



# PAP

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Enzyme immunoassay for the quantitative determination of PAP (prostatic acid phosphatase) in human serum or plasma

**Only for in-vitro diagnostic use**

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Product Number: DNOV068 (96 Determinations)

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## 1. INTRODUCTION

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Prostatic acid phosphatase (PAP) is an enzyme produced by the prostate. It may be found in increased amounts in men who have prostate cancer or other diseases.

The highest levels of acid phosphatase are found in metastasized prostate cancer. Diseases of the bone, such as Paget's disease or hyperparathyroidism, diseases of blood cells, such as sickle-cell disease or multiple myeloma or lysosomal storage disease, such as Gaucher's disease, will show moderately increased levels.

Certain medications can cause temporary increases or decreases in acid phosphatase levels. Manipulation of the prostate gland through massage, biopsy or rectal exam before a test can increase the level.

## 2. INTENDED USE

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Immunoenzymatic colorimetric method (ELISA) for quantitative determination of PAP in human serum or plasma.

## 3. PRINCIPLE OF THE ASSAY

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The PAP assay is based on simultaneous binding of human PAP to two monoclonal antibodies; one is immobilized on the microplate, the other is soluble and conjugated with horseradish peroxidase (HRP). Microtiter strip wells are precoated with anti-PAP IgG antibodies. PAP in samples and standards binds to the immobilised antibodies on the surface of the microtiter wells and the second, soluble anti-PAP antibody-enzyme conjugate binds to the immobile antibody-PAP-complex during the first incubation. Afterwards a bound/free separation is performed by solid-phase washing. The immune complex is visualized by adding Tetramethylbenzidine (TMB) substrate, which gives a blue reaction product. The intensity of this product is proportional to the amount of PAP in samples and standards. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorption at 450 nm is read using an ELISA microwell plate reader.

## 4. MATERIALS

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### 4.1. Reagents supplied

- **Anti-PAP Coated Wells:** 12 breakapart 8-well snap-off strips coated with anti-PAP IgG antibodies; in resealable aluminium foil.
- **Stop Solution:** 1 bottle containing 15 ml sulphuric acid, 0.15 mol/l (avoid any skin contact).
- **Anti-PAP-HRP conjugate:** 1 bottle containing 14 ml of horseradish peroxidase labelled anti-PAP antibodies.
- **TMB Substrate Solution:** 1 bottle containing 15 ml 3, 3', 5, 5'-tetramethylbenzidine (H<sub>2</sub>O<sub>2</sub>-TMB 0.26g/l) (avoid any skin contact).
- **Sample diluent:** 1 bottle containing 11 ml MES buffer, pH 5.0, preservatives
- **Wash Solution 10x:** 1 bottle containing 50 ml of a 10x concentrated wash solution (0.2 M phosphate buffer, Proclin <0.002%)
- **PAP Standards:** 6 bottles, standard 0 contains 3 ml, all other standards 1 ml each. The standards have approx. the following concentrations:
  - Standard 0: 0 ng/ml
  - Standard 1: 1 ng/ml
  - Standard 2: 3 ng/ml
  - Standard 3: 5 ng/ml
  - Standard 4: 15 ng/ml
  - Standard 5: 30 ng/ml

### 4.2. Materials supplied

- 1 Strip holder
- 1 Cover foils
- 1 Test protocol
- 1 Distribution and identification plan

### 4.3. Materials and Equipment needed

- ELISA microwell plate reader, equipped for the measurement of absorbance at 450 nm
- Manual or automatic equipment for rinsing wells
- Distilled water
- Disposable tubes
- Timer

## 5. STABILITY AND STORAGE

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The reagents are stable up to the expiry date stated on the label when stored at 2...8 °C.

## 6. REAGENT PREPARATION

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*It is very important to bring all reagents, samples and standards to room temperature (22...28°C) before starting the test run!*

### 6.1. Coated snap-off Strips

The ready to use break apart snap-off strips are coated with anti-PAP IgG antibodies. Store at 2...8 °C. *Immediately after removal of strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 °C; stability until expiry date. Do not remove the adhesive sheets on the unused strips.*

### 6.2. Anti-PAP-HRP Conjugate

The bottle contains 14 ml of a solution with anti-PAP antibodies conjugated with horse radish peroxidase. It is ready to use.

