Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

Prostaglandin E1 (PGE1) ELISA Kit

05/19

(Catalog # E4716-100; 96 assay; Storage at 4ºC)

I. Introduction:

BioVision's PGE1 ELISA kit uses the Competitive-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with PGE1. During the reaction, PGE1 in the sample or standard competes with a fixed amount of PGE1 on the solid phase supporter for sites on the Biotinylated Detection Ab specific to PGE1. Excess conjugate and unbound sample or standard are washed from the plate, and Avidin conjugated to Horseradish Peroxidase (HRP) are added to each microplate well and incubated. Then a TMB substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of stop solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of PGE1 in the samples is then determined by comparing the OD of the samples to the standard curve.

II. Applications:

- in vitro quantitative determination of PGE1 concentrations in serum, plasma and other biological fluids.
- **Detection Range:** 78.13—5000 pg/mL
- Coefficient of variation is < 10%.
- **Specificity:** This kit recognizes PGE1 in samples. No significant cross-reactivity or interference between PGE1 and analogues was observe
- **Sensitivity:** 46.88 pg/mL

III. Sample Type:

- Plasma, Cell and tissue culture supernatants, Serum, Tissue and cell lysates, Other biological fluids

IV. Kit Contents:

Components	E4716-100	Part Number	Storage Temp
Micro ELISA Plate	8 wells ×12 strips	E4716-100-1	-20°C
Standard	2 Vials	E4716-100-2	-20°C
Biotinylated Detection Ab (100x)	120 µl	E4716-100-3	-20°C
HRP Conjugate (100x)	120 µl	E4716-100-4	-20°C
Standard & Sample Diluent	20 ml	E4716-100-5	4°C
Biotinylated Detection Antibody Diluent	14 ml	E4716-100-6	4°C
HRP Conjugate Diluent	14 ml	E4716-100-7	4°C
Wash Buffer (25x)	30 ml	E4716-100-8	4°C
Substrate Reagent	10 ml	E4716-100-9	4°C
Stop Solution	10 ml	E4716-100-10	4°C
Plate Sealer	5	E4716-100-11	

V. User Supplied Reagents and Equipment:

- Microplate reader with 450 nm wavelength filter
- Deionized or distilled water

VI. Storage Conditions and Reagent Preparation:

An unopened kit can be stored at 4°C for 1 month. **If the kit is not used within 1 month, store the items separately according to the recommended conditions once the kit is received.**

Biotinylated Detection Antibody: Calculate the required amount before the experiment (50µL/well). Centrifuge the stock tube before use, dilute the 100× Concentrated Biotinylated Detection Ab to 1×working solution with Biotinylated Detection Ab Diluent Bring all reagents to room temperature (18~25°C) before use. Follow the Microplate reader manual for set-up and preheat it for 15 min before OD measurement.

Wash Buffer: Dilute 30 mL of Concentrated Wash Buffer with 720 mL of deionized or distilled water to prepare 750 mL of Wash Buffer. Note: if crystals have formed, incubate it in a 40°C water bath and mix it gently until the crystals have completely dissolved.

Storage and Handling: An unopened kit can be stored at 4°C for 1 month. If the kit is not used within 1 month, store the items separately according to the recommended conditions once the kit is received.

HRP Conjugate: Calculate the required amount before the experiment (100 µl/well). Dilute the 100xConcentrated HRP Conjugate to 1x with HRP Conjugate Diluent.

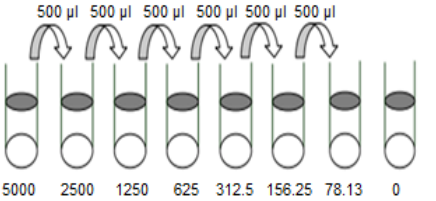
Sample Preparation: Note: Samples should be assayed within 7 days when stored at 4°C, otherwise samples must be divided up and stored at -20°C (≤1 month) or -80°C (≤3 months). Avoid repeated freeze-thaw cycles.

