Prostaglandin E2 (PGE2) ELISA kit

Catalog #: EIA-PGE2

User Manual
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Please read the entire manual carefully before starting your experiment

I. Introduction

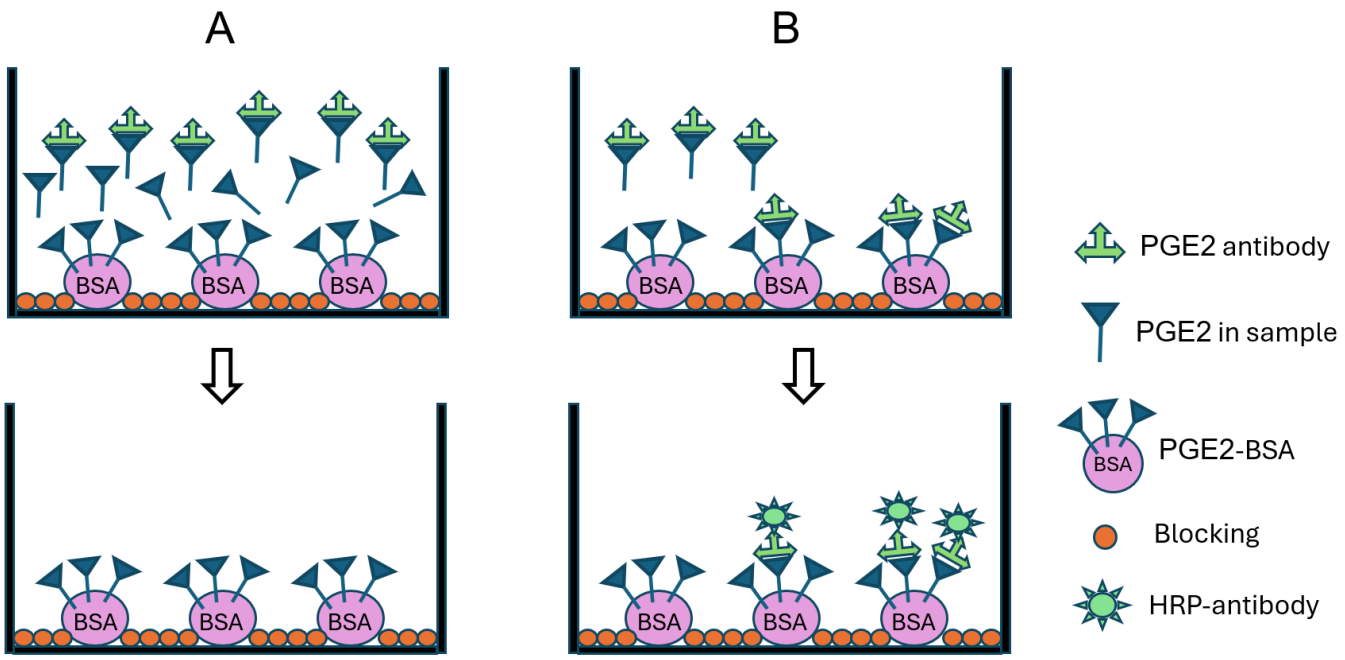
Prostaglandin E2 (PGE2), also known as dinoprostone, has important effects in labor by inducing softening of the cervix and causing uterine contraction, and also stimulating osteoblasts to release factors that stimulate bone resorption by osteoclasts. It is important in the structure and function of the ductus arteriosus in fetuses and newborns. PGE2 also has a variety of functions within the Central Nervous System and Peripheral Nervous System. It contributes to inflammation when bound to EP2 receptors. PGE2 also plays a significant role in vascular smooth muscle tone regulation. Within the kidney, the function of PGE2 is to maintain renal blood flow and glomerular filtration rate (GFR) through localized vasodilation.