### 6.3. Standards

Each of the 6 vials contains standard solution of the concentration mentioned in 4.1. The standards are ready to use. *After first use the standard solutions are still stable for another 6 months if stored at +4 °C.*

### 6.4. TMB Substrate Solution

The bottle contains 15 ml of a tetramethylbenzidine/hydrogen peroxide system. The reagent is ready to use and has to be stored at 2...8°C in the dark. *The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away. After first use the TMB substrate solution is still stable for another 6 months if stored at 2...8 °C.*

### 6.5. Stop Solution

The bottle contains 15 ml 0.15 M sulphuric acid solution (R 36/38, S 26). This ready to use solution has to be stored at 2...8°C.

*After first use stable until expiry date.*

### 6.6. Wash Solution

Dilute the contents of wash solution concentrate (10x) with distilled water to a final volume of 500 mL prior to use. For smaller volumes respect the 1:10 dilution ratio. The diluted wash solution is stable for 30 days at 2...8°C. In concentrated wash solution it is possible to observe the presence of crystals, in this case mix at room temperature until complete dissolution of crystals. For greater accuracy dilute the whole content of the bottle of concentrated wash solution to 500mL on taking care also to transfer crystals with washing of the bottle, then mix until crystals are completely dissolved.

## 7. SPECIMEN COLLECTION AND PREPARATION

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Use human serum or plasma samples with this assay. If the assay is performed within 2 days after sample collection, the specimens should be kept at 2...8°C; otherwise they should be aliquoted and stored deep-frozen (-20 to -70°C). If samples are stored frozen, mix thawed samples well before testing. *Avoid repeated freezing and thawing.*

Samples with concentration of PAP over 30 ng/ml have to be diluted 1:4 with sample diluent.

### 7.1. Precaution

- The reagent contains Proclin 300<sup>R</sup> as a preservative.
- Standards and sample diluent contain Gentamycin as stabiliser.
- Do not use heavily haemolysed or highly lipemic samples.
- Maximum precision is required for dispensation of the reagents.
- This method allows the determination of PAP from 1 ng/ml to 30 ng/ml.
- Avoid exposure of TMB substrate to direct sunlight, metals or oxidants.

## 8. ASSAY PROCEDURE

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### 8.1. Test Preparation

Please read the test protocol carefully **before** performing the assay. Result reliability depends on strict adherence to the test protocol as described. Prior to commencing the assay, the distribution and identification plan for all specimens and standards should be carefully established on the result sheet supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than one plate is used, it is recommended to repeat the dose response curve. Please allocate at least:

1 well	(e.g. A1)	for the substrate blank
2 wells	(e.g. B1+C1)	for standard 0
2 wells	(e.g. D1+E1)	for standard 1
2 wells	(eg. F1+G1)	for standard 2
2 wells	(eg. H1+A2)	for standard 3
2 wells	(eg. B2+C2)	for standard 4
2 wells	(eg. D2+E2)	for standard 5

*It is recommended to determine standards and patient samples in duplicate.*

Perform all assay steps in the order given and without any appreciable delays between the steps.

A clean, disposable tip should be used for dispensing each standard and each patient sample.

1. Dispense 25 µl standards and samples into their respective wells. Add 100 µl conjugate to each well. Leave well A1 for substrate blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 60 min at room temperature (22...28°C).**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µl diluted wash solution. Avoid overflows from the reaction wells. The soak time between each wash cycle should be >5sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

*Note: Washing is critical! Insufficient washing results in poor precision and falsely elevated absorbance values.*

5. Dispense 100 µl TMB Substrate Solution into all wells.
6. **Incubate for exactly 30 min at room temperature (22...28°C) in the dark.**
7. Dispense 100 µl Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution. Shake the microplate gently.  
*Any blue colour developed during the incubation turns into yellow.*
8. Measure the absorbance of the specimen at 450 nm within 30 min after addition of the Stop Solution.

## 8.2. Measurement

Adjust the ELISA Microwell Plate Reader **to zero** using the **substrate blank in well A1**.

