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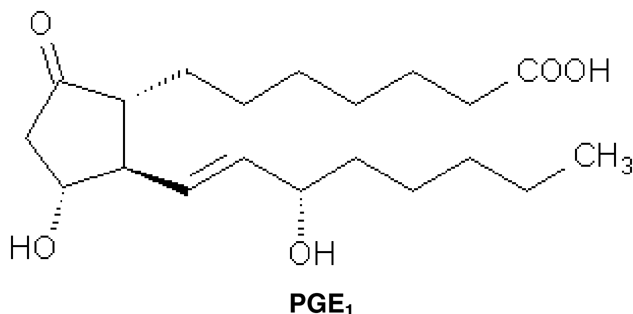
ProductInformation

Prostaglandin E_1 EIA

Product Number **CS0210**
Storage Temperature –20 °C

Technical Bulletin

Product Description
Synonym: PGE_1



PGE_1

Prostaglandin E_1 EIA is a four-hour competitive enzyme immunoassay for the quantitative determination of Prostaglandin E_1 (PGE_1) concentrations in cell culture supernatants, saliva, urine, serum and plasma. PGE_1 present in the samples or standards competes with the fixed amount of PGE_1 conjugated to alkaline phosphatase for the limited number of binding sites on the anti-sheep PGE_1 antibody. During the first incubation, the antigen-antibody complex binds to the anti-sheep 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_1 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_1 (PGE_1) is synthesized from DGLA, dihomolinolenic acid.[3] PGE_1 has been shown to have a number of biological actions including vasodilation, proliferation of vascular smooth muscle cells, and platelet aggregation. It also has been shown to have insulin-like actions. The effects of PGE_1 are induced by receptor-mediated elevation of cAMP. It is the major prostaglandin in semen.[4-8]

Reagents

- **Prostaglandin E_1 (PGE_1) Standard, 1 vial, Product No. P 1996** – 0.5 mL (50,000 pg/mL) in a buffer with preservative.
- **Anti-Sheep IgG-coated 96 well plate, 1 plate, Product No. I 6408**
- **Assay Buffer, 30 mL, Product No. A 4228 –** contains sodium azide. Ready to use.
- **Anti-PGE_1, 5 mL, Product No. P 2121–**from sheep, contains sodium azide, yellow dye. Ready to use.
- **Prostaglandin E_1 (PGE_1)-Alkaline Phosphatase Conjugate, 5 mL, Product No. P 2246-** contains sodium azide, blue dye. Ready to use.
- **p-Nitrophenylphosphate (pNpp) Substrate, 20 mL, Product No. N 6911 -** Ready to use.
- **Wash Buffer Concentrate, 10X, 30 mL, Product No. W 3139 –** TRIS buffered saline with detergents and sodium azide.
- **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 plates 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: indomethacin (Product No. I 8280) or meclofenamic acid (Prod. No. M4531).

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 sheep 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 (Product No. I 8280), or meclofenamic acid (Prod. No. M4531).
- 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 media for blanks, controls and standard dilutions.
- Samples, which normally have low levels of PGE_1, (below assay sensitivity) require extraction.
- Sera and plasma require a 20-fold dilution. As example: take 15 μL sample and add 285 μL 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.

Materials Required for Extraction

- 2 N HCl
- Ethanol
- Hexane
- Ethyl acetate
- 200 mg C18 Reverse Phase Column
- High specific activity tritiated PGE_1 (for determination of extraction efficiency).

Protocol for Sample Extraction

1. Acidify sample to pH 3.5 with 2N HCl. (~ 50 μL HCl per 1 mL of plasma).
2. Equilibrate at 4°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 media.
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.

Please refer to citations 9 and10 for details of extraction protocols.

Reagent Preparation

PGE_1 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 Media mL	Standard from tube #: -mL	Final Standard Concentration pg/mL
0	Standard vial 50,000 pg/mL		
1	0.9 mL	0.1 mL (0)	5,000
2	0.75 mL	0.25 mL (1)	1,250
3	0.75 mL	0.25 mL (2)	312
4	0.75 mL	0.25 mL (3)	78.1
5	0.75 mL	0.25 mL (4)	19.5
6	0.75 mL	0.25 mL (5)	4.9

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

PGE_1 -Alkaline phosphatase conjugate

1. The activity of the PGE_1 conjugate is dependent on the presence of Mg^{2+} and Zn^{2+} ions.
2. The activity of PGE_1 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 10 mL wash concentrate with 90 mL deionized water.
5. Label as **Working Wash buffer**
6. Diluted buffer may be stored at room temperature for up to 3 months or until kit expiration date.

Storage/Stability

- The kit is shipped on dry ice.
- Store unopened kit at <–20 °C until use. Do not store past 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 ºC) 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.
- 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 components and reagents 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.
- Do not use reagents after the kit expiration date.
- Standards and samples can be made up in either glass or plastic tubes.
- 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 at the end of this bulletin 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 150 μL of Assay Buffer to the NSB wells
c. Add 100 μL of Assay Buffer to the zero standard (B_0) wells.
d. Add 100 μL of standards #1-6 to the appropriate wells.
e. Add 100 μL of samples to the appropriate wells
f. Add 50 μL of PGE_1-alkaline phosphatase conjugate to all wells (except the TA and SB).
g. Add 50 μL of Anti-PGE_1 to all wells (except NSB, TA and SB wells).
h. Cover with plate cover and incubate 2 hours at room temperature on orbital shaker set at 500 rpm.
i. All wells should be green, except NSB wells, which are blue.
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_1 conjugate 1:10 dilution to the TA wells.
b. Add 200 μL of pNpp substrate to all wells. Cover.
c. Incubate 2 hours at 37 °C 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.