The RayBio® PGE2 ELISA Kit is a competitive enzyme immunoassay developed for rapid detection and quantitation of PGE2 in serum and plasma samples collected from various species including humans, rats or mice. The quantity of PGE2 is determined by a known standard PGE2 curve. This kit has high sensitivity with LOD of 0.1386 ng/mL. The kit performance was also verified through linearity, stability, precision, and spike & recovery testing. Each kit provides sufficient reagents to perform up to 96 assays, including standards and unknown samples.

II. General Description

The RayBio® PGE2 ELISA Kit is an *in vitro* quantitative assay for detecting PGE2 based on the competitive enzyme immunoassay principle. In this assay, the samples and standards are added to a microplate pre-coated with PGE2. In the plate wells, the endogenous PGE2 in the samples or the standards compete with the pre-coated PGE2 for binding to the anti-PGE2 antibody. After a wash step, any bound PGE2 antibody then interacts with horseradish peroxidase (HRP)-secondary antibody, which catalyzes a color development reaction. The intensity of the colorimetric signal is directly proportional to the amount of bound PGE2 antibody and inversely proportional to the amount of endogenous PGE2 in the standard or samples. A standard curve of known concentration of PGE2 can be established and the concentration of PGE2 in the samples can be calculated accordingly.

III. How It Works



A. High level of endogenous PGE2 in sample. Anti-PGE2 is bound by excess free PGE2 and washed off the plate. **B.** Low to moderate level of endogenous PGE2 in sample. Anti-PGE2 binds to coated PGE2 due to low competition and is then detected by HRP-conjugated secondary antibody.

IV. Storage

The entire kit may be stored at -20°C to -80°C for up to 6 months from the date of shipment. For extended storage, it is recommended to store it at -80°C. **Avoid repeated freeze-thaw cycles.** For prepared reagent storage, see the table below.

V. Reagents

Component	**Size / Description**	**Storage / Stability After Preparation**
PGE2 Microplate	96 wells (12 strips x 8 wells) coated with PGE2	1 month at 4°C*
Assay Diluent B (5X)	15 mL concentrated buffer. Diluent for standards, samples, and antibodies	1 month at 4°C
Standard PGE2	2 vials. 1 vial is enough to run three times	1 week at 4°C
Positive Control	2 vials of Lyophilized Positive Control. 1 vial is enough to run three times	1 month at 4°C
Anti-PGE2 Antibody (1000X)	10 µL concentrated anti-PGE2 Antibody	1 month at 20°C
HRP-conjugated secondary antibody (5000X)	5 µL concentrated antibody	Do not store and reuse
Wash Buffer (20X)	25 mL of 20X concentrated solution	1 month at 4°C
TMB One-Step Substrate Reagent	12 mL of 3,3,5,5'-tetramethylbenzidine (TMB) in buffer solution	1 month at 4°C
Stop Solution	8 mL of 0.2 M sulfuric acid	12 months at 4°C

*Return unused wells to the pouch containing desiccant pack, reseal along entire edge.

VI. Additional Materials Required

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Precision pipettes to deliver 2 µL to 1 mL volumes.
3. Adjustable 1-25 mL pipettes for reagent preparation.
4. 100 mL and 1-liter graduated cylinders.
5. Distilled or deionized water.
6. Tubes to prepare standard or sample dilutions.
7. Orbital shaker.
8. GraphPad Prism or SigmaPlot software.

VII. Reagent Preparation

Keep kit reagents on ice during reagent preparation steps.

