

PGE₂ ELISA kit

Catalog Number: **PGE1**

Store at -20°C.

FOR RESEARCH USE ONLY



Introduction

This competitive ELISA kit is for determination of PGE₂ (Prostaglandin E₂) levels in biological samples. The specificity of the PGE₂ ELISA was investigated using authentic PGE₂ and fatty acids which, based on their structure, might be anticipated to compete with PGE₂ for binding to antibodies against PGE₂. The anti- PGE₂ antibody showed virtually no cross-reactivity with other tested eicosanoids (see Table 1).

Prostaglandin E₂ (PGE₂) is a naturally occurring cyclooxygenase metabolite of the arachidonic acid cascade that mediates many physiological processes¹. Upon activation of the AA pathway, PGE₂ is synthesized *de novo* and released into the extracellular space where it acts as a regulator of systemic blood pressure due to either vasopressor or vasodepressor effects based on its interaction with four distinct receptors². Prostaglandin H₂ is converted to PGE₂ and its formation is an indicator of COX-1 and COX-2 enzyme activity³. PGE₂ is also involved in tumorigenesis and inflammation through activation of Wnt and PPAR α signaling pathways^{4,5,6} and has been shown to regulate hematopoietic stem and progenitor cell function⁷.

Each kit can be used for triplicate analyses of up to 24 samples contains using a 96 well plate format, and contains a vial of PGE₂ standard, a vial of PGE₂ -conjugated horseradish peroxidase (HRP), and buffers for sample and HRP dilutions, and plate washing.

Storage and Stability

This kit will obtain optimal results if all of the components are stored at the proper temperature prior to use. Items should be stored at the designated temperatures upon receipt of this kit.

All components are stored below -20°C and should not be re-frozen and thawed more than necessary.

Precautions

- Please read all instructions carefully before beginning the assay.
- The reagents in this kit have been tested and formulated to perform optimally. This kit may not perform correctly if any of the reagents are replaced or any of the procedures are modified.
- This kit is intended for research use only and is not to be used as a diagnostic.

Materials Provided

Part Number	Item	Description	Quantity
1	PGE2 ELISA Plate	Solid 96-well plate coated with anti-PGE2 antibody in each well	1
2	PGE2 Standard (2 µL)	Stock standard at a concentration of 1 mg/mL	1
3	PGE2-HRP Conjugates (12 µL)	1000 X concentrated solution	1
4	Sample Dilution Buffer (25 mL)	10 X solution of Tris-buffered saline with preservatives	1
5	HRP Buffer (15 mL)	1 X solution of Tris-buffered saline with preservatives	1
6	Wash Buffer Solution (25 mL)	10 X solution of Tris-buffered saline with detergents and preservatives	1
7	TMB Substrate (24 mL)	A solution of TMB (tetra methyl benzidine)	1

Additional Required Materials (Not Provided)

- Plate reader with a 450 nm filter
- An 8-channel adjustable pipetter and an adjustable pipetter
- Storage bottles
- Costar® cluster tubes (1.2 mL) and microcentrifuge tubes
- Deionized water

Procedural Notes

- Remove all of the reagents required, including the TMB, and allow them to equilibrate to room temperature before proceeding with the assay.
- It is necessary to thoroughly mix the concentrated buffer solutions. A stir bar is contained within each buffer solution.

Sample Preparations

There are different protocols for isolating and purifying PGE2 depending on the medium in which it is in. Listed below are the different protocols. For optimal results follow the appropriate protocol based on the biological sample present.

PGE2 measurement in cell supernatant

1. Extraction using ethyl acetate is not necessary. It is recommended that the cell media sample be diluted 4-fold with 1X sample dilution buffer and 100 uL of sample added directly to the ELISA plate well.
2. When calculating the concentration, consider the dilution factor.
3. Perform the ELISA for PGE2 (according to the instructions of the manufacturer).

PGE2 measurement in tissues

1. Homogenize 1 g of tissue, 4 mL of H₂O, and 0.01 mg TPP.
2. Acidify the homogenate by adding 8 µL of acetic acid to each homogenate.
3. Extract with an equal amount of ethyl acetate, vortex thoroughly, spin down, and collect the organic phase. Repeat this extraction twice more and combine all of the organic phases.

