RayBiotech
Empowering your proteomics

3607 Parkway Lane, Suite 200
Peachtree Corners, GA 30092
www.RayBiotech.com | info@raybiotech.com
770-729-2992 | 1-888-494-8555
ISO 13485:2016 cGMP

# Prostaglandin E2 (PGE2) ELISA Kit

Catalog #: EIA-PGE2

**User Manual**
Last Revised: July 26, 2024

## Introduction

The RayBio® Prostaglandin E2 Enzyme Immunoassay (EIA) Kit is an in vitro quantitative assay for detecting Prostaglandin E2 based on the competitive enzyme immunoassay principle.

In this assay, a biotinylated Prostaglandin E2 is spiked into the samples and standards. The samples and standards are then added to the plate, where the biotinylated Prostaglandin E2 competes with endogenous (unlabeled) Prostaglandin E2 for binding to the anti-Prostaglandin E2 antibody. After a wash step, any bound biotinylated Prostaglandin E2 then interacts with horseradish peroxidase (HRP)-streptavidin, which catalyzes a color development reaction. The intensity of the colorimetric signal is directly proportional to the amount of captured biotinylated Prostaglandin E2 and inversely proportional to the amount of endogenous Prostaglandin E2 in the standard or samples. A standard curve of known concentration of Prostaglandin E2 can be established and the concentration of Prostaglandin E2 in the samples can be calculated accordingly.

## Storage / Stability

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 kit components table on the next page.

RayBiotech
Empowering your proteomics

3607 Parkway Lane, Suite 200
Peachtree Corners, GA 30092
www.RayBiotech.com | info@raybiotech.com
770-729-2992 | 1-888-494-8555
ISO 13485:2016 cGMP

## Kit Components

| Name | Catalog # | Size / Qty | Description | Storage / Stability After Preparation |
|---|---|---|---|---|
| *Microplate* | EIA-A2 | 96 wells | Microplate coated with secondary antibody. | 1 month at 4°C* |
| *PGE2 Standard* | EIA-PGE2-STD | 2 vials | PGE2 standard. 1 vial is enough to run each standard in duplicate. | First standard: 2-3 days at 4°C<br>Other dilutions: N/A |
| *Anti-PGE2 Polyclonal Antibody* | EIA-PGE2-CAB | 2 vials | Lyophilized anti-PGE2 antibody | 1 month at 4°C |
| *Biotinylated PGE2* | EIA-PGE2-BP | 2 vials | Biotinylated PGE2, 1 vial is enough to assay the whole plate. | 2-3 days at 4°C |
| *Positive Control* | EIA-PC | 1 vial | Positive Control | 2-3 days at 4°C |
| *HRP-Streptavidin Concentrate* | EIA-HRP | 1 vial | 600 µl 20X concentration HRP-conjugated streptavidin. | Do not store and reuse |
| *Wash Buffer* | EL-ITEMB | 25 ml | 20X concentrated wash buffer | 1 month at 4°C |
| *Assay Diluent B* | EL-ITEME | 15 ml | 5X concentrated assay diluent | 1 month at 4°C |
| *Sample Treatment Buffer 1* | EIA-STBI | 11 ml | Serum/plasma sample treatment buffer 1 | N/A |
| *Sample Treatment Buffer 2* | EIA-STBII | 6 ml | Serum/plasma sample treatment buffer 2 | N/A |
| *TMB One-Step Substrate Reagent* | EL-TMB | 12 ml | 3,3,5,5'-tetramethylbenzidine (TMB) in buffer solution | N/A |
| *Stop Solution* | EL-STOP | 8 ml | 0.2 M sulfuric acid | N/A |

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

## Additional Materials Required

- Microplate reader capable of measuring absorbance at 450 nm
- SigmaPlot software (or similar 4-parameter logistic regression software)

3607 Parkway Lane, Suite 200
Peachtree Corners, GA 30092
www.RayBiotech.com | info@raybiotech.com
770-729-2992 | 1-888-494-8555
ISO 13485:2016 cGMP

- Precision pipettes to deliver 2 µl to 1 ml volumes
- Adjustable 1-25 ml pipettes for reagent preparation
- 100 ml and 1 liter graduated cylinders
- Absorbent paper
- Tubes to prepare standard or sample dilutions
- Orbital shaker
- Distilled or deionized water
- Aluminum foil
- Plastic wrap

## Sample Pre-Treatment

**Note: For serum and plasma samples only**. Sample pretreatment removes potentially interfering proteins and protein-bound PGE2.

