



# ACID PHOSPHATASE

E.C. 3.1.3.2

## Colorimetric Test (Hillmann method)

Cat.No. 101-0311

Size: 18 x 2 ml

Cat.No. 101-0464

Size: 10x10 ml

### PRINCIPLE:

Acid phosphatase activity present in the sample is determined according to the modified method of Hillmann:

Tartrate is used as specific of the prostatic fraction.

### SAMPLE:

Acid phosphatase is extremely labile at room temperature. The sample should therefore be centrifugate immediately after coagulation. The serum should then be cooled and processed as quickly as possible. Do not use hemolytic serum. If the serum is to be examined after some time, its pH value should be adjusted with acetate buffer, Reagent 4 (0.02 ml acetate bufer per 1 ml of serum).

### REAGENT:

1. Buffer  
Citrate buffer, pH 5.2 50 mmol/L
2. Substrate, tablets  
 $\alpha$ -naphthyl phosphate 10 mmol/L  
Fast Red TR 6 mmol/L
3. Tartrate reagent  
Sodium tartrate 2 mmol/L
4. Acetate buffer

Stable up to the expiry date when stored at 2 - 8 °C.

### PREPARATION OF REAGENTS:

Buffer:

Contents ready for use. Stable up to the expiry date when stored at 2 °C - 8 °C.

Dissolve one tablet of Reagent 2 in corresponding volume of buffer (Reagent 1).

The stability of working reagent is 2 days at +2 to +8 °C or 6 hours at room temperature.

### PROCEDURE:

Wavelength: 405 nm  
Cuvette: 1 cm light path  
Temperature: 30 °C / 37 °C  
Zero: Air or distilled water

Pipette into tubes	Total acid phosphatase	Non inhibitor by tartrate fraction
Working reagent	2.0 ml	2.0 ml
Tartrate solution (Reagent 3)	---	20 $\mu$ l
Sample	200 $\mu$ l	200 $\mu$ l

Mix and incubate for 5 min at 30 °C / 37 °C.  
Measure the absorbance increase per minute for 1 to 3 min.  
Calculate  $\Delta A/\text{min}$ .

### CALCULATION:

Total acid phosphatase:

$$\Delta A/\text{min} \times 750 = \text{U/L Total acid phosphatase}$$

Fraction prostatic acid phosphatase:

$$(\Delta A/\text{min}_{\text{Total acid phosph.}} - \Delta A/\text{min}_{\text{Non inhibitor by Tartrate}}) \times 750 = \text{U/L}$$

### EXPECTED VALUES:

Total acid phosphatase		
	30 °C	37 °C
Men up to	4.3 U/L	5.4 U/L
Women up to	3.1 U/L	4.2 U/L
Prostatic phosphatase		
up to	1.5 U/L	1.7 U/L

### LINEARITY:

up to 150 U/L

If the acid phosphatase activity is greater than 150 U/l, repeat the test using a sample diluted 1:10 with saline solution.

### QUALITY CONTROL:

All control sera with mean values determined by this method can be use.

### NOTE:

1. Sample: serum only, do not use plasma
2. Hemolysis will interfere.
3. Serum sample can be stored for 1 week at +2 to +8 °C at pH 5.

### REFERENCES:

1. Hillman, G.Z., Clin.Chem,Clin.Biochem., 1971;9:273