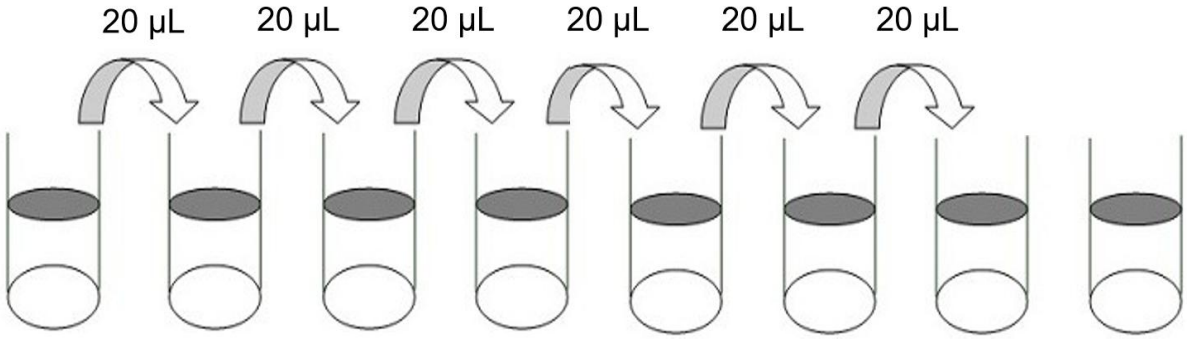
A. Preparation of Plate and Anti-PGE2 Antibody

1. Equilibrate the plate to room temperature before opening the sealed pouch.
2. Label removable 8-well strips as appropriate for your experiment.
3. 5X Assay Diluent B should be diluted 5-fold with deionized or distilled water.
4. Briefly centrifuge the anti-PGE2 antibody vial and dilute 1000-fold with 1X Assay Diluent B for working solution.

B. Preparation of Standards

5. Label 7 microtubes with the following concentrations: S2 (1,000 pg/mL), S3 (100 pg/mL), S4 (10 pg/mL), S5 (1 pg/mL), S6 (0.1 pg/mL), S7 (0.01 pg/mL) and S8 (0 pg/mL). Pipette 180 µL of 1X Assay Diluent B into S2-S8.
6. Briefly centrifuge the vial of PGE2 Standard. Reconstitute with 200 µL of 1X Assay Diluent B. Mix thoroughly. This solution serves as S1.
7. To make the S2 (1,000 pg/mL) standard, pipette 20 µL of the S1 standard into the tube labeled S2 containing 180 µL of 1X Assay Diluent B. Mix thoroughly.

8. Repeat this step with each successive concentration, preparing a dilution series as shown in the illustration below. Each time, add 20 µL of the prior concentration until S7 is reached. Mix each tube thoroughly before the next transfer. 1X Assay Diluent B serves as the blank (S8, 0 pg/mL).



	S1	S2	S3	S4	S5	S6	S7	S8
Diluent Volume	200 µL	180 µL	180 µL	180 µL	180 µL	180 µL	180 µL	180 µL
Conc.	10,000 pg/mL	1,000 pg/mL	100 pg/mL	10 pg/mL	1 pg/mL	0.1 pg/mL	0.01 pg/mL	0 pg/mL

C. Positive Control Preparation

9. Briefly centrifuge the Positive Control vial and reconstitute with 200 µL of 1X Assay Diluent B.

   Positive Control is a mouse serum sample that serves to verify that the kit components are functional. The resulting OD is not used to calculate final concentrations of samples. If no positive competition is observed, please contact RayBiotech Technical Support. Positive Control may be diluted further if desired.

D. Sample Preparation

Serum and plasma samples from humans, mice and rat can be detected. Use immediately or aliquot and store at -20ºC until use. Avoid repeated freeze-thaws. **Recommended Dilution: 2-4-fold for serum and plasma with 1X Assay Diluent B.**

I. Serum: Avoid hemolyzed and lipemic blood samples. Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 5000 x g for 10 minutes at room temperature. Remove the yellow

serum supernatant without disturbing the white buffy layer.

II. Plasma: Avoid hemolyzed and lipemic blood samples. Collect blood with EDTA, heparin or citrate and centrifuge at 5000 g for 10 minutes at room temperature. Remove the plasma layer and avoid disturbing the white buffy layer.

If you have any questions regarding the recommended dilutions, you may contact technical support at techsupport@raybiotech.com

E. Preparation of Wash Buffer and HRP

11. If the Wash Buffer contains visible crystals, warm them to room temperature and mix gently until dissolved.
12. Dilute 20 mL of 20X Wash Buffer Concentrate into deionized or distilled water to yield 400 mL of 1X Wash Buffer.
13. Briefly centrifuge the HRP-Secondary Antibody vial before use. Dilute the HRP-Secondary Antibody concentrate 5000-fold with 1X Assay Diluent B.

