



Revised 3 Aug. 2010 rm (Vers. 5.1)



*Please use only the valid version of the package insert provided with the kit.*

## 1 NAME AND INTENDED USE

The DRG PAP ELISA is a solid phase enzyme-linked immuno-sorbent assay. This test provides quantitative measurement of human prostatic acid phosphatase (PAP) in serum.  
(For Professional Use Only)

## 2 SUMMARY AND EXPLANATION OF TEST

The phosphatases include two main types. The alkaline phosphatase has a pH optimum of about 9 while acid phosphatase (AcP) has its optimal activity at a pH of about 5<sup>1,2</sup>. The AcP, first demonstrated in the urine in 1925, was found to be much more prevalent in male than in female urine<sup>2</sup>. Prostatic acid phosphatase (PAP) enzyme activity was found to be localized in organs of the male genital tract<sup>2,3</sup>. The presence of PAP in sera of patients with prostatic cancer was first demonstrated by Gutman<sup>4</sup>. It was shown that PAP concentration was elevated in many men with primary prostatic carcinoma and metastatic lesions of the prostate<sup>4,5</sup>.

The release of PAP into the blood stream by primary or metastatic carcinoma of the prostate may provide the clinician with a means of following remission or relapse of a prostatic malignancy<sup>5,6</sup>. Therefore, sensitive and accurate measurement of serum PAP is essential in monitoring the effectiveness of various therapeutic treatments<sup>5,9</sup>.

Because moderate elevation of serum PAP is observed to accompany many nonprostatic diseases, a specific assay for determination of PAP is established<sup>7</sup>. The current methodologies for determination of PAP activity using the colorimetric methods<sup>8,9</sup> and immunochemical methods<sup>10</sup> have many limitations.

DRG PAP ELISA provides an enzyme immunoassay system and establishes an ELISA method for quantitative measurement of PAP in human serum<sup>11</sup>.

## 3 PRINCIPLE OF THE ASSAY

DRG PAP ELISA is a solid phase enzyme linked immunosorbent assay (ELISA). The wells are coated with specific anti-PAP antibodies. The Samples, Standards and Controls are incubated in the wells and bound with enzyme conjugate which is a mixture of anti-PAP antibodies with different affinity toward epitopes of PAP molecule and chemically conjugated with horseradish peroxidase. Unbound enzyme conjugate is washed off. The amount of bound peroxidase is proportional to the concentration of the PAP present in the Samples, Standards and Controls. Upon addition of the chromogen substrate, the intensity of color developed is proportional to the concentration of PAP.

## 4 MATERIAL PROVIDED

1. **Micro-well Strips:**  
Rabbit anti-PAP antibodies coated wells, 96 wells.
2. **Enzyme Conjugate** (11 mL):  
Mouse IgG(anti-PAP) conjugated to horseradish peroxidase
3. **Sample Diluent** or Zero Standard (11 mL).
4. Reference **Standard** Set (0.70 mL/each):  
Calibrated to 1, 3, 5, 15, and 30 ng/mL in BSA-containing diluent.
5. **TMB Solution** (11 mL):  
Buffer solution containing hydrogen peroxide and TMB.
6. **Wash Buffer concentrate 100X** (10 mL):  
Prepare working washing solution by adding 10 mL wash buffer concentrate into 990 mL distilled water.
7. **Stop Solution:**  
1 vial, ready to use,  
contains 2 N HCl  
Avoid contact with the stop solution. It may cause skin irritations and burns.
8. Well holder: for securing wells.
9. Package Insert.

(The reagents except TMB and Stop Solution, contain 0.01% Thimerosal as a preservative.)

