

## INTRODUCTION

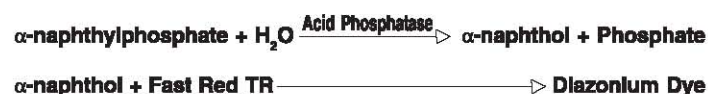
1. AutoZyme Acid Phosphatase is a reagent set for determination of Acid Phosphatase activity based on kinetic method using  $\alpha$ -naphthylphosphate.
2. AutoZyme Acid Phosphatase is a single reagent system using one step procedure.
3. AutoZyme Acid phosphatase can be determined in just 8 minutes at 37°C.
4. AutoZyme Acid Phosphatase is linear upto 75 IU/l.
5. AutoZyme Acid Phosphatase can be used on any Spectrophotometer, Discrete semiautomated and Automated analyzer. Programme can be designed for any specific analyzer upon request.

## PRINCIPLE

In acidic pH of buffer system, Acid Phosphatase hydrolyses  $\alpha$ -naphthylphosphate to  $\alpha$ -naphthol and phosphate. The  $\alpha$ -naphthol is then coupled with Diazotized Fast Red TR to form a Diazo dye which has strong absorbance at 405 nm. The increase in absorbance is directly proportional to the level of acid phosphatase in serum.

The addition of L-Tartrate inhibits prostatic acid phosphatase but does not inhibit other isoenzyme.

The activity of Prostatic Acid Phosphatase is obtained by subtracting the result of Non prostatic Acid phosphatase (Determination with Tartrate) from Total Acid Phosphatase (without tartrate).



## PREPARATION OF WORKING SOLUTION

### Total Acid Phosphatase Working Solution

Dissolve the contents of the substrate bottle with Diluent for Total ACP as per instructions indicated on individual bottle label to prepare working solution.

### Non Prostatic Phosphatase Working Solution

Dissolve the contents of the substrate bottle with Diluent for Non Prostatic ACP as per instructions indicated on individual bottle label to prepare working solution.

The Stabilizer reagent (Acetate Buffer) is ready-to-use as provided.

NOTE : ACP = Acid Phosphatase

## REAGENT STORAGE & STABILITY

The kit should be stored at 2 - 8°C and is stable till the expiry date indicated on the label.

The working solutions of — Non Prostatic Acid Phosphatase & Total Acid Phosphatase are stable for 7 days at 2 - 8°C.

## COMPONENTS & CONCENTRATION OF WORKING SOLUTION

Component	Concentration	
	Total ACP	Non Prostatic ACP
• Buffer, pH 5.3	80 mmol/l	80 mmol/l
• $\alpha$ - naphthylphosphate	> 5.0 mmol/l	> 5.0 mmol/l
• Fast Red TR	> 1.0 mmol/l	> 1.0 mmol/l
• Tartrate	—	150 mmol/l

## SPECIMEN COLLECTION & PRESERVATION

Serum sample must be completely free from haemolysis. Acid Phosphatase is very unstable at the pH of serum. Immediately after separation of the serum from the clot, stabilize the serum by the addition of 20  $\mu$ l of stabilizer reagent (Acetate buffer) to every 1 ml of serum. The enzyme activity will be stable for 3 days at 2-8°C. Oxalate & fluoride as anticoagulants will interfere with the assay

## PROCEDURE

- Reaction type ..... Kinetic
- Reaction direction ..... Increasing
- Wavelength ..... 405 nm.
- Flowcell temperature ..... 37°C
- Zero setting with ..... Distilled water
- Delay time ..... 300 seconds
- No. of readings ..... 4
- Interval ..... 60 seconds
- Blank absorbance limit ..... < 0.500
- Sample volume ..... 0.1 ml (100  $\mu$ l)
- Reagent volume ..... 1.0 ml
- Factor ..... 743
- Linearity ..... 75 IU/l

### Manual assay procedure

Prewarm at 37°C the required amount of working solutions before use.

Perform the assay as given below :

### Total Acid Phosphatase

Specimen ..... 0.1 ml (100  $\mu$ l)  
Total ACP working solution ..... 1.0 ml

### Non Prostatic Acid Phosphatase

Specimen ..... 0.1 ml (100  $\mu$ l)  
Non Prostatic ACP working solution ..... 1.0 ml

For each assay, mix thoroughly and transfer the assay mixture immediately to the thermostated cuvette and start the stop watch simultaneously. Record the first reading at 300<sup>th</sup> second and subsequently three more readings with 60 seconds interval at 405 nm.

### Calculation:

#### A) Total Acid Phosphatase & Non Prostatic Acid Phosphatase

Conc. in IU/l = 743 x  $\Delta$  Abs. / min

#### B) Prostatic Acid Phosphatase

Conc. in IU/l = Total Acid Phosphatase - Non Prostatic Acid Phosphatase



