Prostaglandin E2 Competitive ELISA Kit

Prostaglandin E2 Competitive ELISA Kit is an Enzyme Immunoassay kit for the quantification of Prostaglandin E2 in serum, saliva, urine and cell culture supernatants.

Catalog number: ARG82995

Package: 96 wells

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Prostaglandin E2 (PGE2), also known as dinoprostone, is a naturally occurring prostaglandin with oxytocic properties that is used as a medication. Dinoprostone is used in labor induction, bleeding after delivery, termination of pregnancy, and in newborn babies to keep the ductus arteriosus open. In babies it is used in those with congenital heart defects until surgery can be carried out. It is also used to manage gestational trophoblastic disease. It may be used within the vagina or by injection into a vein.

PGE2 synthesis within the body begins with the activation of arachidonic acid (AA) by the enzyme phospholipase A2. Once activated, AA is oxygenated by cyclooxygenase (COX) enzymes to form prostaglandin endoperoxides. Specifically, prostaglandin G2 (PGG2) is modified by the peroxidase moiety of the COX enzyme to produce prostaglandin H2 (PGH2) which is then converted to PGE2.

Common side effects of PGE2 include nausea, vomiting, diarrhea, fever, and excessive uterine contraction. In babies there may be decreased breathing and low blood pressure. Caution should be taken in people with asthma or glaucoma and it is not recommended in those who have had a prior C-section. It works by binding and activating the prostaglandin E2 receptor which results in the opening and softening of the cervix and dilation of blood vessels. [Provided by Wikipedia: Prostaglandin E2]

PRINCIPLE OF THE ASSAY

This assay employs the competitive quantitative enzyme immunoassay technique. A highly specific antibody (anti-mouse) has been pre-coated onto a microplate. PGE2 containing samples or standards and AP conjugated-PGE2 are given into the wells of the microtiter plate. The PGE2 mouse monoclonal antibody is also add into the well. AP conjugated-PGE2 and free PGE2 compete for the antibody binding sites. After incubation, the wells are washed with Wash Buffer to remove unbound material. A pNpp substrate is added to the wells and color develops in inversely proportion to the amount of free PGE2 present in the samples. The color development is stopped by the addition of STOP solution and the intensity of the color is measured at a wavelength of 405 nm. The concentration of PGE2 in the samples is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Upon received, standard and AP conjugated-PGE2 must be stored at -20°C. Store all other components at 4°C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody Coated microplate	8 X 12 strips	4°C
Standard (50ng/ml)	0.5 ml	-20°C
AP conjugated-PGE2	5 ml (ready to use)	-20°C
PGE2 Antibody	5 ml (ready to use)	4°C
Assay Buffer	27 ml (ready to use)	4°C
20X Wash Buffer	27 ml	4°C
pNpp substrate	20 mL (ready to use)	4°C (protect from light)
STOP solution	5 mL (ready to use)	4°C
Plate sealer	1 ea	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 405 nm (570-590 nm as optional reference wave length)
- Deionized or distilled water
- Mixer or Ultra-Turrax
- Microplate shaker
- Pipettes and pipette tips
- Microtiter plate washer

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Upon received, standard and AP conjugated-PGE2 must be stored at -20°C. Store all other components at 4°C. Use the kit before expiration date.
- Prior to beginning the assay procedure, bring all reagents and required number of strips to room temperature (20-25°C).
- Remove the number of strips required and return unused strips to the pack and reseal.
- Briefly spin down the all vials before use.
- If crystals are observed in the 20X Wash Buffer, warm to 37°C until the crystals are completely dissolved.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Take care not to contaminate the pNpp Substrate.
- Do NOT return leftover pNpp Substrate to bottle. Do NOT contaminate the unused pNpp Substrate.
- Change pipette tips between the addition of different reagent or samples.
- Taping the well strips together with lab tape can be done as an extra precaution to avoid plate strips from coming loose during the procedure.
- Run both standards and samples in at least duplicates.

SAMPLE COLLECTION & STORAGE INFORMATION

The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design.

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 1000 x g for 10 minutes at 4°C.

Saliva- Collect saliva using a collection device (e.g. Salivette), centrifuge 10,000 x g for 2 min at 4°C. Collect saliva and assay immediately or aliquot and store samples at -20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles. The collection device should not have protein binding or filtering features.

Urine - Collect the urine by micturating directly into a sterile container. Remove impurities by centrifugation at 10,000 x g for 1 min. Collect the supernatants and assay immediately or aliquot and store samples at-20°C up to 1 month or -80°C up to 6 months.

Cell culture supernatant: Centrifuge at 300 x g for 10 minutes at 4°C to remove the cell debris.

Note:

1. Do not use haemolytic, icteric or lipaemic specimens.
2. Samples containing sodium azide should not be used in the assay.
3. After collection, sample should be assayed immediately or store at ≤-20°C. Avoid repeated freeze and thaw.
4. For tissue, urine and plasma samples, prostaglandin synthetase inhibitors such as indomethacin or meclofenamic acid at concentrations up to 10 µg/mL should be added to either the tissue homogenate or urine and

plasma samples.

