ACID PHOSPHATASE KIT

(α Naphthylphosphate Kinetic method)

For the determination of Acid Phosphatase activity in serum.

(For Invitro Diagnostic Use Only)

Summary

Acid Phosphatase (ACP) is an enzyme of the Hydrolase class of enzymes and acts in an acidic medium. It is widely distributed and found in high concentrations in the liver, RBC's and the prostate. Increased levels of the prostatic fraction are associated with prostatic carcinomas. Increased levels of the non prostatic fraction are associated with liver diseases, hyperparathyroidism, and Paget's disease.

Principle

ACP at an acidic pH hydrolyses α Naphthylphosphate to form α Naphthol and Inorganic Phosphate. The α Naphthol formed is coupled with Fast Red TR salt to form a diazo dye complex. The rate of formation of this complex is measured as an increase in absorbance which is proportional to the ACP activity in the sample. Tartrate inhibits prostatic ACP and the testing in it's presence is done to find the non prostatic ACP. The difference between the activities of the total and non prostatic ACP gives the activity of the prostatic ACP.

$$\alpha\text{ Naphthylphosphate} + H_2O \xrightarrow{ACP} \alpha\text{ Naphthol} + \text{Phosphate}$$

$$\alpha\text{ Naphthol} + \text{Fast Red TR Salt} \longrightarrow \text{Diazo dye complex}$$

Normal reference values

Serum (male) : upto 4.2 U/L at 30°C / upto 4.7 U/L at 37°C

(female) : upto 3.0 U/L at 30°C / upto 3.7 U/L at 37°C

Prostatic ACP : upto 1.5 U/L at 30°C / upto 1.6 U/L at 37°C

It is recommended that each laboratory establish its own normal range representing its patient population.

Contents

	10 x 2 ml	30 x 2 ml
L1 : Buffer Reagent	25 ml	75 ml
T1 : Substrate Tablets	10 Nos.	30 Nos.
L2 : Tartrate Reagent	2 ml	2 ml
L3 : Acetate Buffer	2 ml	2 ml

Storage / stability

Contents are stable at 2-8° C till the expiry mentioned on the labels.

Reagent Preparation

Reagents L2 and L3 are ready to use.

The Buffer (L1) when retrieved from 2-8° C may appear turbid. However the turbidity clears up on attaining R.T. In case the turbidity persists a little warming of the Buffer to

Working reagent : Dissolve 1 Substrate tablet (T1) in 2.2 ml of Buffer Reagent (L1). Allow the tablet to hydrate for around 5 min. and then shake to dissolve. This working reagent is stable for at least 3 days when stored at 2-8°C. The Working Reagent may be used for the Total ACP assay or the Non Prostatic ACP assay as required.

Sample material

Serum. Free from hemolysis.

ACP, especially the prostatic fraction, is unstable in a collected sample hence the serum should be separated from the clot, as soon as possible, and assayed. In case of a delay in testing the serum should be acidified to a pH of 5.0 with 0.02 ml Acetate Buffer (5M) provided for each ml of serum.

Procedure

Wavelength / filter : 405 nm

Temperature : 30°C / 37°C

Light path : 1 cm

Total ACP Assay :

Pipette into a clean dry test tube labelled as Test (T) :

Addition Sequence	(T) (ml)
Working Reagent	1.0
Sample	0.1

Mix well and read the initial absorbance A_0 after 5 minutes & repeat the absorbance reading after every 1, 2, & 3 minutes. Calculate the mean absorbance change per minute (ΔA / min.)

Non Prostatic ACP Assay : (Tartrate Inhibited)

Pipette into a clean dry test tube labelled as Test (T) :

Addition Sequence	(T) (ml)
Working Reagent	1.0
Tartrate Reagent	0.02
Incubate at the assay temperature for 1 minute and add	
Sample	0.1

Mix well and read the initial absorbance A_0 after 5 minutes & repeat the absorbance reading after every 1, 2, & 3 minutes. Calculate the mean absorbance change per minute (ΔA / min.)

Calculations

ACP Activity in U/L = ΔA / min x 750

Prostatic ACP Activity in U/L = Total ACP - Non Prostatic ACP

Linearity

The procedure is linear upto 75 U/L at 37°C. If the absorbance change (ΔA / min.) exceeds 0.100, dilute the sample 1 + 4 with normal saline (NaCl 0.9%) and repeat the assay (Results x 5).

Notes

Samples having a high activity show a very high initial absorbance. If this is suspected then dilute the sample and repeat the assay.

The working reagent should have an absorbance below 0.800 against distilled water at 405 nm. Discard the reagent if the absorbance is above 0.800.

It has been seen that in a collected sample ACP, especially the prostatic form, may loose around 50% of its activity in an hour at R.T.

References

Hillman, G.C., (1971) Klin. Biochem, 9 , 273-274.

System Parameters

Reaction	:	Kinetic
Wavelength	:	405 nm
Zero Setting	:	Distilled water
Incub. Temp.	:	30^0C / 37^0C
Incub. Time	:	---
Delay Time	:	300 sec.
Read Time	:	180 sec.
No. of read.	:	4
Interval	:	60 sec.
Sample Vol.	:	0.10 ml
Reagent Vol.	:	1.00 ml
Standard	:	---
Factor	:	750.0
React. Slope	:	Increasing
Linearity	:	75 U/L
Units	:	U/L

ACP(Kin).01(P)

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