



BIOLABO
www.biolabo.fr

MANUFACTURER:
BIOLABO SAS,

Les Hautes Rives
02160, Maizy, France

ACID PHOSPHATASE Kinetic method

Reagent for quantitative determination of total (ACP) and prostatic acid phosphatase (P ACP) activity [EC 3.1.2] in human serum.

REF 82560	R1	10 x 15 mL	Citrate Buffer
	R2	5 x 15 mL	Citrate/Tartrate Buffer
	R3	10 x 15 mL	Substrate
	R4	1 x 5 mL	Stabiliser

TECHNICAL SUPPORT AND ORDERS

Tel : (33) 03 23 25 15 50

Fax: (33) 03 23 256 256



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 intent of detecting or monitoring carcinoma of the prostate. The frequency of increased ACP 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 tumour markers measurements such as PSA determination (Prostatic Specific Antigen) be combined with both patient clinical examinations.

P ACP activity in serum then allows confirming and evaluating a positive diagnosis of prostatic carcinoma.

PRINCIPLE (4) (5)

Hillmann modified method. Reaction scheme is as follows:



The rate at which the diazo compound is formed, measured at 405 nm, is proportional to the ACP activity in the specimen.

The NP ACP activity (Non prostatic ACP activity, tartrate resistant) is measured in the presence of Tartrate. The difference between the assay of the ACP and the one of the NP ACP gives the P ACP activity.

REAGENTS COMPOSITION

Vial R1 CITRATE BUFFER Concentration in the test

Citrate buffer pH 5.4 150 mmol/L
1,5-Pentanediol 114 mmol/L
Surfactant, preservative

Vial R2 CITRATE/TARTRATE BUFFER

Na Tartrate 75 mmol/L
Citrate buffer pH 5.4 150 mmol/L
1,5-Pentanediol 114 mmol/L
Surfactant, preservative

Vial R3 SUBSTRATE

α -naphthyl phosphate 12.5 mmol/L
Fast Red TR Salt 1.6 mmol/L

(diazo 2, chloro 5 toluene)

Concentration in vial R4

Vial R4 STABILISER (corrosive)

Acetic acid 1.5 mol/L

C, R34: Corrosive, causes burns

S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

S36/37/39: Wear suitable protective clothing, gloves and eyes/face protection.

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.
 - 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.

REAGENTS PREPARATION

- Vial R3: Use a non-sharp instrument to remove aluminium cap.
- Vial R4 is ready for use.
- ACP Reagent: Add promptly the contents of vial R3 (Substrate) into vial R1 (Citrate Buffer) Mix gently and wait for complete dissolution before using reagent (approximately 2 minutes).
- NP ACP Reagent: Add promptly the contents of vial R3 (Substrate) into vial R2 (Citrate/Tartrate Buffer) Mix gently and wait for complete dissolution (approximately 2 minutes).

STABILITY AND STORAGE

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

- Unopened, reagents are stable until expiry date indicated on the label of the kit when stored and used as described in the insert.
- Once reconstituted, ACP and NP ACP reagents are stable for 10 days when free from contamination.
- Discard any ACP or NP ACP reagents if cloudy or if the absorbance measured at 405 nm > 0.600 (see § **MANUAL PROCEDURE**).
- Don't use working reagent after expiry date stated on the label of the Kit.

SPECIMEN COLLECTION AND HANDLING (2)

Unhemolysed serum. Separate from the clot as soon as possible after collection and promptly assayed. Acidify at pH 5.4-6.2, adding a drop (20 μ L) of vial R4 (Stabiliser) for 1 mL of serum.

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

Acid Phosphatase activity is stable in the acidified serum for:

- 7 days at 2-8°C.

INTERFERENCES (2) (3) (6) (7)

Oxalate and fluorides inhibit Acid Phosphatase activity.

Discard any icteric specimens

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

CALIBRATION

Results will depend on the accuracy of the instrument calibration, the time counting, the respect of reagent/specimen ratio and the temperature control.

- Use the theoretical calibration factor (§ **CALCULATION**)
- Or **REF** 95015 BIOLABO Multicalibrator (calibration value determined with validated statistical technics and metrologically controlled instrument)
- or a multiparametric 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, calibration factor.
- 4.If control is still out of range, use a new vial of reagent and reassay.
 - 1.If control is still out of range, please contact BIOLABO technical support or your local Agent.

