

Acid phosphatase.α-Naphtyl phosphate.Kinetic
Quantitative Determination of acid phosphatase
 Only for *in vitro* use in clinical laboratory
 Store at 2-8°C

Ref.: ACP-031

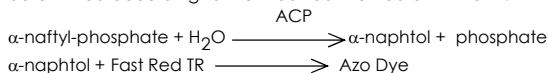
19 x 2 mL

ACID PHOSPHATASE



PRINCIPLE OF THE METHOD

Hillmann method: Phosphatase acid activity present in the sample is determined according to the modified method of Hillmann.



α-naphtol reacts with a diazoted compound forming a colour with a maximum of absorbance at 405 nm.
 Tartrate is used as specific of the prostatic fraction.

CLINICAL SIGNIFICANCE

Acid phosphatase is an enzyme present in almost all weaves of the organism, being particularly high in prostate, stomach, liver, muscle, spleen, erythrocytes and platelets.
 High levels of acid phosphatase are found in prostatic pathologies as hypertrophy, prostatitis or carcinoma. In hematological disorders, bones or liver diseases as well as in Paget's or Gaucher's diseases.
 Decreased serum acid phosphatase has no clinical significance^{1,4,5}.
 Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

REAGENTS

R 1 Buffer	Sodium citrate pH 5.2	50 mmol/L
R 2 Substrate	α-Naphtyl phosphate Fast Red TR	10 mmol/L 6 mmol/L
R 3 Tartrate	Sodium tartrate	2 mmol/L
R 4	Acetic acid	0.5 mol/L

PREPARATION

- Working reagent (WR):
 Ref: 1001121.
 Dissolve (→) one tablet of R 2 Substrate in one vial of R 1 Buffer.
 Ref: 1001122
 Dissolve (→) one tablet of R 2 Substrate in 15 mL of R 1 Buffer.
 Cap and mix gently to dissolve contents.
 Stability: 2 days at 2-8°C or 6 hours at room temperature.
- R 3 and R 4: Ready to use. (R 4 Included in Ref.:1001121).

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C, protected from light and contaminations prevented during their use.
 Do not use the tablets if appears broken.
 Do not use reagents over the expiration date.

Signs of reagent deterioration:

- Presence of particles and turbidity.
- Blank absorbance (A) at 450 nm ≥ 0,44.

ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 405 nm.
- Thermostatic bath at 30°C or 37°C (± 0,1°C)
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment.

SAMPLES

Serum¹. Use only clear and unhemolyzed serum, separated from the clot as soon as possible. Do not use plasma.
 Acid phosphatase is very labile; stabilize by adding 50 µL of acetic acid (R.4) per mL of the sample. Stability: 7 days at 2-8°C.

PROCEDURE

1. Assay conditions:
 Wavelength: 405 nm
 Cuvette: 1 cm light path
 Constant temperature 30°C / 37°C
2. Adjust the instrument to zero with distilled water or air.
3. Pipette into a cuvette:

	ACP Total (T)	ACP Non Prostatic (No P)
WR (mL)	1.0	1.0
R 3 (µL)	--	10
Sample (µL)	100	100

4. Mix, incubate for 5 minute.
5. Read initial absorbance (A) of the sample, start the stopwatch and read absorbance at 1 minute intervals thereafter for 3 minutes.
6. Calculate the difference between absorbance and the average absorbance differences per minute (ΔA/min).

CALCULATIONS

$\Delta A/\text{min} \times 750 = \text{U/L of ACP (T)}$
 $750 \times (\Delta E/\text{min ACP (T)} - \Delta E/\text{min ACP Non inhibitor by Tartrate}) = \text{U/L of ACP prostatic.}$

Units: One international unit (IU) is the amount of enzyme that transforms 1 µmol of substrate per minute, in standard conditions. The concentration is expressed in units per litre of sample (U/L).

QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures.
 If control values are found outside the defined range, check the instrument, reagents and technique for problems.
 Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES^{4,5}

	30°C	37°C
Total acid phosphatase:		
Men :	< 4.3 U/L	< 5.4 U/L
Women:	< 3.1 U/L	< 4.2 U/L
Prostatic acid phosphatase	< 1.5 U/L	< 1.7 U/L

These values are for orientation purpose; each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS (Total ACP)

Measuring range: From detection limit of 0,13 U/L to linearity limit of 150 U/L.

If the results obtained were greater than linearity limit, dilute the sample 1/2 with NaCl 9 g/L and multiply the result by 2.

Precision:

	Intra-assay (n=20)		Inter-assay (n=20)	
Mean (U/L)	23.67	2.56	23.6	2.6
SD	0.22	0.07	0.22	0.07
CV (%)	0.95	2.90	0.92	2.76

Sensitivity: 1 U/L = 0.0034 A/min.

Accuracy: Results obtained using BSM reagents did not show systematic differences when compared with other commercial reagents.

The results obtained using 50 samples were the following:

Correlation coefficient (r): 0.99

Regression equation: $y = 0.9977x + 0.1486$.

The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

Hemolysis interferes due the high concentration of acid phosphatase in red cells¹. A list of drugs and other interfering substances with acid phosphatase determination has been reported by Young et. al^{2,3}.

NOTES

BSM has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

BIBLIOGRAPHY

1. Abbott L. et al. Acid phosphatase. Kaplan A et al. Clin Chem The C.V. Mosby Co. St Louis, Toronto, Princeton 1984; 1079-1083.
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