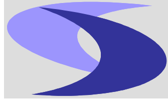

Signosis
Innovative Plate Assay Solutions

Human PAP ELISA Kit (Colorimetric)

Catalog Number EA-9042 **(For Research Use Only)**

Introduction

Prostatic acid phosphatase (PAP) is an enzyme expressed by prostate epithelium and pain-detecting spinal cord neurons. This protein is upregulated in men with prostate cancer, especially if it has metastasized. Furthermore, high levels of PAP may be found in other diseases such as Paget's disease, sickle-cell disease, and multiple myeloma. There has been interest in using PAP as a biomarker for prostate cancer due to its potential role in prognosticating intermediate and high-risk cases.

Principle of the assay

The wells of the plate are coated with capture antibody specific to PAP. In this assay, the test sample initially reacts with the solid phase capture antibody, resulting in PAP being bound to the well. The wells are then washed to remove unbound proteins, and biotin-linked antibodies are added to bind to the immobilized PAP. After washing away the unbound antibodies, Streptavidin-HRP conjugate is added to form a complex with the antibody-bound PAP. After incubation, the wells are washed to remove unbound Streptavidin-HRP conjugate. TMB substrate is then added and forms a blue color when the HRP-linked antibodies are detected. The reaction is then terminated with Stop Solution, which changes the color from blue to yellow. The PAP concentration in each well is directly proportional to its color intensity and can be quantified by measuring its optical density at 450 nm (OD450) in a microplate reader.

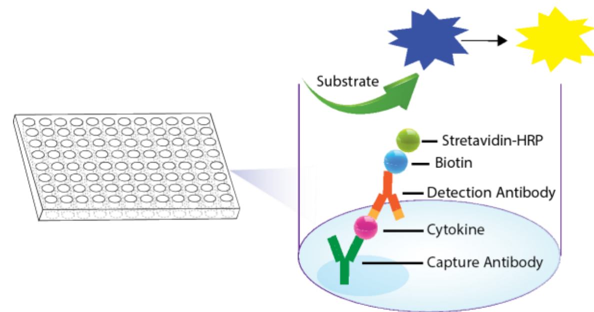

Diagram of Human PAP ELISA

Materials provided with the kit

Component	Qty	Store at
One clear plate coated with antibody against PAP	1	4°C
Biotin-labeled anti-PAP antibody	200 µL	-20°C
Streptavidin-HRP conjugate	50 µL	4°C
1x Diluent buffer	40 mL	4°C
5x Assay wash buffer	40 mL	4°C
Substrate	10 mL	4°C
Stop solution	5 mL	4°C

Material required but not provided

- Microplate reader
- Distilled H2O

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Reagent preparation before starting experiment

- Dilute the 5x Assay wash buffer to 1x buffer
  - 40 ml 5x Assay wash buffer
  - 160 ml ddH2O
- Dilute 50 times of biotin labeled antibody with 1X Diluent buffer.
  (AVOID FREEZE/THAW OF ANTIBODY MIX)
- Dilute 200 times of streptavidin-HRP with 1x Diluent buffer.

Sample preparation before starting experiment

- For **cell culture medium samples**, add 100 ul directly to the well or dilute 2-fold with 1X Diluent buffer.
- For **cell lysate samples**, use cell lysis buffer (Catalog# EA-0001). Follow protocol on Cell Lysate Buffer User Manual on our website.
- For **serum or plasma samples**, we recommend a 1:10 to 1:20 dilution with 1X diluent buffer. When serum-containing conditional media is required, be sure to use serum as control.

Assay procedure

1. Take the plate from the aluminized bag. Seal the unused wells with a film.
2. Prepare 2.5 ml sample and add 100 µl of sample per well to one section and incubate for **2 hours** at room temperature with gentle shaking.
   Optional: If you want to have a blank reading, you can design one well as a blank well by adding diluent buffer instead of your sample.
3. Invert the plate over an appropriate container and expel the contents forcibly. Wash the plate by adding 200 µl of 1x Assay wash buffer. Repeat the washing process two times for a total of three washes. Complete removal of liquid at each wash by firmly tapping the plate against a pile of clean paper towels.
4. Add 100 µl of diluted biotin-labeled antibody to each well and incubate for 1 hour at room temperature with gentle shaking.
5. Repeat the aspiration/wash as in step 3.
6. Add 100 µl of diluted streptavidin-HRP conjugate to each well and incubate for 45 min at room temperature with gentle shaking.
7. Repeat the aspiration/wash as in step 3.
8. Add 100 µl substrate to each well and incubate for 30-40 minutes at least.

   Note: Substrate incubation time may vary due to different antibodies reactivity. Stronger signals (Strong blue color) could be stopped early after 5 minutes. Weaker signals should be incubated for 10-30 minutes.

9. Add 50 µl of Stop solution to each well. The color in the wells should change from blue to yellow.
10. Determine the optical density of each well with a microplate reader at 450 nm within 30 minutes.