1. Add 200 µl of sample and 50 µl sample treatment buffer I to a microcentrifuge tube. The tubes will have a precipitate. Mix well.
2. Incubate for 15 minutes at room temperature.
3. Centrifuge at ≥ 12,000 x g for 4 minutes.
4. Carefully remove and retain the supernatant. The supernatant may be cloudy until the addition of treatment buffer II.
5. Add 37.5 µl of sample treatment buffer II to 125 µl of the retained supernatant. Mix well.
6. The concentration read off the standard curve must be multiplied by the dilution factor, 1.6.

## Reagent Preparation

**Note:** Keep kit reagents on ice during reagent preparation steps.

### A. Preparation of Microplate and Anti-PGE2 Antibody

1. Equilibrate plate to room temperature before opening the sealed pouch.
2. Label removable 8-well strips as appropriate for your experiment.
3. Assay Diluent B should be diluted 5-fold with deionized or distilled water.
4. Briefly centrifuge the anti-PGE2 antibody vial. Then add 55 µl of 1X Assay Diluent B to prepare the antibody concentrate. Pipette up and down to mix gently.

3607 Parkway Lane, Suite 200
Peachtree Corners, GA 30092
www.RayBiotech.com | info@raybiotech.com
770-729-2992 | 1-888-494-8555
ISO 13485:2016 cGMP

5. The antibody concentrate should then be diluted 100-fold with 1X Assay Diluent B. This is your anti-PGE2 antibody working solution, which will be used in step 2 of assay procedure.

## B. Preparation of Biotinylated PGE2 Peptide

6. Briefly centrifuge the vial of Biotinylated PGE2 before use.

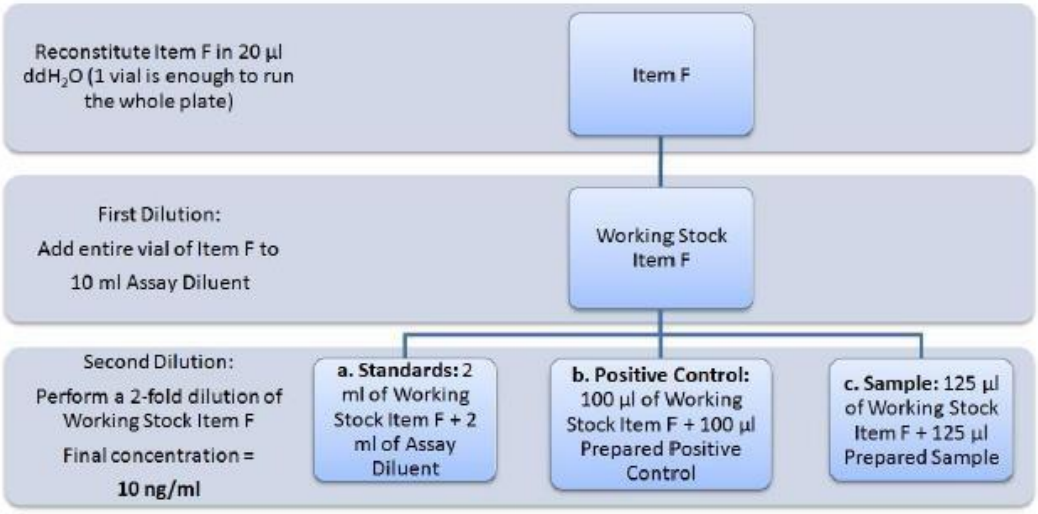
7. See the image below for proper preparation of Biotinylated PGE2. Transfer the entire contents of biotinylated peptide vial into a tube containing 10 ml of 1X Assay Diluent B. This is your working stock. Pipette up and down to mix gently. *The final concentration of biotinylated PGE2 will be 20 ng/ml.*

    a. Second Dilution of Biotinylated PGE2 Peptide for standards: Add 2 ml of working stock to 2 ml of 1X Assay Diluent B. The final concentration of biotinylated PGE2 will be **10 ng/ml.**

    b. Second Dilution of Biotinylated PGE2 Peptide for Positive Control: Add 100 µl of working stock biotinylated peptide to 100 µl of prepared Positive Control. (See section D for Positive Control preparation) The final concentration of biotinylated PGE2 will be **10 ng/ml.**

    c. Second Dilution of Biotinylated PGE2 Peptide for samples: Add 125 µl of working stock biotinylated peptide to 125 µl of prepared sample (See section E for sample preparation). This is a 2-fold dilution of your sample. The final concentration of biotinylated PGE2 will be **10 ng/ml.**