VIII. Assay Procedure

1. Remove the appropriate number of microtiter wells of PGE2 coated microplate from foil pouch and place them into the well holder. Return any unused wells to the foil pouch with desiccant pack, reseal along entire edge, and store at -20ºC.

2. Keep kit reagents on ice during reagent preparation steps. It is recommended that all standards and samples be run at least in duplicate.

3. Add 50 µL of each standard (S1-S8), Positive Control and sample to appropriate wells. Incubate at room temperature for 10 minutes on an orbital shaker.

4. Add 50 µL of the diluted Anti-PGE2 Antibody to each well. Cover wells and incubate at room temperature for 1 hour on an orbital shaker.

5. Discard the solution and wash wells 5 times with 1X Wash Solution Buffer (200-300 µL each). Washing may be done with a multichannel pipette or an automated plate washer. Complete removal of liquid at each step is essential to good assay performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

6. Add 100 µL of prepared HRP-Secondary antibody solutions (see Reagent Preparation step 13) to each well. Incubate for 1 hour at room temperature with gentle shaking.

7. Discard the solution and wash 5 times as directed in Step 5.

8. Add 100 µL of TMB One-Step Substrate Reagent to each well. Incubate for 2-10 minutes at room temperature in the dark with gentle shaking (1-2 cycles/sec).

9. Add 50 µL of Stop Solution to each well. Read at 450 nm immediately. Results should be read immediately (color will fade over time).

Note: Reliable standard curves are obtained when OD values do not exceed 2.5 units for the blanks (S8).

IX. Assay Procedure Summary

1. Prepare all reagents, samples and standards as instructed.

2. Add 50 µL standards or samples to appropriate well. Incubate for 10 minutes at room temperature.

3. Add 50 µL anti-PGE2 to appropriate well. Incubate 1 hour at room temperature. And wash 5 times.

4. Add 100 µL prepared HRP-Secondary antibody solution. Incubate 1 hour at room temperature. And wash 5 times.

5. Add 100 µL TMB One-Step Substrate Reagent to each well. Incubate 2-10 minutes at room temperature.

6. Add 50 µL Stop Solution to each well. Read at 450 nm immediately.

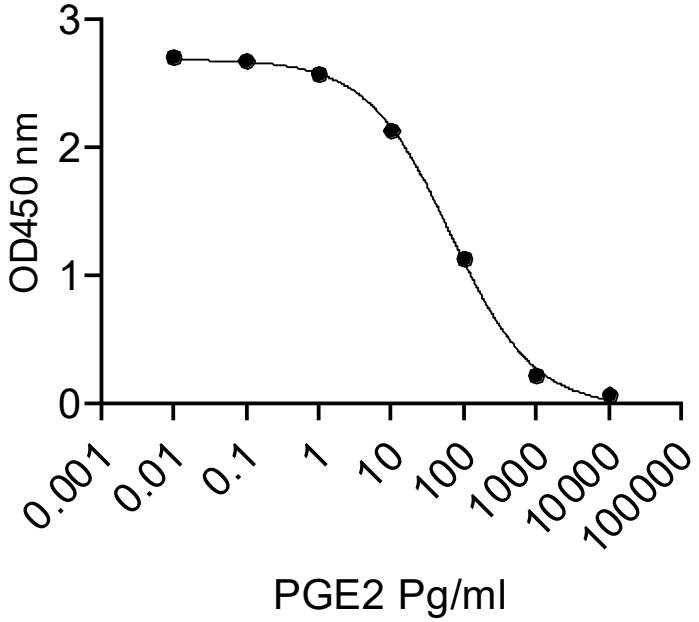
X. Calculation of Results

Calculate the mean absorbance for each set of duplicate stands, controls, and samples and subtract the blank optical density. Plot the standard curve using GraphPad Prism or Sigma Plot software (or other software which can perform four-parameter logistic regression models). If the samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

The measurement range is 0.01 – 10000 pg/mL. Any sample reading lower than the highest standard should be diluted with dilution buffer in higher dilution and re-assayed. Dilution factors need to be taken into consideration in calculating the PGE2 concentration.

