BIOLABO
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ACID PHOSPHATASE
End Point Method (PNPP)

Reagent for quantitative determination of total and prostatic phosphatase activity [EC 3.1.2] in human serum.

REF 3300060	**R1**	1 x 120 mL	Citrate Buffer
	R2	1 x 60 mL	Tartrate Buffer
	R3	4 x 10 mL	Substrate
	R4	1 x 40 mL	Stop Reagent
	R5	1 x 5 mL	Stabiliser

TECHNICAL SUPPORT AND ORDERS
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CE

IVD IN VITRO DIAGNOSTIC USE

CLINICAL SIGNIFICANCE (1)

Determination of phosphatase acid activity in serum is almost always directed toward the prostatic enzyme with the entent of detecting or monitoring carcinoma of the prostate. The frequency of increased PAP activity is variable according to cancer stage. This frequency passes from 11% for grade A to 57% for grade D. Indeed, it is recommended that tumor markers measurements such as PSA determination (Prostatic Specific Antigen) be combined with both patient clinical examinations.
PAP activity in serum then allows confirming and evaluating a positive diagnosis of prostatic carcinoma.

PRINCIPLE (4) (5)

Method developped by Fishman and Al, optimised by Richterich and Al. In an acid medium, acid phosphatase hydrolyses paranitrophenyl-phosphate (PNPP) to paranitrophenol and phosphate. When L-Tartrate is present, only the non prostatic acid phosphatase (PANP) is active. The reaction is stopped in alkaline environment. The intensity of the colored complex which absorbance is proportional to the acid phosphatase activity is measured at 405 nm. The difference between the assay of the PAT and the one of the PANP gives the PAP activity.

REAGENTS COMPOSITION

Vial R1 **CITRATE BUFFER**

Citrate buffer pH 4.6	64	mmol/L
Preservative		

Vial R2 **TARTRATE BUFFER**

Citrate buffer pH 4.6	64	mmol/L
L-Tartrate	25	mmol/L
Preservative		

Vial R3 **SUBSTRATE**

Paranitrophenylphosphate	30	mmol/L

Vial R4 **STOP REAGENT (irritating)**

Sodium hydroxide	1,0	mol/L
EDTA	20	mmol/L

Vial R5 **STABILISER (corrosive)**

10% acetic acid	36.8	mmol/L

C, R35: Corrosive. Causes severe burns
S36/37/39: Wear suitable protective clothing, gloves and eyes/face protection

REAGENTS PREPARATION

- Vials R1, R2, R4, R5 are ready for use.
- Vial R3: add promptly to the contents of the vial the amount of demineralised water stated on the label.

Mix gently and wait for complete dissolution before using reagent (approximately 2 minutes).

Vial R3: Use a non-sharp instrument to remove aluminium cap.

SAFETY CAUTIONS

BIOLABO reagents are designated for professional in vitro diagnostic use.

- Verify the integrity of the contents before use.
- Use adequate protections (overall, gloves, glasses). Do not pipette with mouth.
- Avoid contact with skin and eyes. If spilt, thoroughly wash affected areas with plenty of water.
- Reagents contain sodium azide (concentration < 0.1%) which may react with copper and lead plumbing. Flush with plenty of water when disposing.
- Material Safety Data Sheet is available upon request.
- Waste disposal: Respect legislation in force in the country.

All specimens should be handled as potentially infectious, in accordance with good laboratory practices using appropriate precautions. Respect legislation in force in the country.

STABILITY AND STORAGE

Store at 2-8°C, well recap in the original vial and away from light.

- Reagents are stable until expiry date indicated on the label of the kit when stored and used as described in the insert.
- Reconstituted substrate (vial R3) is stable at least for 30 days when free from contamination.
- Don't use reconstituted substrate after expiry date stated on the label.

Discard any reagent if cloudy or if reagent blank at 405 nm > 0.200 (see § **MANUAL PROCEDURE**).

SPECIMEN COLLECTION AND HANDLING (2)

Unhemolysed serum. Separate from the clot as soon as possible after collection and promptly assayed. Acifify at pH 4.5-6.2 ,adding a drop of vial R5 (Stabiliser) for 1 mL of serum.

Acid Phosphatase activity is stable in the acidified serum for:

- 7 days at 2-8°C.

INTERFERENCES (2) (3)

Oxalate and fluorides inhibit Acid Phosphatase activity.

Bilirubin: under-estimation above 30 mg/L.

Haemolysis: acid phosphatase activity overestimated.

Acid Phosphatase activity in serum is labile (activity decreases of 50% in 8 hours).

For a more comprehensive review of factors affecting this assay refer to the publication of Young D.S.

MATERIAL REQUIRED BUT NOT PROVIDED

1. Basic medical analysis laboratory equipment.
2. Normal and pathological control sera.
3. Demineralised water for the preparation of the reagent R3.

CALIBRATION

Results will depend on the accuracy of the instrument calibration, the time counting, the respect of reagent/specimen ratio and the temperature control.
It is recommended to use the theoretical calibration factor (§ **CALCULATION**) or a calibrator traceable to a reference method or material.