"Item F" = Biotinylated PGE2 Peptide



3607 Parkway Lane, Suite 200
Peachtree Corners, GA 30092
www.RayBiotech.com | info@raybiotech.com
770-729-2992 | 1-888-494-8555
ISO 13485:2016 cGMP

## C. Preparation of Standards

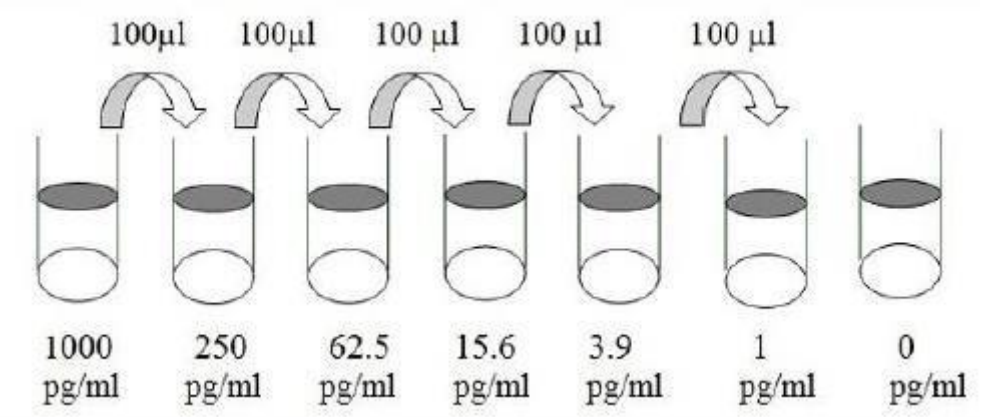
8. Label 5 microtubes with the following concentrations: 250 pg/ml, 62.5 pg/ml, 15.625 pg/ml, 3.9 pg/ml, 1 pg/ml, and 0 pg/ml. Pipette 300 µl of biotinylated PGE2 peptide working solution (prepared in step 7 a) into each tube.

*It is very important to make sure the concentration of biotinylated PGE2 is 10 ng/ml in all standards.*

9. Briefly centrifuge the vial of PGE2 Standard. Pipette 792 µl of 10 ng/ml biotinylated PGE2 working solution (prepared in step 7 a) to the vial. Mix thoroughly. This solution serves as the first standard (1000 pg/ml PGE2 standard, 10 ng/ml biotinylated PGE2).

10. To make the 250 pg/ml standard, pipette 100 µl of the 1000 pg/ml PGE2 standard into the tube labeled 250 pg/ml. Mix thoroughly.

11. Repeat this step with each successive concentration, preparing a dilution series as shown in the illustration below. Each time use 300 µl of biotinylated PGE2 and 100 µl of the prior concentration until the 1 pg/ml is reached. Mix each tube thoroughly before the next transfer.




## D. Positive Control Preparation

12. Briefly centrifuge the Positive Control vial and reconstitute with 125 µl.

13. Refer to step 7b. This is a 2-fold dilution of the positive control. The final concentration of biotinylated PGE2 should still be 10 ng/ml.

3607 Parkway Lane, Suite 200
Peachtree Corners, GA 30092
www.RayBiotech.com | info@raybiotech.com
770-729-2992 | 1-888-494-8555
ISO 13485:2016 cGMP

The Positive Control serves as a system control to verify that the kit components are working. The resulting OD will not be used in any calculations; if no positive competition is observed please contact RayBiotech Technical Support. The Positive Control may be diluted further if desired.

## E. Sample Preparation

14. If you wish to perform a 2-fold dilution of your sample, proceed to step 7c. If you wish to perform a higher dilution of your sample, dilute your sample with the 1x Assay Diluent B before performing step 7c.