5. If the sample needs to be diluted, dilute the cell culture supernatant with culture medium and dilute other types of samples with Assay buffer.
6. Cell culture supernatants can be used directly without dilution, and other type of samples can be diluted 1:10 before assay. (It is recommended to do pre-test to determine the suitable dilution factor).
7. Samples containing mouse IgG may interfere with the assay.

REAGENT PREPARATION

- **1X Wash Buffer:** Dilute **20X** Wash Buffer into **distilled water** to yield 1X Wash Buffer. (E.g., add 25 mL of 20X Wash Buffer into 475 mL of distilled water to a final volume of 500 mL) The 1X Wash Buffer is stable for up to 3 months at 2-25°C.
- **AP-conjugated PEG2**: Allow the AP-conjugated PEG2 to warm to room temperature before assay. Any unused AP-conjugated PEG2 should be aliquoted and re-frozen at **≤-20°C**.
- **Standards:** Allow the **50 ng/mL** of PGE2 standard stock solution to warm to room temperature. Label seven 12 x 75 mm glass tubes (or plastic tubes) from #1 to #7. Dilute standard by **Assay Buffer** or **Cell Culture Media** (for cell culture supernatants samples). Dilute each Standard as follow;

Standard tube	PGE2 (pg/mL)	Assay Buffer or medium (µL)	Standards (µL)
S1	2500	950	50 (50 ng/ml standard stock)
S2	1250	500	500 of S1
S3	625	500	500 of S2
S4	313	500	500 of S3
S5	156	500	500 of S4
S6	78.1	500	500 of S5
S7	39.1	500	500 of S6
S0 (B0)	0	500	0

Note:

1. Diluted Standards for cell culture supernatant with culture medium and dilute other type of samples with Assay Buffer.
2. Used the diluted standards within 1 hour after preparation.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-25°C) before use (Bring all reagents 30 min before use). Standards and samples should be assayed in duplicates. The recommended reaction well settings and samples are summarized below.

	Blank	NSB	B_0	TA	Standard	sample
Well	1	1	1	1	7 X 2	*N X 2

Note:

N: sample number

Blank: Background absorbance

NSB: Non-Specific Binding (No PGE2 Antibody)

B_0: Maximum Binding

TA: Total Activity (streptavidin-HRP activity)

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it. Store unused wells at 4°C.
2. Add **100 µL** of **standard diluent** (Assay Buffer or Tissue Culture Media) into the NSB and the Bo (0 pg/mL Standard) wells.
3. Add **100 µL** of **each diluted Standard** to the Standard wells. Add **100 µL** of **each sample** to the sample wells.
4. Add **50 µL** of **Assay Buffer** into the NSB wells.
5. Add **50 µL** of **AP conjugated-PGE2** to each well except Blank and TA well.
6. Add **50 µL** of **PGE2 Antibody** into the appropriate wells except Blank, NSB (Non-specific binding) and TA (Total activity) well.

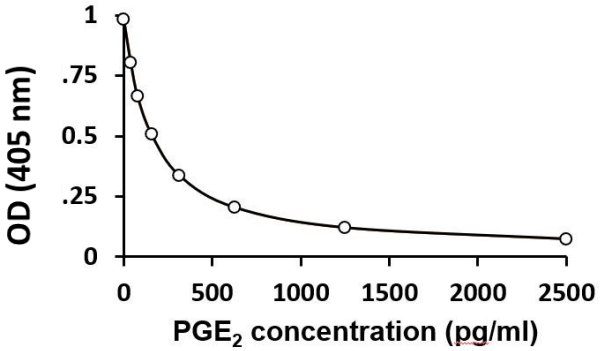
   NOTE: Every well used should be **Green** in color except the NSB wells which

should be **Blue**. The Blank and TA wells are empty at this point and have no color.

7. Cover plate with the plate sealer and incubation for **120 minutes** at **RT** on a microplate shaker (~**500 rpm**).
8. Aspirate each well and wash, repeating the process 2 times for a total **3 washes.** Wash by filling each well with **1× Wash Buffer (400 µL)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
9. Add **5 µL** of the **AP-conjugated PGE2** to the TA wells.
10. Add **200 µL** of the **pNpp Substrate solution** to every well. Incubate at **room temperature for 45 minutes** without shaking.
11. Immediately Add **50 µL** of **Stop Solution** to each well, including the blank wells.
12. 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.

EXAMPLE OF TYPICAL STANDARD VALUES

The following figures demonstrate typical results with the Prostaglandin E2 Competitive ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.



CALCULATION OF RESULTS

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) x 100

3. (OD_{sample} or $OD_{standard}$ / OD_{B0}) X 100% for B/B_0%

4. Use B/B_0% value or Average Net OD of S1 to S7 on the vertical (Y) axis and concentration on the horizontal (X) axis to construct a standard curve.

5. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
6. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)
7. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

QUALITY ASSURANCE

Sensitivity

The sensitivity of Prostaglandin E2 Competitive ELISA kit is 13.4 pg/mL.

Specificity

Substance	Cross Reactivity (%)
PGE1	70
PGE3	16.3
PGF1α	1.4
PGF2α	0.7
6-keto-PGF1α	0.6
PGA2	0.1
PGB1	0.1
PGD2	< 0.1
TXB2	< 0.1
Arachidonic acid	< 0.1

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 10.7% and CV value of inter-assay precision was 4%.

Recovery

101-123%