Acid Phosphatase α-Naphtyl Phosphate. Kinetic Quantitative determination of Acid Phosphatase (ACP)

IVD *For In-Vitro diagnostic and professional use only*

2°C 8°C Store at 2-8°C

INTENDED USE

For the determination of Total Acid Phosphatase concentration in human serum.

PRINCIPLE

Hillmann method: Acid Phosphatase hydrolyses at pH 5. 0 the α -naftyl-phosphate or inorganic phosphate to α -naphtol.

$$\text{α -naphtyl-phosphate} + H_2O \xrightarrow{\text{ACP}} \text{α- naphtol} + \text{Phosphate}$$

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

α -naphtol reacts with a diazoted chromogen forming a coloured with a maximum of absorbance at 405 nm.

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. Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

REAGENTS

R1 Buffer	Sodium citrate pH 5.2	50 mmol/L
R 2 Substrate	α -naphtyl-phosphate Fast Red TR	10 mmol/L 6 mmol/L

ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 405 nm.
- Thermostatic bath at 30°C o 37°C (± 0.1°C).
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment.

PREPARATION

Working reagent (WR):

Dissolve one tablet of R 2 Substrate in 2ml of R 1 Buffer. Cap and mix gently to dissolve contents.

Stability: 2 days at 2-8°C or 6 hours at room temperature.

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.

SAMPLES

Serum. 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	1cm light path
Constant temperature	30 , 37°C

2. Adjust the instrument to zero with distilled water or air.
3. Pipette into a cuvette:

	ACP Total (T)
WR (mL)	1.0
Sample (µL)	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)

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

- 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

	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

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 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)	26.3	57.5	29.3	63.0
SD	0.15	0.19	1.70	2.48
CV (%)	0.58	0.34	5.82	3.94

Sensitivity:

1 U/L = 0.00156 Δ Abs.

Accuracy:

Results obtained using Atlas reagents did not show systematic differences when compared with other commercial reagents. The results obtained using 50 samples were the following:

Correlation coefficient $(r)^2$: 0.970510.

Regression equation: y= 0.828963x + 1.06196.

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

INTERFERENCES

Hemolysis interferes due the high concentration of acid phosphatase in red cells. A list of drugs and other interfering substances with acid phosphatase determination has been reported.

REFERENCES

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REF	Catalogue Number		Temperature limit
IVD	*In Vitro* diagnostic medical device		Caution
Σ	Contains sufficient for <n> tests and Relative size		Consult instructions for use (IFU)
LOT	Batch code		Manufacturer
	Fragile, handle with care		Use-by date
	Manufacturer fax number		Do not use if package is damaged
	Manufacturer telephone number		Date of Manufacture
	Keep away from sunlight		Keep dry