    EXAMPLE (to make a 4-fold dilution of sample):

    a. Dilute sample 2-fold (62.5 µl of sample + 62.5 µl of 1X Assay Diluent B).

    b. Perform step 7c (125 µl of working solution Biotinylated PGE2 Peptide + 125 µl of sample prepared above).

    The total volume is 250 µl, enough for duplicate wells on the microplate.

It is very important to make sure the final concentration of the biotinylated PGE2 is **10 ng/ml.**

**Note:** Optimal sample dilution factors should be determined empirically, however you may reference below for recommended dilution factors for pretreated serum:

Human=4X Mouse=2X Rat=2X

If you have any questions regarding the recommended dilutions, you may contact technical support at 770-729-2992 or techsupport@raybiotech.com.

## F. Preparation of Wash Buffer and HRP

15. If 20X Wash Concentrate contains visible crystals, warm to room temperature and mix gently until dissolved.
16. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1X Wash Buffer.
17. Briefly centrifuge the HRP-Streptavidin vial before use.
18. Dilute the HRP-Streptavidin concentrate 20-fold with 1X Assay Diluent B.

# Assay Procedure

3607 Parkway Lane, Suite 200
Peachtree Corners, GA 30092
www.RayBiotech.com | info@raybiotech.com
770-729-2992 | 1-888-494-8555
ISO 13485:2016 cGMP

1. Keep kit reagents on ice during reagent preparation steps. It is recommended that all standards and samples be run at least in duplicate.
2. Add 100 µl of Anti-PGE2 Antibody (See Reagent Preparation step 5) to each well. Incubate for 1.5 hours at room temperature on a horizontal orbital microplate shaker set at 500 rpm.
3. Discard the solution and wash wells 4 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.
4. Add 100 µl of each standard (See Reagent Preparation Section C), Positive control (See Reagent Preparation Section D) and sample (See Reagent Preparation Section E) to appropriate wells. Be sure to include a blank well (Assay Diluent only). Cover well and incubate for 2.5 hours at room temperature on the shaker set at 500 rpm.
5. Discard the solution and wash 4 times as directed in Step 3.
6. Add 100 µl of prepared HRP-Streptavidin solution (See Reagent Preparation step 18) to each well. Incubate for 45 minutes at room temperature on the shaker set at 500 rpm. It is recommended that incubation time should not be shorter or longer than 45 minutes.
7. Discard the solution and wash 4 times as directed in Step 3.
8. Add 100 µl of TMB One-Step Substrate Reagent to each well. Incubate for 30 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.

## Assay Procedure Summary

1. Prepare all reagents, samples and standards as instructed.
2. Add 100 µl anti-PGE2 to each well. Incubate 1.5 hours at room temperature or overnight at 4ºC.
3. Add 100 µl standard or sample to appropriate well. Incubate 2.5 hours at room temperature or overnight at 4ºC.
4. Add 100 µl prepared Streptavidin solution. Incubate 45 minutes at room temperature.
5. Add 100 µl TMB One-Step Substrate Reagent to each well. Incubate 30 minutes at room temperature.

3607 Parkway Lane, Suite 200
Peachtree Corners, GA 30092
www.RayBiotech.com | info@raybiotech.com
770-729-2992 | 1-888-494-8555
ISO 13485:2016 cGMP

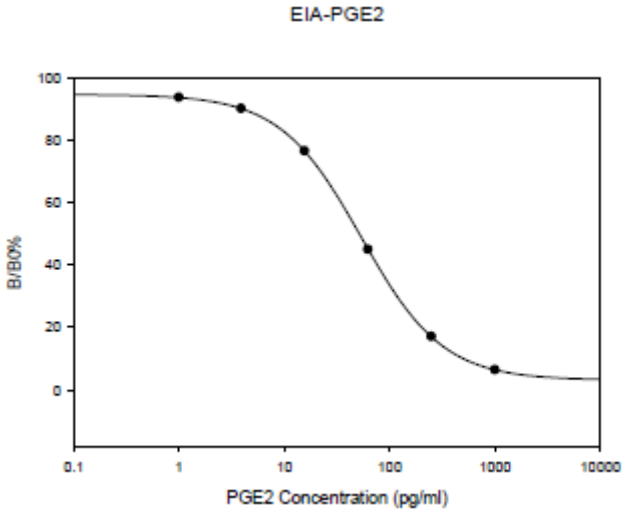
6. Add 50 µl Stop Solution to each well. Read at 450 nm immediately.