A. Typical Data

These standard curves are for demonstration only. A standard curve must be run with each assay.



B. Sensitivity

The limit of detection (LOT, Blank-3SD) is 0.1386 pg/mL.

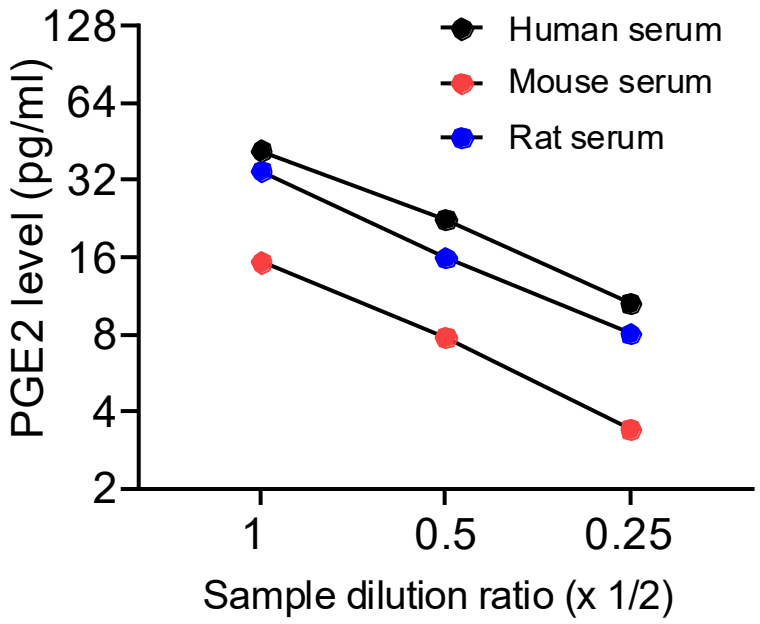
C. Precision

Intra-assay (Within-Run) CV% < 10%.

Inter-assay (Run-to-Run) CV% < 15%.

D. Linearity

Human, mouse and rat serum samples were serially diluted from 2-8-fold (n=3). The recovery ranges from 87.81 to 107.63%.



Sample	Dilution	Observed (pg/mL)	Expected (pg/mL)	Recovery (%)
Human serum	0.5	41.756		
	0.25	22.472	20.878	107.633
	0.125	10.598	11.236	94.326
Mouse serum	0.5	15.317		
	0.25	7.785	7.658	101.649
	0.125	3.418	3.892	87.806
Rat serum	0.5	34.858		
	0.25	15.916	17.429	91.318
	0.125	8.029	7.958	100.898

XIV. Troubleshooting Guide

Problem	Cause	Solution
Poor standard curve	○ Inaccurate pipetting ○ Improper standard dilution	○ Check pipettes. ○ Briefly centrifuge and dissolve the powder thoroughly by gently mixing
Low signal	○ Improper preparation of standard and/or biotinylated antibody ○ Too brief incubation times Inadequate reagent volumes or improper dilution	○ Briefly spin down vials before opening. Dissolve the powder thoroughly. ○ Ensure sufficient incubation time. ○ Check pipettes and ensure correct preparation.
Large CV	○ Inaccurate pipetting Air bubbles in wells	○ Check pipettes. ○ Remove bubbles in wells
High background	○ Plate is insufficiently washed. ○ Contaminated wash buffer	○ Review the manual for proper washing. If using a plate washer, ensure that all ports are unobstructed. ○ Make fresh wash buffer
Low sensitivity	○ Improper storage of the ELISA kit ○ Stop solution	○ Follow storage recommendations in sections IV and V. Keep substrate solution protected from light. ○ Add stop solution to each well before reading plate

RayBio® ELISA Kits

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