

*Conjugate for TA must be diluted 1:10 in Assay Buffer: 450 µL Assay Buffer + 50µL conjugate

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