## Calculations 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 SigmaPlot software (or other software which can perform four-parameter logistic regression models), with standard concentration on the x-axis and percentage of absorbance (see calculation below) on the y-axis. Draw the best-fit curve through the standard points.

Percentage absorbance = (B-blank OD)/(B0-blank OD)
B = OD of sample or standard
B0 = OD of zero standard (total binding)

### A. Typical Data




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

### B. Sensitivity

The minimum detectable concentration of PGE2 is 12.3 pg/ml.

### C. Standard Curve Range

1 - 1,000 pg/ml

3607 Parkway Lane, Suite 200
Peachtree Corners, GA 30092
www.RayBiotech.com | info@raybiotech.com
770-729-2992 | 1-888-494-8555
ISO 13485:2016 cGMP

## D. Reproducibility

Intra-Assay: CV<10%
Inter-Assay: CV<15%

## E. Assay Diagram

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Blank | Blank | SA1 | SA1 | SA9 | SA9 | SA17 | SA17 | SA25 | SA25 | SA33 | SA33 |
| Total Binding | Total Binding | SA2 | SA2 | SA10 | SA10 | SA18 | SA18 | SA26 | SA26 | SA34 | SA34 |
| Standard1 | Standard1 | SA3 | SA3 | SA11 | SA11 | SA19 | SA19 | SA27 | SA27 | SA35 | SA35 |
| Standard2 | Standard2 | SA4 | SA4 | SA12 | SA12 | SA20 | SA20 | SA28 | SA28 | SA36 | SA36 |
| Standard3 | Standard3 | SA5 | SA5 | SA13 | SA13 | SA21 | SA21 | SA29 | SA29 | SA37 | SA37 |
| Standard4 | Standard4 | SA6 | SA6 | SA14 | SA14 | SA22 | SA22 | SA30 | SA30 | SA38 | SA38 |
| Standard5 | Standard5 | SA7 | SA7 | SA15 | SA15 | SA23 | SA23 | SA31 | SA31 | SA39 | SA39 |
| Pos Control | Pos Control | SA8 | SA8 | SA16 | SA16 | SA24 | SA24 | SA32 | SA32 | SA40 | SA40 |

Key:
Blank = Buffer Only
Total Binding = Biotin-PGE2 only
Standard 1 = 1000 pg/ml
Standard 2 = 250 pg/ml
Standard 3 = 62.5 pg/ml
Standard 4 = 15.625 pg/ml
Standard 5 = 3.9 pg/ml
Standard 6 = 1 pg/ml
Pos Control = Positive Control

## F. Specificity

This EIA kit is designed to detect human, mouse, and rat PGE2.

RayBiotech
Empowering your proteomics

3607 Parkway Lane, Suite 200
Peachtree Corners, GA 30092
www.RayBiotech.com | info@raybiotech.com
770-729-2992 | 1-888-494-8555
ISO 13485:2016 cGMP

# Troubleshooting Guide

| *Problem* | *Cause* | *Solution* |
|---|---|---|
| *Poor standard curve* | • Inaccurate pipetting<br>Improper standard dilution | • Check pipettes<br>• Briefly centrifuge standard and dissolve the powder thoroughly by gently mixing |
| *Low signal* | • Improper preparation of standard and/or biotinylated antibody<br>• Too brief incubation times<br>Inadequate reagent volumes or improper dilution | • Briefly spin down vials before opening. Dissolve the powder thoroughly.<br>• Ensure sufficient incubation time; assay procedure step 2 may be done overnight<br>• Check pipettes and ensure correct preparation |
| *Large CV* | • Inaccurate pipetting Air bubbles in wells | • Check pipettes<br>• Remove bubbles in wells |
| *High background* | • Plate is insufficiently washed<br>• Contaminated wash buffer | • Review the manual for proper wash. If using a plate washer, ensure that all ports are unobstructed<br>• Make fresh wash buffer |
| *Low sensitivity* | • Improper storage of the ELISA kit<br>• Stop solution | • Follow storage recommendations. Keep substrate solution protected from light.<br>• Add stop solution to each well before reading plate |