**5 MATERIALS REQUIRED BUT NOT PROVIDED**

1. Micro-well reader.
2. Disposal tips and pipettor of 25 µL and 100 µL.
3. Quality controls such as Bio-Rad Lyphochek were recommended

**6 WARNING AND PRECAUTION**

1. DRG PAP ELISA is designed for in Vitro Diagnostic Use only.
2. The Components in the kits are intended for usage as an integral unit. The components from different lots should not be mixed, and not be used beyond expiration date.
3. The material should be used in a designated work area, the bench surface should be cleaned with detergent and the contaminated materials should be disposed properly.
4. Some components have been tested using FDA-approved methods and has been found negative for antibody to human immuno-deficiency virus (HIV-I, HIV-II), antibody to Hepatitis C and Hepatitis B surface antigen (HBsAg). No known test method can offer total assurance that HIV-I, HIV-II, Hepatitis B & C virus or other infectious agents are absent. Handle these reagents as if they were potentially infectious. Information on handling human serum is provided in the CDC/NIH manual ABiosafety in Microbiological and Biomedical Laboratories® (U.S.A. HHS publication No. (NIH 88-8395).
5. Avoid microbial contamination of reagents when removing aliquots from the vials.

**7 STORAGE AND STABILITY**

1. Store the kits at 2-8 °C in a refrigerator.
2. Keep micro-wells in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit. TMB Solution should be colorless; if the solution turns blue, it must be replaced. Do not expose these reagents to strong light during storage usage. If reagent is turbid or contains precipitates, contact DRG technical support.

**8 SPECIMEN AND COLLECTION**

Collect blood by venipuncture, allow to clot, and separate the serum by centrifugation at room temperature.

Serum is required for the DRG PAP ELISA, and do not add sodium azide as preservative.

PAP is unstable at 4 °C and 20 °C<sup>8</sup>. Samples should not be stored at room temperature or 4 °C for more than 24 hours.

Serum samples are recommended to be frozen for longer storage. Avoid repeated freezing and thawing of serum samples.

**9 PREPARATION FOR ASSAY**

1. Bring all reagents and samples to room temperature (24 °C ± 3 °C) and shake gently before beginning the test. Have all reagents and samples ready before the start of the assay. Once the test is begun it must be performed without any interruption to get the most reliable and consistent results.
2. Use new disposable tips for each specimen.

**10 ASSAY PROCEDURE**

1. Secure the desired number of coated wells in the holder.
2. Dispense 25 µL of references, controls or serum samples into the appropriate wells.
3. Dispense 100 µL of enzyme conjugate into wells.  
Incubate for 30 minutes at room temperature.
4. Remove incubation mixture and rinse the wells 5 times with wash buffer (300 µL/well /each rinse).
5. Dispense 100 µL of TMB Solution into each well and incubate for 15 minutes at room temperature.
6. Stop reaction by adding 50 µL of Stop Solution into each well.
7. Read O.D. at 450 nm with a microwell reader in 5 minutes.

**11 PROCEDURE NOTE**

1. Wash the microwells and remove washing buffer thoroughly.
2. Pipet all reagents and samples into bottom of the well. Vortex-mixing or shaking is not required.
3. Absorbance is a function of the time and temperature of incubations. It is recommended to have reagents, samples and needed wells ready for ensure the equal elapsed time for each pipetting without interruption.
4. For the same reason run no more than 20 patient samples with a set of reference standards in duplicate for each assay.
5. If a serum specimen contains greater than 30 ng/ml of PAP, the sample must be diluted with sample diluent and re-assayed as described in the assay procedure.

**12 QUALITY CONTROL**

Good laboratory practices include the use of control specimens to ensure that all reagents and protocols are performing properly. DRG PAP ELISA does not include serum controls. Bio-Rad Lyphochek series control serum is recommended.



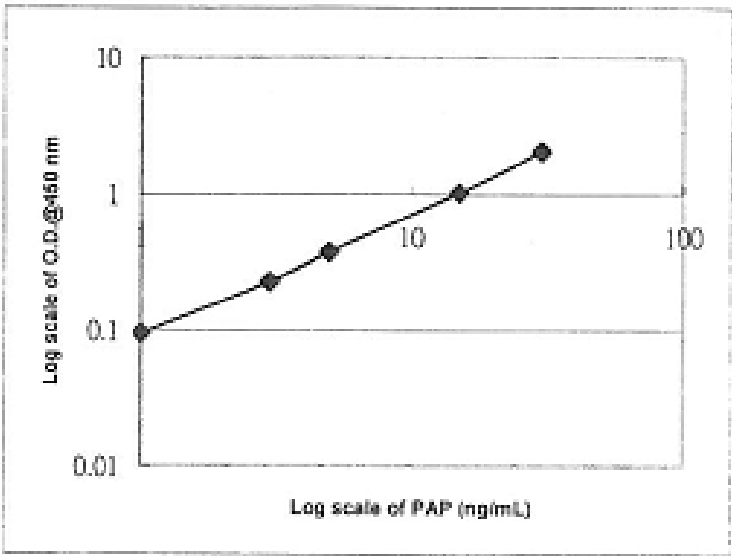
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### 13 CALCULATION OF RESULTS