*If - due to technical reasons - the ELISA reader cannot be adjusted to zero using the substrate blank in well A1, subtract the absorbance value of well A1 from all other absorbance values measured in order to obtain reliable results!*

**Measure the absorbance** of all wells at **450 nm** and record the absorbance values for each standard and patient sample in the distribution and identification plan.

Where applicable calculate the **mean absorbance values** of all duplicates.

## 9. RESULTS

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### 9.1. Calculation of results

Calculate the mean absorbance for each point of the standard curve and each sample. Plot the mean value of absorbance of the standards against concentration. Draw the best-fit curve through the plotted points. (e.g. Four Parameter Logistic or Cubic Spline ).

Interpolate the values of the samples on the standard curve to obtain the corresponding values of the concentrations expressed in ng/ml.

### 9.2. Reference values

Normal sample	< 3ng/ml
Benign prostatic hypertrophy	> 3 ng/ml
Prostatic carcinoma	>3 ng/ml

## 10. QUALITY CONTROL

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Each laboratory should assay controls at normal, high and low levels range of PAP for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

If computer controlled data reduction is used to calculate the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

## 11. SPECIFIC PERFORMANCE CHARACTERISTICS

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### 11.1. Precision

#### Intra Assay Variation

Within run variation was determined by replicate determination (16x) of two different control sera in one assay. The within assay variability is ≤ 2.7 %.

#### Inter Assay Variation

Between run variation was determined by replicate measurements of three different control sera in different lots. The between assay variability is ≤ 15.1 %.

## 11.2. Sensitivity

The lowest detectable concentration of PAP that can be distinguished from the standard zero is 0.15 ng/ml at the 95 % confidence limit.

## 11.3. Recovery

The recovery of 1.43 – 2.85 – 5.70 – 11.40 – 22.80 ng/ml of PAP added to a sample gave a mean recovery of 105.09 %.

## 11.4. Correlation with Referenz method

The NovaTec PAP ELISA was compared to another reference method. Serum samples of 28 patients (4 positive and 24 negative samples) were analysed according in both test systems.

The linear regression curve was calculated

(PAP NovaTec)= 2.47 (PAP Reference) - 0.68

r<sup>2</sup> = 0.971

## 11.5. Hook effect

The PAP ELISA, a competitive enzyme immunoassay, shows no Hook Effect up to 60 ng/ml.

## 12. LIMITATIONS OF THE PROCEDURE

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Bacterial contamination or repeated freeze-thaw cycles of the specimen may affect the absorbance values.

## 13. PRECAUTIONS AND WARNINGS

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- In compliance with article 1 paragraph 2b European directive 98/79/EC the use of the in vitro diagnostic medical devices is intended by the manufacturer to secure suitability, performances and safety of the product. Therefore the test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the test kits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive. Nevertheless, all materials should still be regarded and handled as potentially infectious.
- Do not interchange reagents or strips of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate without splashing accurately to the bottom of wells.

WARNING:	In the used concentration Proclin 300 <sup>R</sup> has hardly any toxicological risk upon contact with skin and mucous membranes!
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WARNING:	Sulphuric acid irritates eyes and skin. Keep out of the reach of children. Upon contact with the eyes, rinse thoroughly with water and consult a doctor!
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### 13.1. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

## 14. LITERATURE

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## 15. ORDERING INFORMATION

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Prod. No.:

DNOV068

PAP Determination (96 Determinations)



# SCHEME OF THE ASSAY

PAP

## Test Preparation

Prepare reagents and samples as described.  
Establish the distribution and identification plan for all specimens and controls on the result sheet supplied in the kit.  
Select the required number of microtiter strips or wells and insert them into the holder.

## Assay Procedure

	Substrate blank	Standard 0-5	Sample
Standard 1-5	-	25 µl	-
Sample	-	-	25 µl
Conjugate	-	100 µl	100 µl
Cover wells with foil supplied in the kit <b>Incubate for 60 min at room temperature (22...28°C)</b> Wash each well three times with 300 µl diluted wash solution			
TMB Substrate	100 µl	100 µl	100 µl
<b>Incubate for exactly 30 min at room temperature in the dark</b>			
Stop Solution	100 µl	100 µl	100 µl
Shake the microplate gently Photometric measurement at 450 nm			

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