COD 11548 40 mL
STORE AT 2-8ºC
Reagents for measurement of ACP concentration Only for *in vitro* use in the clinical laboratory

ACID PHOSPHATASE (ACP)

BioSystems

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NAPHTYL PHOSPHATE/PENTANEDIOL

PRINCIPLE OF THE METHOD

Acid phosphatase (ACP) catalyzes in acid medium the hydrolysis of the phosphate group from α-naphtyl phosphate. The α-naphtol formed reacts with a diazonium salt (Fast Red TR) originating a chromogen. The catalytic concentration is determined from the rate of chromogen formation, measured at 405 nm[1]. Pentanediol accelerates the reaction by acting as phosphate acceptor. Tartrate in used as a specific inhibitor of the prostatic fraction[1,2].

$$\alpha\text{ - Naphtyl - phosphate} + H_2O \xrightarrow{ACP} \alpha\text{ - Naphtol} + Pi$$

$$\alpha\text{ - Naphtol} + \text{Fast Red TR} \longrightarrow \text{Azoic chromogen}$$

CONTENTS AND COMPOSITION

AT. Reagent. 1 x 45 mL. Sodium citrate 110 mmol/L, 1,5-pentanediol 220 mmol/L, pH 5.2.

AI. Reagent. 1 x 22.5 mL. Sodium citrate 110 mmol/L, 1,5-pentanediol 220 mmol/L, sodium tartrate 110 mmol/L, pH 5.2.

B1. Reagent. 4 for 10 mL. α-Naphtyl phosphate 12.5 mmol/L, after dissolution.

B2. Reagent. 4 for 10 mL. Fast Red TR 1.25 mmol/L, after dissolution.

C. Reagent. 1 x 5 mL. Acetic acid 0.6 mol/L.

STORAGE

Store at 2-8ºC.

Reagents are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

– Reagents: Presence of particulate material, turbidity, absorbance of the blank over 0.450 at 405 nm (1 cm cuvette).

WARNING AND PRECAUTIONS

Exercise the normal precautions required for handling all laboratory reagents. Safety data sheet available for professional user on request. Disposal of all waste material should be in accordance with local guidelines. Any serious incident that might occur in relation to the device shall be reported to BioSystems S.A.

REAGENT PREPARATION

Working Reagent. Add 10 mL of Reagent AT (total ACP) or 10 mL of Reagent AI (non prostatic ACP) to the Reagent B1. Cap and shake until dissolved. Next, add this solution to the Reagent B2. Cap and shake until dissolved. Stable for 10 days at 2-8ºC.

ADDITIONAL EQUIPMENT

– Analyzer, spectrophotometer or photometer with cell holder thermostatable at 37ºC and able to read at 405 nm.

– Cuvettes with 1 cm light path.

SAMPLES

Serum collected by standard procedures. Acid phosphatase is unstable in serum. Measure immediately or add 1 drop of Reagent C per mL serum. Acid phosphatase in acidified serum is stable for 6 days at 2-8ºC. Hemolysed samples are not suitable for testing.

PROCEDURE

1. Bring the Working Reagent and the instrument to reaction temperature.
2. Pipette into a cuvette: (Note 1)

Working Reagent	1.0 mL
Sample	0.1 mL

3. Mix and insert the cuvette into the photometer. Start the stopwatch.
4. After 5 minutes, record initial absorbance and at 1 minute intervals thereafter for 3 minutes.
5. Calculate the difference between consecutive absorbances, and the average absorbance difference per minute (ΔA/min).

CALCULATIONS

The ACP concentration in the sample is calculated using the following general formula:

$$\Delta A/min \times \frac{Vt \times 10^6}{\varepsilon \times l \times Vs} = U/L$$

The molar absorbance (ε) of the azoic chromogen at 405 nm is 13033, the lightpath (l) is 1 cm, the total reaction volume (Vt) is 1.1, the sample volume (Vs) is 0.1, and 1 U/L are 16.67 nkat/L. The following formulas are deduced for the calculation of the catalytic concentration:

ΔA/min	x 844 = U/L x 14061 = nkat/L

Prostatic ACP concentration = Total ACP – Non Prostatic ACP

REFERENCE VALUES

Reaction temperature	37ºC
Total, up to	10 U/L = 167 nKat/L
Prostatic, up to	3.5 U/L = 58 nKat/L

Values at 37ºC are obtained from those at 30ºC by using a conversion factor. These ranges are given for orientation only; each laboratory should establish its own reference ranges.

QUALITY CONTROL

It is recommended to use the Biochemistry Control Serum level I (cod. 18005, 18009 and 18042) and II (cod. 18007, 18010 and 18043) to verify the performance of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

METROLOGICAL CHARACTERISTICS

– Detection limit: 0.8 U/L = 13 nkat/L

– Linearity limit: 150 U/L = 2500 nkat/L. For higher values dilute sample 1/2 with distilled water and repeat measurement as soon as possible.

– Repeatibility (within run):

Mean Concentration	CV	n
37 U/L = 617 nkat/L	2.1 %	20
93 U/L = 1550 nkat/L	1.1 %	20

– Reproducibility (run to run):

Mean Concentration	CV	n
37 U/L = 617 nkat/L	2.6 %	25
93 U/L = 1550 nkat/L	1.9 %	25

– Sensitivity: 1.185 ΔmA·L/U·min = 0.071 ΔmA·L/nkat·min

– Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents. Details of the comparison experiments are available on request.

– Interferences: Lipemia (triglycerides < 5 g/L) do not interfere. Bilirubin (>2.5 mg/dL) interfere. Other drugs and substances may interfere[3].

These metrological characteristics have been obtained using an analyzer. Results may vary if a different instrument or a manual procedure are used.

DIAGNOSTIC CHARACTERISTICS

Acid phosphatase catalyzes the hydrolysis of organic phosphate monoesters at acid pH. The major tissue sources of blood ACP are prostate, spleen, liver, kidney and other cellular elements.

Determination of ACP concentration in serum is almost always directed toward the prostatic enzyme with the intent of detecting or monitoring prostatic pathologies (hypertrophy, prostatitis, carcinoma)[4,5].

Elevations in total ACP concentration in serum may also be due to other causes: hematological disorders (idiopathic thrombocytopenic, myelocytic leukemia); metastasic breast cancer; bone disease (Paget's disease, metastasic carcinoma of bone); various liver diseases (hepatitis, obstructive jaundice); acute renal impairment; Niemann-Pick disease and Gaucher's disease[4,5].

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

NOTES

1. These reagents may be used in several automatic analysers. Instructions for many of them are available on request.

BIBLIOGRAPHY

1. Escribano J. Kinetic analysis of chemical reactions coupled to an enzymic step. Application to acid phosphatase assay with Fast Red. *Biochem. J* 1984; 223: 633-638.
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3. Young DS. Effects of drugs on clinical laboratory tests, 5th ed. AACC Press, 2000.
4. Friedman and Young. Effects of disease on clinical laboratory tests, 4th ed. AACC Press, 2001.
5. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 4th ed. Burtis CA, Ashwood ER, Bruns DE. WB Saunders Co, 2005.

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