1. Plot the concentration (X) of each reference standards against its absorbance (Y) on a full logarithmic graph paper.
2. Obtain the PAP value of patient by reference to the standard curve as follows: (These data are for demonstration purpose only and must not be used in place of data generated for each assay).

Well No.	Description (ng/mL)	Absorbance 450 nm	PAP (ng/mL)
A1	0	0.000	
B1	(Blank)	0.000	
A2	1	0.102	
B2		0.089	
A3	3	0.215	
B3		0.241	
A4	5	0.371	
B4		0.383	
A5	15	1.019	
B5		1.039	
A6	30	2.054	
B6		2.060	
A7	PATIENT A	1.653	23.19
B7		1.455	



### 14 EXPECTED VALUE

1. Normal serum concentrations of prostatic acid phosphatase in men have been reported to range from zero to 5 ng/mL<sup>12</sup>. Elevated serum concentrations have been observed frequently in patients with prostatic carcinoma and metastatic lesions of the prostate<sup>4, 5, 12</sup>.
2. A clinical study of the DRG PAP ELISA was conducted and results are summarized as following:
  - a. Serum samples from 100 apparently healthy men were assayed, and 99% of the observations were less than 3 ng/mL.



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- b. Serum samples from 82 patients with benign Hypertrophy (BPH) were assayed and 79% of observations were less than 3 ng/mL.
  - c. Serum samples from 51 patients with prostatic carcinoma (PC) were assayed and 80% of observations were higher than 3 ng/mL.
3. Elevated PAP levels can be an indication of the presence of prostate cancer, they can also be the result of some other prostate diseases such as BPH; therefore, the test must be used in conjunction with a digital rectal examination (DRE) and that if either test is positive. Confirmatory testing with transrectal ultrasound and biopsy is needed to diagnose prostate cancer. Conversely, low PAP levels do not necessarily indicate an absence of prostate cancer.

15 LIMITATION

- 1. For diagnostic purpose, the PAP values should be used as an adjunct to other data available to the physician.
- 2. The DRG ELISA kit is designed to avoid “hook effect” up to 1000 ng/mL.
- 3. Samples with PAP level above 30 ng/mL should be diluted to obtain accurate value.

16 PERFORMANCE CHARACTERISTICS

16.1 ACCURACY

Recovery studies were performed by mixing an aliquot of pooled serum and PAP Standards. The PAP values were measured and percentage of recovery determined.

Initial Values ng/mL	Concentration Spiked ng/mL	Expectd Value ng/mL	Observed Values ng/mL	Recovery (%)
A: 6.5	5	5.7	5.8	102
B: 6.5	30	18.2	18.1	99
C: 21	5	13	12.9	99
D: 21	30	25.5	25.8	101

16.2 PRECISION

Intra-assay and inter-assay coefficient of variation were evaluated at three different pooled serum samples.

Intra-assay	Pool A	Pool B	Pool C
N	10	10	10
Mean (ng/mL)	1.01	17.2	29.1
S.D. (ng/mL)	0.09	0.99	1.8
C.V. (%)	8.9	5.7	6.2

Inter-assay	Pool A	Pool B	Pool C
N	10	10	10
Mean (ng/mL)	1.11	18.7	30.1
S.D. (ng/mL)	0.06	0.98	1.91
C.V. (%)	5.4	5.2	6.3

16.3 SPECIFICITY

The test is specific for human PAP.  
Cross-reactivity with other serum proteins, human hormones and the tumor makers were not found.



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**16.4 MINIMAL DETECTABLE CONCENTRATION**

The minimal detectable concentration of PAP is defined as that concentration of prostatic acid phosphatase which corresponds to the absorbance that is two standard deviations greater than the mean absorbance value of 20 replicate determinations of the zero diluent.

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