

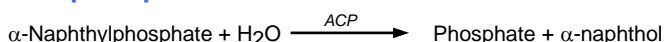
<b>Cat. No.13011</b> <b>For 15 tests</b>	R1	3 Powder for	5 ml
	R2	1 X	15 ml
	R3	1 X	1 ml
	R4	1 X	1 ml

## Acid Phosphatase

Kinetic colorimetric method

### Powder Reagent

#### Test principle



Acid phosphatase hydrolyzes  $\alpha$ -naphthylphosphate to yield  $\alpha$ -naphthol which reacts immediately with Fast Red TR to produce a dye measured at 405 nm. The rate of increase in absorbance at 405 nm is proportional to the acid phosphatase activity. If L-tartrate is added to the sample, prostatic acid phosphatase is inhibited, but all the other acid phosphatase in the serum react. Therefore, the test is run both in the presence and absence of L-tartrate. The difference in activity between the two assays is equal to the prostatic acid phosphatase activity in serum.

#### Concentrations of reagents

<b>Reagent R1 (after reconstitution)</b>		
Fast Red TR (2-amino-5-chlorotoluene-1,5-naphthalene disulphonate)	1.6	mmol/L
$\alpha$ -Naphthylphosphate	12	mmol/L
<b>Reagent R2</b>		
Citrate buffer pH = 5.3	1.0	mmol/L
<b>Reagent R3</b>		
Buffer pH = 5.3		
Sodium L-tartrate	200	mmol/L
<b>Reagent R4: ACP stabilizer</b>		
Acetate buffer pH = 5.0	5	mol/L

#### Stability and preparation of working reagent

**Reagent R1:** powder.

**Reagent R2:** liquid.

**Reagent R3:** liquid, ready to use.

**Reagent R4:** liquid, ready to use.

All reagents are stable up to expiry date given on the label when stored at +2 → +8 °C.

#### Working Reagent:

Add 5 mL of reagent R2 to the vial of reagent R1. Mix gently, allow five minutes for reconstitution, then remix before use.

Stability: 14 days at 2 - 8 °C.

#### Notes:

- Freshly reconstituted reagent R1 should be clear to slightly pink in color. If the absorbance of the reagent at 405 nm without serum added is greater than 0.300 the reagent should be discarded.
- If crystallization of reagent R3 (L-tartrate) occurs, warm at 40 - 50 °C until dissolved.

#### Specimen collection and handling

- Fresh non-hemolyzed serum is the specimen of choice.
- Don't use plasma. Fluoride, oxalate and heparin anticoagulants inhibit acid phosphatase activity.
- Acid phosphatase is extremely labile at the normal pH of serum, therefore, specimens should be treated as follows:
  - Serum should be separated from red cells immediately.
  - Add 20  $\mu$ l of acetate stabilizing reagent (R4) to each 1 ml of serum. Acetate stabilizing reagent should also be added to control serum in the same ratio: 20  $\mu$ l to 1ml.
- Acid phosphatase in serum is stable for 8 hours at 2 - 8 °C. without stabilizer and 8 days at 2 - 8°C with stabilizer.

#### Calibrator

MediCal U Cat. No. 15011

#### Quality control

Meditrol N Cat. No. 15171

Meditrol P Cat. No. 15181

#### Procedure

Wavelength	Hg 405
Spectrophotometer	405 nm
Cuvette	1 cm light path
Temperature	37°C
Measurement	against air or distilled water
Reaction	kinetic - increase

#### Assay: Total and Non-Prostatic acid phosphatase

**Incubate working reagent at 37 °C before use:**

	Total	Non-Prostatic
Working Reagent	1000 $\mu$ l	1000 $\mu$ l
Reagent R3 (L-tartrate)	--	100 $\mu$ l
Sample	100 $\mu$ l	100 $\mu$ l

Mix, incubate for 5 min. at 37°C. Read change in the absorbance per 1 min. for 5 min.

Determine the mean absorbance change per 1 min ( $\Delta A$ /min).

#### Calculation

**Total acid phosphatase:** Activity (U/L) =  $\Delta A$ /min. <sub>sample</sub> X 853

**Non-Prostatic acid phosphatase:** Activity (U/L) =  $\Delta A$ /min. <sub>sample</sub> X 860

**Prostatic acid phosphatase** = Total acid phosphatase - Non-Prostatic acid phosphatase  
(without tartrate) (with tartrate)

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**Note:** It is recommended that each laboratory (as per instrument performance) could make its own factor (F) by using a calibrator according to the following formula :

$$F = \frac{Conc_{calibrator}}{\Delta A / \min_{Calibrator}}$$

### Linearity

Up to 60 U/L.

If the result higher than 40 U/L. Repeat the test using diluted serum (1+2) with sodium chloride solution (0.9 %) and multiply the result by 3.

### Interferences

1. Bilirubin will interfere in the reaction and will lower the acid phosphatase values.
2. Hemolysed specimens contain large amounts of red cell acid phosphatase and should not be used.
3. EDTA will falsely elevate acid phosphatase values.
4. Young *et. al.*, provide a list of drugs and other substances that interfere with the determination of acid phosphatase activity.

### Precaution

Don't ingest any material, in case of contact flush with water.

### Reference range

Serum total acid phosphatase	2.5 – 11.7	U/L
Serum prostatic acid phosphatase	0.2 – 3.5	U/L

### References

1. Hillman, G. F., *Klin. Chem. Klin. Biochem.* 3 : 273 (1971).
2. Doe, R.P., Mellinger, G.T., and Seal, U.S.: *Clin. Chem.* 11: 943 (1965).
3. Kaplan, L.A., Pesce, A.J., (Editors), *Clinical Chemistry: Theory, Analysis, and Correlation*, The C.V. Mosby Co., p. 1087 (1984).
4. Young, D.S., *Effects of Drugs on Clinical Laboratory Tests* fifth edition 2000, AACC Press, Washington.