PGE_2 ELISA Kit

Store at–20°C

Catalog Number: EHPGE2

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Product description

A competitive immunoassay for the quantitative determination of Prostaglandin E_2 in biological fluids. The assay is based on the competition between PGE_2 in the standard or sample and Alkaline Phosphatase conjugated PGE_2 (PGE_2-AP) for a limited amount of PGE_2 monoclonal antibody bound to an Anti-Mouse IgG precoated 96-well plate. As the concentration of PGE_2 in the sample increases, the amount of PGE_2-AP captured by the coating antibody decreases. Thus, there is an inverse relationship between optical density (OD) and the amount of analyte in the sample.

Contents and storage

Upon receipt, store the kit at ≤ –20°C. Avoid repeated freeze-thaw cycles.

Description	Size
Anti-Mouse IgG Precoated 96-well Strip Plate	1 plate
Reagent Diluent	30 mL
PGE_2 Antibody	6 mL
PGE_2-AP Conjugate	6 mL
PGE_2 Standard (50,000 pg/mL PGE_2)	0.5 mL
20X Wash Buffer	30 mL
Substrate Solution	20 mL
Stop Solution	5 mL
Plate Sealer	1 each

Additional required materials

- Deionized or distilled water
- Precision pipettes (for volumes between 5 µL and 1,000 µL)
- Repeater pipettes (for dispensing 50 µL and 200 µL)
- Disposable beaker for diluting buffer concentrates
- Graduated cylinders
- 12 × 75 mm glass tubes
- Microplate shaker
- Absorbent paper for blotting
- Microplate reader capable of reading at 405 nm, preferably with correction between 570 and 590 nm.

General guidelines

- Do not mix components from different kit lots or use reagents beyond the kit expiration date.
- Pre-rinse the pipette tip with the reagent, use fresh pipette tips for each sample, standard or reagent.
- Care must be taken to minimize contamination by endogenous alkaline phosphatase (AP). Contaminating AP activity, especially in the substrate solution, may lead to high blanks.
- Care should be taken not to touch pipette tips and other items that are used in the assay with bare hands.
- Prior to addition of substrate, ensure that there is no residual wash buffer in the wells. Any remaining wash buffer may cause variation in assay results.

Assay compatibility

- The PGE_2 ELISA is compatible with PGE_2 samples in a wide range of matrices after dilution in Reagent Diluent.
- The assay is also compatible with samples in the majority of tissue culture media, including those containing fetal bovine serum provided the standard is also diluted into the tissue culture media and not Reagent Diluent.
- Samples containing mouse IgG may interfere with the assay.

Prepare 1X Wash Buffer

1. Allow the 20X Wash Buffer to reach room temperature and mix to redissolve any precipitated salts.
2. Dilute 5 mL of 20X Wash Buffer with 95 mL of deionized or distilled water. Label as 1X Wash Buffer.

   The diluted buffer is stable for up to 3 months at room temperature.

Sample preparation guidelines

- Add 10 µg/mL prostaglandin synthetase inhibitors (e.g., indomethacin or meclofenamic acid) to tissue homogenate, urine and plasma samples.
- Perform extraction procedure on samples with very low levels of PGE_2 (see "Extraction procedure example").
- Because conditions may vary, it is recommended that each investigator determine the optimal dilution to be used for each application.

Sample type	Dilution
Tissue culture media	Neat
Human urine	1:10
Human saliva	1:10
Human serum (male or female)	1:10
Whole blood	1:10

Extraction procedure example

1. Add 2M HCl to acidify plasma, urine, or tissue homogenate to pH 3.5. Use approximately 50 µL of HCL per 1 mL of plasma. Incubate at 2°C to 8°C for 15 minutes. Centrifuge samples for 2 minutes to remove any precipitate.
2. Wash the C18 reverse phase column (200 mg) with 10 mL of ethanol followed by 10 mL of deionized water.
3. Apply the sample under a slight positive pressure to obtain a flow rate of about 0.5 mL/minute. Wash the column with 10 mL of water, followed by 10 mL of 15% ethanol, and finally 10 mL hexane. Elute the sample from the column by addition of 10 mL ethyl acetate.
4. If proceeding immediately to analysis, evaporate solvent in samples under a nitrogen stream. Add at least 250 µL of Reagent Diluent to the dried sample. Vortex well, then incubate for five minutes at room temperature. Repeat twice more.

