



## Product Information

### ANTI-HUMAN PROSTATIC ACID PHOSPHATASE DEVELOPED IN RABBIT

Product Number **P 5664**

#### Product Description

Antiserum is developed in rabbit using human prostatic acid phosphatase (PAP) antigen purified from prostatic fluid as the immunogen.

By immunoelectrophoresis, the antibody shows two lines versus seminal fluid and is negative versus normal human serum. Antibody titer, sensitivity and specificity have been evaluated in a double antibody radioimmunoassay system utilizing an iodinated tracer. The product has also been tested on regularly fixed, paraffin-embedded prostatic tissue using immunoperoxidase staining.

After dilution, the antiserum may be used for detection and quantitation of human prostatic acid phosphatase in plasma or serum by radioimmunoassay, enzyme immunoassay or in immunohistological methods. Estimate of prostatic acid phosphatase activity in serum has served in the diagnosis of the prostatic carcinoma since 1938. However, since enzymatic activity is sensitive to many factors, the enzymatic assay resulted in 26% false positives while radioimmunoassay resulted in only 5%. The immunological reactivity of prostatic acid phosphatase is more consistent than the enzymatic activity. The use of immunoperoxidase stains for the localization of Prostatic Acid Phosphatase is well established. Comparison of multiple human markers in serum of patients with prostatic cancer has been documented.

#### Reagents

The product is provided as undiluted antiserum containing 0.1% sodium azide as a preservative.

#### Precautions and Disclaimer

Due to the sodium azide content a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazards and safe handling practices.

#### Product Profile

1. RIA: Minimum 1:5,000

Determined by second antibody-polyethylene glycol (PEG) RIA. Dilute antibody in 0.01 M phosphate buffered saline containing 0.1% BSA.

2. Immunohistology: Minimum 1:400

Determined by immunoperoxidase staining of formalin-fixed, paraffin-embedded human prostate sections using Mouse Extravidin® Peroxidase Staining Kit (Product Code EXTRA-2).

#### Sensitivity

Sensitivity is defined as the 90% intercept of a B/B<sub>0</sub> standard curve. In the above system the sensitivity has been found to be 200 pg PAP/tube.

#### RIA Affinity Constant

The affinity constant (K<sub>a</sub>) is determined by a Scatchard plot using the described RIA system.

K<sub>a</sub> = 1-10 x 10<sup>10</sup> L/mole.

#### Storage/Stability

Store undiluted antiserum at -20 °C in working aliquots. For continuous use, store at 2-8 °C for up to one month. Repeated freezing and thawing is not recommended.

#### RIA Protocol

##### Reagents

- PBS: 0.01 M phosphate buffered saline, pH 7.4, containing 0.15 M NaCl, 0.1% NaN<sub>3</sub> and 0.1% BSA.
- Anti-prostatic acid phosphatase serum: dilute with buffer according to recommended working dilution.
- Radiolabeled tracer: Prostatic acid phosphatase <sup>125</sup>I with specific activity of 20-80 μCi/μg 200-700 pg/tube in buffer.
- Standards: prepare in the following concentrations: 0.1, 2.5, 5, 10, 25 and 50 ng/ml of human prostatic acid phosphatase isoenzyme no. 2.

- (E) Second Antibody: Goat Anti-Rabbit IgG (whole molecule), (Product No. R 0881).
  - (F) EDTA Solution: 0.1M ethylenediaminetetraacetic acid (EDTA) disodium salt (Product Code ED2SS) in distilled water. Adjust pH to 7.8.
  - (G) EDTA-second antibody mixture reagent: Mix equal volumes of the second antibody solution and the EDTA solution.
  - (H) PEG Solution: 6% polyethylene glycol (PEG), approximate molecular weight 8,000 (Product No. P 2139) in PBS (D).
  - (I) Normal rabbit serum solution: Prepare a solution of 2% normal rabbit serum in PBS
- 5. Peroxidase anti-rabbit IgG (w.m.) Affinity Isolated Antibodies (AIA) adsorbed with human serum proteins (Product No. A 0545).
  - 6. Substrate Chromogen: 3-amino 9-ethyl carbazole (AEC)(Product Code AEC-101).
  - 7. Mayer's hematoxylin.
  - 8. Glycerol gelatin.

#### Procedure

1. In polypropylene test tubes add 0.2 ml standard Prepare a zero control and a blank tube.
2. Add 0.2 ml anti-prostatic acid phosphatase antiserum to all tubes except the blank. To the blank add 0.2 ml buffer.
3. Vortex and incubate 4 hours at room temperature.
4. Add 0.1 ml <sup>125</sup>I-PAP solution to all tubes.
5. Prepare two empty tubes for the total count. Add 0.1 ml <sup>125</sup>I-PAP solution to these.
6. Vortex and incubate all tubes at room temperature overnight.
7. Add 0.2 ml 2% NRS solution into all tubes except total tubes.
8. Add 0.2 ml EDTA-second antibody mixture reagent to all tubes except total tubes.
9. Add 0.5 ml PEG solution.
10. Vortex the tubes.
11. Centrifuge for 15 minutes at 3,000 rpm at 4 °C.
12. Remove supernatant from each tube and determine the amount of radioactivity present in the precipitate.

#### **Immunoperoxidase Staining Protocol**

##### **MATERIALS:**

1. Formalin fixed paraffin embedded 5µm sections of human prostate.
2. 3% Hydrogen peroxidase (in H<sub>2</sub>O).
3. Normal goat serum 1:5 (in PBS).
4. Rabbit anti-prostatic acid phosphatase (Product No. P 5664)

#### METHOD:

1. Deparaffinize and hydrate sections.
2. Block endogenous peroxidase with 3% H<sub>2</sub>O<sub>2</sub> for 5 min.
3. Rinse in PBS.
4. Block non-specific staining by incubation with normal goat serum in PBS 1:5 for 30 min.
5. Rinse in PBS.
6. Apply anti prostatic acid phosphatase (1:100-1:400 dilution in PBS) for 30 min.
7. Rinse in PBS.
8. Apply peroxidase labeled APA to rabbit IgG (1:100 in PBS) for 30 min.
9. Rinse in PBS.
10. Apply AEC for 15 min.
11. Rinse with H<sub>2</sub>O.
12. Counterstain lightly with Mayer's hematoxylin.
13. Rinse with H<sub>2</sub>O.
14. Coverslip with liquid glycerol gelatin.

REMARKS: All steps are carried out at room temperature.

#### **Bibliography**

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