- **Serum:** Allow samples to clot for 2 hours at room temperature or overnight at 4°C before centrifugation for 15 min at 1000xg at 2~8°C. Collect the supernatant to carry out the assay. Blood collection tubes should be disposable and endotoxin free.
- **Plasma:** Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 min at 1000xg at 2~8°C within 30 min of collection. Collect the supernatant to carry out the assay. Hemolysed samples are not suitable for ELISA assay!
- **Cell lysates:** For adherent cells, gently wash the cells with moderate amount of pre-cooled PBS and dissociate the cells using

trypsin. Collect the cell suspension into a centrifuge tube and centrifuge for 5 min at 1000xg. Discard the medium and wash the cells 3 times with pre-cooled PBS. For each 1×10^6 cells, add 150-250 µL of pre-cooled PBS to keep the cells suspended. Repeat the freeze-thaw process several times until the cells are fully lysed. Centrifuge for 10 min at 1500xg at 4°C. Remove cell pellet, collect the supernatant for assay. Avoid repeated freeze-thaw cycles.

- **Tissue homogenates:** Hemolysed blood may affect the results, so the tissues should be minced into small pieces and rinsed in ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then homogenized in PBS (tissue weight (g): PBS (mL) volume=1:9) with a glass homogenizer on ice. To further break down the cells, sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 5 min at 5000xg to get the supernatant Cell culture supernatant or other biological fluids: Centrifuge samples for 20 min at 1000xg at 2~ 8°C. Collect the supernatant for assay.

Standard Preparation:



Centrifuge the standard at 10,000xg for 1 min. Add 1.0 mL of Standard and Sample Diluent, let it stand for 10 min and invert it gently several times. After it dissolves fully, mix it thoroughly with a pipette. This reconstitution produces a working solution of 5000 pg/mL. Then make serial dilutions as needed. The recommended dilution gradient is as follows: 5000, 2500, 1250, 625, 312.5, 156.25, 78.13, 0 pg/mL. Take 7 tubes; add 500 uL of Standard & Sample Diluent to each tube. Pipette 500 uL of the 5000 pg/mL stock solution to the first tube and mix up to produce a 2500 pg/mL working solution. Transfer 500 µl of the solution into the other tube to form 2-fold serial dilutions of the highest standards to make the standard curve within the range of this assay.

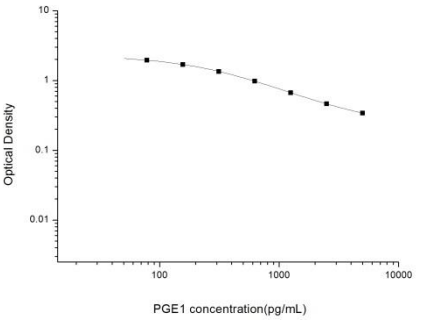
VII. Assay protocol:

Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay. It is recommended that all standards and samples be run at least in duplicate. A standard curve must be run with each assay.

a) Add 50 µl of each **standard or samples** into appropriate wells.
b) Immediately add 50 µl of **Biotinylated Detection Antibody** working solution to each well. Cover the plate with the sealer provided in the kit. Incubate for 45 min at 37°C. Note: solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall and formation of bubbles.
c) Aspirate the solution from each well add 350 µl of **1x wash buffer** to each well. Soak for 1~2 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times. Note: a microplate washer can be used in this step and other wash steps.
d) Add 100 µl of **HRP Conjugate** working solution to each well. Cover with the Plate sealer. Incubate for 30 min at 37°C.
e) Aspirate the solution from each well, repeat the wash process for five times as conducted in step c.
f) Add 90 µl of **Substrate Reagent** to each well. Cover with a new plate sealer. Incubate for about 15 min at 37°C. Protect the plate from light. Note: the reaction time can be shortened or extended according to the actual color change, but not more than 30 min.
g) Add 50 µl of **Stop Solution** to each well. Note: adding the stop solution should be done in the same order as the substrate solution.

Measurement: Determine the optical density (OD value) of each well at once with a microplate reader set to 450 nm.

Calculation: Determine the average of the duplicate readings for each standard and samples. Plot a four-parameter logistic with standard concentration on the x-axis and OD values on the y-axis. If the samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. If the OD of the sample is under the lowest limit of the standard curve, retest the samples with appropriate dilution. The actual concentration is the concentration obtained by calculation multiplied by the dilution factor.



Typical data: As the OD values of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), the operator should establish a standard curve for each test. Typical standard curve and data are provided below for reference only.

Conc. (ng/ml)	5000	2500	1250	625	312.5	156.25	78.13	0
OD	0.342	0.462	0.669	0.977	1.347	1.695	1.952	2.29

VII. Related Products:

Prostaglandin E2 (PGE2) ELISA Kit (E4637)

FOR RESEARCH USE ONLY! Not to be used on humans.