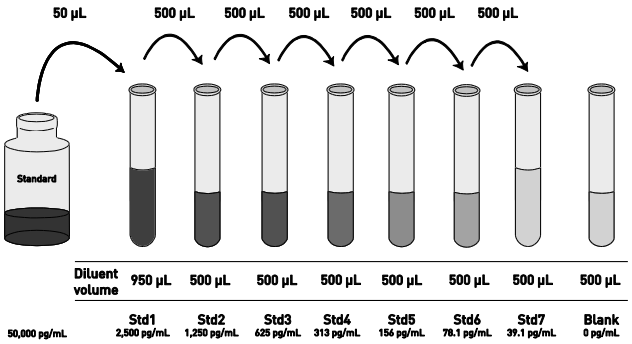
   If performing analysis later, store samples in ethyl acetate at –80°C until the immunoassay is to be run. Evaporate the organic solvent under a stream of nitrogen prior to running the assay and reconstitute as above.

Standard preparation guidelines

- Run standard curves in the appropriate matrix.
- For samples in tissue culture media, dilute standards in the same type of media instead of Reagent Diluent. A small change in binding is associated with running standards and samples in media.
- Use the diluted standards within 60 minutes.

Dilute standards

1. Allow the PGE_2 standard solution to warm to room temperature
2. Label seven 12 × 75mm glass tubes #1 through #7.
3. Add 950 µL of Reagent Diluent to Tube #1.
4. Add 500 µL Reagent Diluent to Tubes #2 to #7.
5. Add 50 µL of the 50,000 pg/mL standard to Tube #1 and vortex thoroughly.
6. Make serial dilutions of the standard as described below in the dilution diagram.



ELISA procedure

Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store at 2 to 8°C for future use. Allow kit components to come to room temperature for at least 30 minutes before use.

Run all standards and samples in duplicate.

1. Add 100 µL of standard diluent (Reagent Diluent or tissue culture media) into the NSB and the B_0 (0 pg/mL Standard) wells.
2. Add 100 µL of Standards #1 through #7 into the appropriate wells.
3. Add 100 µL of the Samples into the appropriate wells.
4. Add 50 µL of Reagent Diluent into the NSB wells.
5. Add 50 µL of the blue PGE_2-AP conjugate into each well, except the Total Activity (TA) and blank wells.
6. Add 50 µL of the yellow PGE_2 antibody into each well, except the Blank, TA and NSB wells.

   NOTE: Every well used should be GREEN in color except the NSB wells which should be BLUE. The Blank and TA wells are empty at this point and have no color.
7. Cover with a plate sealer and incubate the plate at room temperature on a plate shaker for 2 hours at ~500 rpm.
8. Empty the contents of the wells and wash by adding 400 µL of the 1X Wash Buffer to every well. Repeat the wash 2 more times for a total of 3 washes.
9. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
10. Add 5 µL of the blue PGE_2-AP conjugate to the TA wells.
11. Add 200 µL of the Substrate Solution to every well. Cover with a plate sealer and incubate at room temperature for 45 minutes without shaking.
12. Add 50 µL of Stop Solution to every well and read the plate immediately.
13. Blank the plate reader against the Blank wells, read the optical density at 405 nm preferably with correction between 570 and 590 nm. If the plate reader is not able to be blanked against the Blank wells, manually subtract the mean optical density of the Blank wells from all readings.

Performance characteristics

The following parameters for this kit were determined using the guidelines listed in the National Committee for Clinical Laboratory Standards (NCCLS) Evaluation Protocols.