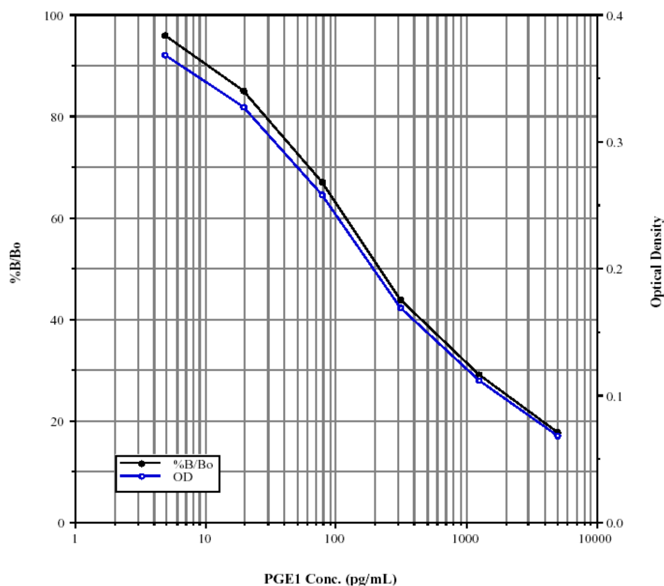
Results

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 (nm) 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_oOD and multiplying by 100.
5. Calculate the concentration of PGE_1 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

pg/mL	Average OD nm	PGE_1 pg/mL	% B/B0
NSB	0.063	--	--
		--	--
O (B_0)	0.427	0.364	100
4.9	0.424	0.361	99
19.5	0.378	0.315	86.4
78.1	0.313	0.250	68.5
312	0. 241.	0.178	48.8
1,250	0.163	0.100	27.5
5,000	0.126	0.063	17.3



Typical Standard Curve for PGE_1 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_1 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

Substrate Blank (O.D.)	= 0.112
TA (TA x 10)	= 2.2
% NSB (NSB/TA x 100)	= 0.05%
% Bo (Bo/TA x 100)	= 16.6%
Quality of Fit	= 0.999

Performance Characteristics

Sensitivity

Sensitivity was determined by subtracting two standard deviations from the mean optical density value of sixteen zero standard (B_0) replicates and calculating the corresponding concentration. The sensitivity of the PGE1 assay is typically less than 6.06 pg/mL.

Linearity

To assess the linearity of the assay, Assay Buffer spiked with PGE_1 was assayed using serial 2-fold dilutions.

Dilution	Observed pg/mL	Expected pg/mL	% Observed: Expected
Neat	2136	--	
1:2	1059	1068	99
1:4	552	534	103
1:8	231	267	87
1:16	144	134	107
1:32	76	67	113
1:64	28	33	85

Precision

Intra-assay precision was determined by taking samples containing low, medium and high concentrations of PGE_1 and running these samples 16 times in the same assay.
Inter-assay precision was determined by measuring three samples with low, medium and high concentrations of PGE_1 in 8 different assays.

	PGE_1 pg/mL	Intra Assay %CV	Inter Assay %CV
Low	53.3	4.5	
Medium	246	9.5	
High	1103	13.7	
Low	49.2		9.3
Medium	214		10.9
High	737		6.2

Recovery

The recovery of PGE_1 spiked into samples in various matrices was evaluated.

Sample	**% Recovery**
Tissue Cultures	92.0
Human Saliva	107
Human Urine	110
Human Serum	87
Human Plasma	108

Cross reactivity

The cross reactivities for a number of related compounds were determined by dissolving the cross reactant (purity checked by N.M.R. and other analytical methods) in Assay Buffer at concentrations from 0.4 to 40,000 pg/mL. These samples were measured in the PGE_1 assay and the PGE_1 concentration at 50% B/Bo 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	6.50%
PGE^3	2.22%
13,14-dihydro-PGE_1	1.50%
PGE_0	1.45%
15-keto-PGE_1	1.15%
13,14-dihydro-15-keto-PGE_1	0.19%
$PGF_{1\alpha}$	0.14%
$PGF_{2\alpha}$	0.04%
6-keto-$PGF_{1\alpha}$	<0.1%
6-trans-PGE_1	<0.1%
PGA_2	<0.1%
PGD_2	<0.1%
13,14-dihydro-15-keto-PGF_2	<0.1%
Thromboxane B2	<0.1%

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PGE_1 Assay Flow Chart (CS0210)

Well ID	Blank A1, B1	TA C1, D1	NSB E1, F1	B_0 G1, H1	Standards A2-C3	Samples D3-H12
Assay Buffer			150 μL	100 μL		
Standard and/or Sample					100 μL	100 μL
Conjugate			50 μL	50 μL	50 μL	50 μL
Anti-PGE_1 Antibody				50 μL	50 μL	50 μL

Incubate 2 hours @ RT with shaking
Wash 3X, blot dry

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

Incubate 2 hours @ 37 ºC 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 Standard Diluent: 450 μL Standard Diluent + 50μL conjugate

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