EXPECTED VALUES (2)

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 < 6.6 IU/L < 0.110 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.

PERFORMANCE CHARACTERISTICS

TOTAL ACID PHOSPHATASE

Within-run N = 20	Low level	High level	Beetwen run N = 40	Low level:	High level:
Mean IU/L	4.36	82.4	Mean IU/L	4.7	22.7
S.D. IU/L	0.09	0.66	S.D.IU/L	0.24	0.83
C.V. %	2.1	0.8	C.V. %	5.1	3.7

PROSTATIC ACID PHOSPHATASE

Within-run N = 40	Low level:	High level:	Beetwen run N = 40	Low level	High level
Mean IU/L	2.17	77.1	Mean IU/L	2.18	10.8
S.D. IU/L	0.08	0.1	S.D.IU/L	0.14	0.58
C.V. %	3.7	0.13	C.V. %	6.5	5.4

Detection limit: approximately 1.0 IU/L

Comparison with commercially available reagent:

ACP: $y = 0.979x - 3.05$ $r = 0.992$

NP ACP: $y = 1.03x + 0,47$ $r = 0.971$

LINEARITY

ACP: The assay is linear up to 150 IU/L (2.5 µKat/L).

NP ACP: The assay is linear up to 75 IU/L (1.25 µKat/L).

Above, dilute specimen with saline solution and re-assay taking into account the dilution factor to calculate the result. Linearity limit depend on the specimen/reagent ratio.

MANUAL PROCEDURE

Let stand reagents and specimens at room temperature.

Pipette into 30 or 37°C thermostated 1 cm path length cuvette:	Assay 1	Assay 2
ACP Reagent	1 mL	
NP ACP Reagent		1 mL
Serum or calibrator	100 µL	100 µL

Mix. Record initial absorbance at 405 nm after 5 minutes, and ΔAbs /min every minutes during 3 minutes.

Note:

Specific procedures are available upon request for automated instruments. Please contact BIOLABO technical support.

CALCULATION

Calculate the result as follows:

Total Acid phosphatase (Assay 1):

Non Prostatic Acid Phosphatase (Assay 2):

With theoretical factor:

$$UI/L = (\Delta Abs/min) \times 730$$

With seric multicalibrator:

$$ACP \text{ Activity} = \frac{(\Delta Abs /min) \text{ Assay}}{(\Delta Abs/min) \text{ Calibrator}} \times \text{Calibrator activity}$$

Prostatic acid phosphatases:

$$P \text{ ACP Activity} = \text{Assay 1 Activity} - \text{Assay 2 Activity}$$

$$\mu\text{kat/L} = \frac{UI/L}{60}$$

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
- (4) HILLMANN G. *Fortlaufende photometrische Messung der sauren Prostataphosphatase-Aktivität.* -Z. Clin. Chem. u. Klin. Biochem. -1971, vol. 9, p.273-274.
- (5) VASSAULT A., PHUNG H. T., AUBRY C., GOUDARD M., et les membres de la commission "Enzymologie" (Maire I., président) de la SFBC (1991)-*Recommandations pour la mesure de la concentration catalytique des phosphatases acides dans le sérum humain à 30°C.* Inf. Sci. Biol. -1991, vol. 17, n°5, p.327-340.
- (6) SCHIELE F., ARTHUR Y, FLOCH A.Y., et SIEST G., *Total, tartrate resistant, and tartrate inhibited acid phosphatases in serum: biological variations and reference limits.*-Clin. Chem.-1988, vol.34, n°4, p.685-690.
- (7) SMALL C. W., McNUTT P -*Interferences in the direct kinetic determination of acid phosphatase activity.*-Clin. Chem.-1984, vol.30, n°4, p.594-595.
- (8) Junge W, Thormeyer I, Schlottmann A et al. *Determination of Reference Values for Acid Phosphatase using a New Photometric Assay.* Pecs, Hungary: 3e Alpe-Adria Congress on Clinical Chemistry and Laboratory Medicine. September 7-9, 1994