4. Dry the organic phase with argon or nitrogen gas.
5. **Saponification if needed (see below)**
6. Dissolve the dried residue from above step #4 with ethanol or DMF. (Add approximately 20 μ L of ethanol or DMF to reconstitute the dried-up residue.)
7. Dilute further with 1x Sample Dilution Buffer: Add approximately 0.5 mL of 1x Sample Dilution Buffer and centrifuge at 10,000 rpm for five minutes at room temperature. The supernatant will be used for ELISA.
8. Perform the ELISA for PGE2 (according to the instructions of the manufacturer).

Saponification (to cleave fatty acid from glycerol backbone):

1. Dissolve dried fatty acids (obtained from 3X ethyl acetate extractions) in 2 mL of 20% KOH solution (make working solution: 1 mL of 2 M KOH + 4 mL methanol so that the final conc. of KOH = 0.4 N).
2. Vortex and incubate for 1 h at 50°C.
3. Add 1.5 X H₂O to the solution and adjust pH with 20% formic acid to pH~5.
4. Re-extract the solution with ethyl acetate (1 part aqueous solution + 1 part ethyl acetate) and dry.
- 5.

PGE2 measurement in plasma or serum

Below are two protocols for measuring PGE2 in plasma or serum using starting volumes of 1.0 or 1.8 mL. Starting volumes below 1.0 mL can also be used (i.e. for mouse studies) for measuring PGE2 by making appropriate adjustments to volumes and dilutions. Please contact customer service at 313-961-1606 for assistance.

I. Protocol for 1.0 mL plasma or serum

1. Combine 1.0 mL of plasma (adjusted with approximately 12 μ L of acetic acid to pH 4) and 1.0 mL of ethyl acetate. Vortex thoroughly. Centrifuge at 2000 rpm for ten minutes at 22°C. Three phases should result:
 - i. Upper organic phase – ethyl acetate phase (lipoproteins)
 - ii. Interphase – proteins
 - iii. Lower phase – aqueous phase
2. Collect the upper organic phase (a) and set aside.
3. Discard the interphase. Transfer the lower phase with a glass pipette to a new tube, and repeat the ethyl acetate extraction step 2 more times.
4. Evaporation of pooled organic phase: There should be approximately 3 mL of the ethyl acetate phase (a). Dry the pooled organic phase in a Speedvac to get the extracted sediment (b).
5. Saponification (to cleave fatty acid from glycerol backbone): Dissolve the dried residues (b) in 2 mL of 20% KOH solution (for preparation see PGE2 measurement in cells). Vortex thoroughly and incubate for 1 h at 50°C. This will yield an aqueous solution (c).
6. Dilute 2 mL of the aqueous solution (c) with 3 mL of H₂O. Adjust the pH using 20% formic acid (132 μ L) to pH~5.5. Add ethyl acetate (1 part aqueous solution (c) + 1 part ethyl acetate), vortex thoroughly, and centrifuge at 2000 rpm for ten minutes at 22°C. Repeat the procedure twice more using an equal volume of ethyl acetate per sample. Collect the upper phase containing saponified lipids.
7. Dry the pooled ethyl acetate upper phase (d) and dry in a Speedvac, yielding the dried sample-sediment (e). Store the sediment (e) at -20°C. For ELISA assay, dissolve the sediment (e) in 20 μ L of ethanol, then add 380 μ L of 1X Sample Dilution Buffer, pH 7.4. *(Please note that the 10X Sample Dilution Buffer that is supplied with the ELISA kit must be diluted 10-fold).*
8. When calculating the concentration, consider the dilution factor. In this case, 400 μ L total sample volume from 1.0 mL plasma (2.5-fold concentration) you must divide your calculated result by 2.5.
9. Perform the ELISA for PGE2 (according to the instructions of the manufacturer).