Sensitivity

The minimum detectable dose of PGE_2 is 13.4 pg/mL. This was calculated by determining the average optical density bound for sixteen (16) wells run as B_0, and comparing to the average optical density for sixteen (16) wells run with Standard #7. The detection limit was determined as the concentration of PGE_2 measured at two (2) standard deviations from the zero along the standard curve.

Calculations

Several options are available for the calculation of the concentration of PGE_2 in the samples. It is recommended that the data be analyzed by a 4-parameter logistic curve fitting program.

1. Calculate the average net Optical Density (OD) bound for each standard and sample by subtracting the average NSB OD from the average OD bound:

   Average Net OD = Average Bound OD - Average NSB OD
2. Calculate the binding of each pair of standard wells as a percentage of the maximum binding wells (Bo), using the following formula:

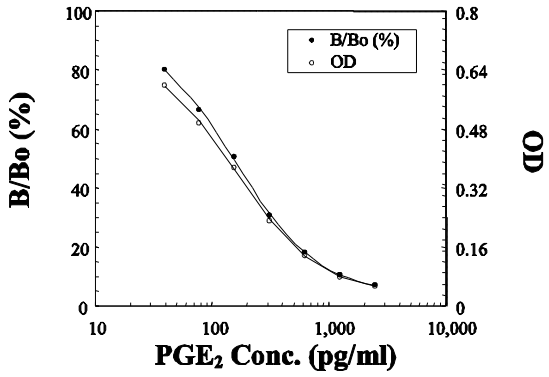
   Percent Bound = (Net OD/Net Bo OD) × 100
3. Plot both the Percent Bound and the Net OD versus Concentration of PGE_2 for the standards. Sample concentrations may be calculated off of Net OD values using the desired curve fitting.

Make sure to multiply sample concentrations calculated off the curve by the dilution factor used during sample preparation to get starting sample concentration.

Samples that read at concentrations outside of the standard curve range will need to be re- analyzed using a different dilution.

Typical standard curve

A typical standard curve is shown below. This curve must not be used to calculate PGE_2 concentrations; a standard curve must be run with every assay.



Linearity

A sample containing 50,000 pg/mL PGE_2 was serially diluted in Reagent Diluent over the range of the assay. Linear regression analysis of samples versus the expected concentration yielded a line with a slope of 1.161 and 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 multiple times (n=24) in the same assay. Inter-assay precision was determined by measuring them in multiple assays (n=8).

The precision numbers listed below represent the percent coefficient of variation for the concentrations of PGE_2 determined in these assays as calculated by a four-parameter logistic curve fitting program.

Intra-assay	PGE_2 (pg/mL)	%CV
Low	116	8.9
Medium	492	5.8
High	2,416	17.5

Inter-assay	PGE_2 (pg/mL)	%CV
Low	111	3.0
Medium	419	5.1
High	1,902	3.9

Cross-reactivity

The cross-reactivities for a number of related compounds were determined by dissolving the cross reactant in Reagent Diluent at concentrations from 500,000 to 39 pg/mL. These samples were then measured in the PGE_2 assay and the measured PGE_2 concentration at 50% B/B_0 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%
$PFG_{2\alpha}$	0.7%
6-keto-$PGF_{1\alpha}$	0.6%
PGA_2; PGB_1	0.1%
13,14-dihydro-15-keto-$PGF_{2\alpha}$; 6,15-keto-13-dihydro-$PGF_{1\alpha}$; Thromboxane B_2; 2-Arachidonoylglycerol; Anandamide; PGD_2; Arachadonic Acid	<0.1%

Sample recovery

PGE_2 concentrations were measured in a variety of different samples including tissue culture media, human saliva, serum, and urine. PGE_2 was spiked into the undiluted samples of these matrices, which were then diluted with the appropriate diluent and then assayed in the kit. The following results were obtained:

Sample	% Recovery	Recommended Dilution*
Tissue Culture Media	104.4	None
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

* See Sample Preparation Guidelines on page 2 for details.

15 January 2016