QUALITY CONTROL

- BIOLABO EXATROL-N (level I) REF 95010
- BIOLABO EXATROL-P (level II) REF 95011
- Other control sera assayed referring to the same method.
- External quality control program.

It is recommended to control in the following cases:

- At least once a run.
- At least once within 24 hours.
- When changing vial of reagent.
- After maintenance operations on the instrument.

If control is out of range, apply following actions:

1. Repeat the test with the same control.
2. If control is still out of range, prepare a fresh control serum and repeat the test.
3. If control is still out of range, verify analysis parameters: wavelength, temperature, specimen/reagent ratio, time counting, and calibration factor.
4. If control is still out of range, use a new vial of reagent and reassay.
5. If control is still out of range, please contact BIOLABO technical support or your local Agent.

EXPECTED VALUES (2) (8)

α-naphtyl phosphate Methode (2)

Prostatic Acid Phosphatase (30°C or 37°C)	
0-0.8 IU/L	0-0.01 µkat/L

See § REFERENCES (8)

Total Acid Phosphatase (37°C)		
Men	< 6.6 IU/L	< 0.110 µkat/L
Women	< 6.5 IU/L	< 0.108 µkat/L

Prostatic Acid Phosphatase (37°C)		
Men	< 3,5 U/L	< 0,058 µkat/L

Each laboratory should establish its own normal ranges for the population it serves.

LINEARITY

The assay is linear up to 80 IU/L (1.33 µKat/L).
If absorbance is > 1.400, dilute specimen with saline solution and reassay taking into account the dilution factor to calculate the result. Linearity limit depend on the specimen/reagent ratio.

PERFORMANCE CHARACTERISTICS

TOTAL ACID PHOSPHATASE

Within-run N = 20	Low level	High level
Mean IU/L	9.2	50.4
S.D. IU/L	0.13	0.77
C.V. %	1.4	1.5

Beetwen run N = 40	Low level:	High level:
Mean IU/L	8.9	34.1
S.D.IU/L	0.35	1.0
C.V. %	4	2.9

PROSTATIC ACID PHOSPHATASE

Within-run N = 40	Low level:	High level:
Mean IU/L	3.3	11.8
S.D. IU/L	0.12	0.70
C.V. %	3.6	5.9

Beetwen run N = 40	Low level	High level
Mean IU/L	3.05	8.55
S.D.IU/L	0.16	0.67
C.V. %	5.1	7.8

Detection limit: approximately 0.7 IU/L

Sensitivity for 1 IU/L (0.017 µKat/L): approximately 0.018 Abs at 405 nm.

MANUAL PROCEDURE

Let stand reagents and specimens at room temperature.

For each serum, pipette into 3 test tubes:	Serum Blank	Assay 1 (A1)	Assay 2 (A2)
Vial R1 (Citrate Buffer)	1 mL	1 mL	
Vial R2 (Tartrate Buffer)			1 mL
Serum		100 µL	100 µL
Mix. Let stand for 5 minutes at 30°C or 37°C Add contents of vial R3 as follows and simultaneously start a timer:			
Vial R3 (Substrate)	200 µL	200 µL	200 µL
Mix. Let stand for exactly 15 minutes at 30°C or 37°C. Add:			
Vial R4 (STOP Reagent)	200 µL	200 µL	200 µL
Serum	100 µL		
Mix. Read A1 (Assay 1) and A2 (Assay 2) absorbance at 405 nm against serum blank.			

Note:

Reagent Blank: Prepare an additional tube replacing specimen with demineralised water. Add other reagents as indicated in the board. This measure is used only as validation of the quality of the reagent and do not occurred in the procedure (§ **STABILITY AND STORAGE**).

CALCULATION

Calculate the result as follows:

Total acid phosphatase:

With theoretical factor:

IU/L = A1 x 54

µkat/L = A1 x 0.90

With seric multicalibrator

$$\text{PAT Activity} = \frac{A1_{\text{Assay}}}{A1_{\text{Calibrator}}} \times \text{Calibrator Concentration}$$

Prostatic acid phosphatase:

With theoretical factor:

IU/L = (A1 – A2) x 54

µkat/L = (A1 – A2) x 0.90

With seric multicalibrator

$$\text{PAP Activity} = \frac{(A1 - A2)_{\text{Assay}}}{(A1 - A2)_{\text{Calibrator}}} \times \text{Calibrator Concentration}$$

REFERENCES

(1) *TIETZ N.W. Text book of clinical chemistry, 3rd Ed. C.A. Burtis, E.R. Ashwood, W.B. Saunders (1999) p. 711-715*
(2) *Clinical Guide to Laboratory Test, 4th Ed., N.W. TIETZ (2006) p. 912-915*
(3) *YOUNG D.S., Effect of Drugs on Clinical laboratory Tests, 4th Ed. (1995) p. 3-498*
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(5) *RICHTERICH R., COLOMBO J. P., WEBER H., Schweizerich medizinische Wochenscrift, 92 (1962), p.1496-1500.*
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