II. Protocol for 1.8 mL plasma or serum *(Following this procedure, the user will have approximately 70 μ L of material left-over from step 8. This material can be stored at -20°C and be used for a second measurement following a 5X dilution).*

1. Combine 1.8 mL of plasma (adjusted with approximately 20 μ L of acetic acid to pH 4) and 1.8 mL of ethyl acetate. Vortex thoroughly. Centrifuge at 2000 rpm for ten minutes at 22°C. Three phases should result:

- i. Upper organic phase – ethyl acetate phase (lipoproteins)
 - ii. Interphase – proteins
 - iii. Lower phase – aqueous phase
2. Collect the upper organic phase (a) and set aside.
 3. Discard the interphase. Transfer the lower phase with a glass pipette to a new tube, and repeat the ethyl acetate extraction step 2 more times.
 4. Evaporation of pooled organic phase: There should be approximately 5-6 mL of the ethyl acetate phase (a). Dry the pooled organic phase in a Speedvac to get the extracted sediment (b).
 5. Saponification (to cleave fatty acid from glycerol backbone): Dissolve the dried residues (b) in 2 mL of 20% KOH solution (for preparation see PGE2 measurement in cells). Vortex thoroughly and incubate for 1 h at 50°C. This will yield an aqueous solution (c).
 6. Dilute 2 mL of the aqueous solution (c) with 3 mL of H₂O. Adjust the pH using 20% formic acid (132 μ L) to pH~5.5. Add ethyl acetate (1 part aqueous solution (c) + 1 part ethyl acetate), vortex thoroughly, and centrifuge at 2000 rpm for ten minutes at 22°C. Repeat the procedure twice more using an equal volume of ethyl acetate per sample. Collect the upper phase containing saponified lipids.
 7. Dry the pooled ethyl acetate upper phase (d) and dry in a Speedvac, yielding the dried sample-sediment (e). Store the sediment (e) at -20°C. For ELISA assay, dissolve the sediment (e) in 20 μ L of ethanol, then add 130 μ L of 1X Sample Dilution Buffer.
 8. For the competitive PGE2 ELISA, the above 150 μ L sample needs to be further diluted: Dilute 1:4 (e.g., 80 μ L sample + 320 μ L 1x Sample Dilution Buffer). Check the final pH (should be pH 7.4). When calculating the concentration, consider the dilution factor. In this case, 150 μ L total sample volume from 1.8 mL plasma (12-fold concentration) and then, 80 sample in 400 μ L SDB (5-fold dilution). Since, the samples are concentrated 2.4-fold; to get the actual concentration, you must divide by 2.4.
 9. Perform the ELISA for PGE2 (according to the instructions of the manufacturer).

PGE2 measurement in urine.

1. Extraction using ethyl acetate is not necessary. It is recommended that the urine sample be diluted 4-fold with 1X sample dilution buffer and 100 μ L of sample added directly to the ELISA plate well.
2. When calculating the concentration, consider the dilution factor.

Assay Preparations

The solid 96-well plate and TMB solution are provided ready to use. The preparations of other assay reagents are detailed below.

Wash Buffer: Mix the solution with a stir bar, applying low heat until a clear colorless solution is obtained. Dilute the entire contents of the Wash Buffer Concentrate (25 mL) with 225 mL of deionized water to yield a final volume of 250 mL of 1 X Wash Buffer. This can then be refrigerated for the entire life of the kit.

HRP Conjugate: Dilute 1 vial of the PGE2-HRP conjugate (0.012 mL) with 12.00 mL of 1 X HRP buffer. One vial makes enough conjugate for one plate. The conjugate must be used the same day and should not be stored for later use.

