Acid phosphatase.α-Naphtyl phosphate.Kinetic Ref.: ACP-031
Quantitative Determination of acid phosphatase
Only for *in vitro* use in clinical laboratory
Store at 2-8ºC 19 x 2 mL

ACID PHOSPHATASE

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

$$\alpha\text{-naftyl-phosphate} + H_2O \xrightarrow{ACP} \alpha\text{-naphtol} + \text{phosphate}$$

$$\alpha\text{-naphtol} + \text{Fast Red TR} \longrightarrow \text{Azo Dye}$$

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 phatologies 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-Naftyl 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 2-8ºC 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 2-8ºC, 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 30ºC o 37ºC (± 0.1ºC)
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment.

SAMPLES
Serum[1]. 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 2-8ºC.

PROCEDURE
1. Assay conditions:
   Wavelength: . . . . . 405 nm
   Cuvette: . . . . . 1 cm light path
   Constant temperature . . . . . .30ºC / 37ºC
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
ΔA/min x 750 = U/L of ACP (T)
750 x (ΔE/min ACP (T) - ΔE/min ACP Non inhibitor by Tartrate) = 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]

	30ºC	37ºC
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 cells[1]. 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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4. Burtis A et al. Tietz Textbook of Clinical Chemistry, 3rd ed AACC 1999.
5. Tietz N W et al. Clinical Guide to Laboratory Tests, 3rd ed AACC 1995.