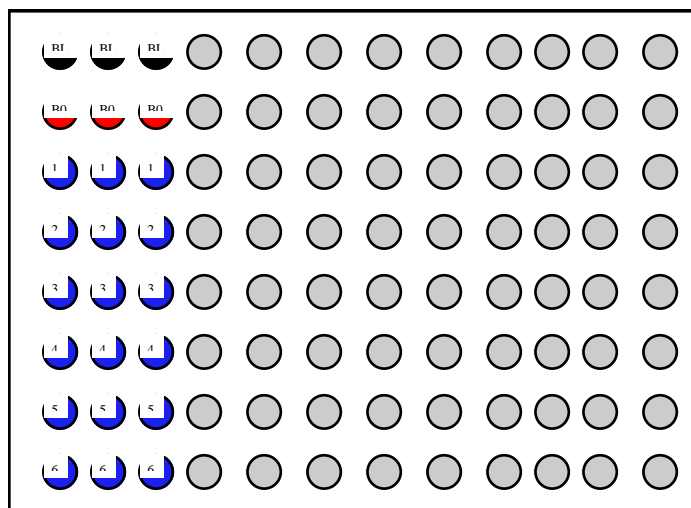
Standards: Label 5 microtubes as Standard 1 through Standard 5. Dilute the entire contents of Sample Dilution Stock buffer (25 mL) with 225 mL deionized water to yield a final volume of 250 mL of 1 X Sample Dilution Buffer. Add 0.9 mL of the Sample Dilution Buffer to the microtubes for Standards 1 to 5. Spin down the enclosed

PGE2 standard vial (2 μ L, filled with inert gas) and add 1.998 mL of Sample Dilution Buffer to obtain 2 mL of solution. Label this Standard 6. Add 0.1 mL of the Standard 6 to the microtube labeled Standard 5 and mix thoroughly. Next, add 0.1 mL of Standard 5 into the microtube labeled Standard 4 and mix thoroughly. Continue to serially dilute the standards using 1:10 dilutions for the remaining standards.

Samples: Samples can be directly diluted into the 1 X Sample Dilution Buffer if it is in solution. For extracted and dried samples, it is recommended to dissolve the dried-up samples with a minimal amount of ethanol of N, N-dimethyl-formamide (DMF, 10 μ L to 20 μ L) and vortex well. Before ELISA assay, add 100 μ L of 1 X Sample Dilution Buffer to make the stock sample solution ready for quantification with ELISA. The stock sample solution can be further diluted to a proper range of concentration for ELISA test.

Performing the Assay

Plate Setup: Each plate must contain a minimum of three blank wells (B_L), three maximum binding wells (B_O), and a six point standard curve (S_1 - S_6). Each sample should be assayed in triplicate. A suggested plate format is shown below:



Standard Dilutions Table = B_L = B_O = S_1 — S_6 =Samples

Standards	Final Concentration (pg/mL)	Add Sample Dilution Buffer (mL)	Serial Dilutions Procedure
No. 6	1,000,000	1.998	2 μ L of stock solution.
No. 5	100,000	0.9	Add 0.1 mL of No. 6
No. 4	10,000	0.9	Add 0.1 mL of No. 5
No. 3	1,000	0.9	Add 0.1 mL of No. 4
No. 2	100	0.9	Add 0.1 mL of No. 3
No. 1	10	0.9	Add 0.1 mL of No. 2

Assay Procedure

Step 1: Load 200 microliters of Sample Dilution Buffer into the blank (B_L) wells and 100 microliters of Sample Dilution Buffer into the maximum binding (B_O) wells.

Step 2: Load 100 microliters of each of the standards into the appropriate wells.

Step 3: Load 100 microliters of each of the samples into the appropriate wells.

Step 4: Load 100 microliters of the diluted PGE2-HRP conjugate in the B_O wells, the standard wells, and the sample wells. Do NOT add HRP conjugate into the B_L wells.

Step 5: Incubate the plate at room temperature for two hours.

Step 6: Wash the plate three times with 400 microliters of the diluted Wash Buffer per well.

Step 7: After the last of the three wash cycles pat the plate dry onto some paper toweling.

Step 8: Add 200 microliters of the TMB substrate to all of the wells (including B_L wells).

Step 9: Incubate the plate at room temperature for 15-30 minutes.

Step 10: Add 50 microliters of 2 N sulfuric acid to all of the wells.

Step 11: Read the plate at 450 nm.

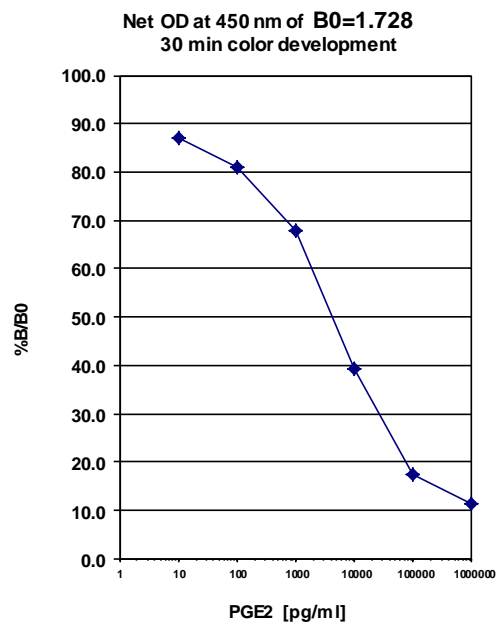
Calculating the Results

Most plate readers provide data reduction software that can be used to plot the standard curve and determine the sample concentrations. If your plate reader does not have this option, then a data reduction program can be used (4 parameter of log-log curve fit).

If you do not have these options, the results can be obtained manually as follows:

1. Average the absorbance readings from the blanks and subtract that value from each well of the plate to obtain the corrected readings. (Note: Some plate readers do this automatically. Consult the user manual of your plate reader.)
2. Average the corrected absorbance readings from the B_O wells. This is your maximum binding.
3. Calculate the %B/B_O for Standard 1 by averaging the corrected absorbance of the two S₁ wells, divide the average by the maximum binding, then multiply by 100. Repeat this formula for the remaining standards.
4. Plot the %B/B_O versus the concentration of PGE2 from the standards using semi-log paper.
5. Calculate the %B/B_O for the samples and determine the concentrations, utilizing the standard curve.
6. Multiply the concentrations obtained for each of the samples by their corresponding dilution factor.

Typical Results



Standard	Concentration	O.D.	%B/B ₀
No. 1	10 pg/mL	1.498	87.1
No. 2	100 pg/mL	1.386	80.9
No. 3	1,000 pg/mL	1.155	68.0
No. 4	10,000 pg/mL	0.643	39.3
No. 5	100,000 pg/mL	0.250	17.4
No. 6	1,000,000 pg/mL	0.144	11.4

The data shown here is an example of typical results obtained using the Detroit R & D PGE2 ELISA kit. These results are only a guideline, and should not be used to determine values from your samples. The user must run their own standard curve every time.

B_L wells = 0.059
 B_0 wells = 1.728

Specificity of anti-14,15-DHET IgG

The specificity of the PGE2 ELISA was investigated using authentic PGE2 and a panel of eiconsanoids.

PGE2	100.00 %
20-hydroxy PGE2	1.16 %
11-deoxy PGE2	<0.01 %
20-HETE	<0.01 %
15(S) HETE	<0.01 %
12 (S) HETE	<0.01 %
8-isoprostane	<0.01 %
11,12-DHET	<0.01 %
14,15-DHET	<0.01 %

Troubleshooting

No color present in standard wells.

- The HRP conjugate was not added. Redo the assay and add the conjugate at the proper step.
- The HRP conjugate was not incubated for the proper time. Redo the assay and incubate for the proper time.

No color in any wells, including the TA wells.

- The TMB substrate was not added. Add substrate.
- The TMB substrate was not incubated for the proper time. Continue incubation until desired color is reached.

The color is faint.

- One or all of the incubation times were cut short. Redo the assay with the proper incubation times.
- The TMB substrate was not warmed up to room temperature. Redo the assay making sure all reagents are at room temperature.
- The lab is too cold. Be sure the lab temperature is between 21-27°C and redo the assay.

The background color is very high.

- The TMB substrate has been contaminated. Redo the assay with a fresh bottle of substrate.

Scattered O.D. obtained from the sample.

- Redo assay using an 8-channel pipetman making sure that 8 channels are equal volume while loading.

References

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Warranty

Detroit R&D, Inc., makes no warranty of any kind expressed, or implied, including, but not limited to the warranties of fitness for a particular purpose and merchantability.



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