

# ACID PHOSPHATASE

AC 0120 TC

20 x 6 ml

## SUMMARY OF TEST

Non specific acid phosphatase activity is widely distributed throughout the living world. The enzyme secreted by human prostate gland has attracted most attention. Because of its clinical importance, extensive characterization and structural studies have now been carried out. Since acid phosphatase is also produced in other tissues, the prostatic isoenzyme must be distinguished from the non prostatic for accurate diagnosis. Elevated levels of non prostatic acid phosphatase have been observed in patients with Paget's disease, hyperparathyroidism with skeletal involvement, and in cancers that have invaded the bones.

Numerous phosphate compounds have been proposed as substrates for measuring acid phosphatase activity, such as phenylphosphate, p-nitrophenylphosphate, and thymolphthalein phosphate.  $\alpha$ -Naphthylphosphate was proposed by Babson et al. as a specific substrate for prostatic acid phosphatase. However, Amador et al. demonstrated that this compound could be hydrolyzed by enzymes derived from other tissues. Hillman proposed a method in 1971 that included diazotized 2-amino-5-chlorotoluene (Fast Red TR) that formed a diazo dye that absorbed strongly at 405 nm. L-tartrate was used as a specific inhibitor of prostatic acid phosphatase to establish differentially the amount of prostatic isoenzyme. The above kinetic method is specific, fast, simple and can easily be adapted to automated instrumentation.

## PRINCIPLE OF THE METHOD

The enzyme acid phosphatase (EC 3.1.3.2, Orthophosphoric-monoester Phosphohydrolase - acid optimum) releases  $\alpha$ -Naphthol from the substrate  $\alpha$ -Naphthylphosphate, which is coupled with Fast Red TR to produce a colored complex that absorbs light at 405 nm. The reaction can be quantitated photometrically because the coupling reaction is instantaneous.

L-Tartrate inhibits prostatic acid phosphatase but does not interfere with the reaction mechanism. Therefore, if testing is performed in the presence or absence of L-Tartrate, the difference between the results of the two assays is the level of prostatic acid phosphatase in the serum.

## KIT COMPONENTS

### For in vitro diagnostic use only.

The components of the kit are stable until expiration date on the label.

Keep away from direct light sources.

### Reagent A 20 x 6 ml (powder)

Composition:  $\alpha$ -naphthylphosphate 3mM, sodium citrate 60 mM, Fast Red TR 1 mM, pH 5.3.

### Reagent B 1 x 5 ml (powder)

Composition: sodium L-tartrate 2 M, citric acid 70 mM, sodium citrate 10 mM, pH 5.3.

### Reagent C 1 x 5 ml (liquid)

Composition: acetate buffer 5M, pH 5.0

Store all components at 2-8°C.

## MATERIALS REQUIRED BUT NOT SUPPLIED

Current laboratory instrumentation. Spectrophotometer UV/VIS with thermostatic cuvette holder. Automatic micropipettes. Glass or high quality polystyrene cuvettes. Saline solution.

## REAGENT PREPARATION

Reagent A: reconstitute with 6 ml of deionized water. Swirl to dissolve.  
Stability: 1 day at 15-30°C or 7 days at 2-8°C.

Reagent B: reconstitute with 5 ml of deionized water. Swirl to dissolve.  
Stability: until expiration date stated on the label at 2-8°C.  
If crystallization of components occurs, warm at moderate temperature (40-50°C) until dissolved.

Reagent C: ready to use.

Stability: until expiration date stated on the label at 2-8°C.

Unopened vials are stable until stated expiration date on the vials at 2-8°C.

## PRECAUTIONS

Reagent may contain some non-reactive and preservative components. It is suggested to handle carefully it, avoiding contact with skin and swallow.

Perform the test according to the general "Good Laboratory Practice" (GLP) guidelines.

## SPECIMEN

Serum, must be separated from clot within 2 hours from collection.

Acid phosphatase activity is extremely labile at room temperature. Stabilization of the enzyme can only be achieved by acidifying with the acetate buffer provided. Add 20  $\mu$ l of buffer per 1.0 ml of serum then mix. Treated serum samples will remain stable for seven days when kept refrigerated at 2-8°C.

Do not use plasma. Some anticoagulants inhibit acid phosphate activity and/or cause turbidity.

## TEST PROCEDURE - TOTAL ACP

Wavelength:	405 nm
Lightpath:	1 cm
Temperature:	37°C
dispense in cuvette reagent A:	1 ml
preincubate at 37°C for 5 minutes.	
add sample:	100 $\mu$ l
Mix, execute a first reading of absorbance after 5 minutes, incubating at 37°C. Perform other 5 readings at 60 seconds intervals. Calculate the $\Delta A/min$ .	

## TEST PROCEDURE - NON PROSTATIC ACP

Wavelength:	405 nm
Lightpath:	1 cm
Temperature:	37°C
dispense in cuvette reagent A:	1 ml
dispense in cuvette reagent B:	10 $\mu$ l
preincubate at 37°C for 5 minutes.	
add sample:	100 $\mu$ l
Mix, execute a first reading of absorbance after 5 minutes, incubating at 37°C. Perform other 5 readings at 60 seconds intervals. Calculate the $\Delta A/min$ .	

## RESULTS CALCULATION

Perform calculation in units per litre, multiplying the  $\Delta A/min$  by the factor as it is indicated.

### TOTAL ACID PHOSPHATASE (Total-ACP)

Calculation in U/l:  $\Delta A/min \times 853$   
Calculation in  $\mu$ kat/l:  $U/l \times 0.0167 = \mu$ kat/l

### NON PROSTATIC ACID PHOSPHATASE (NP-ACP)

Calculation in U/l:  $\Delta A/min \times 860$   
Calculation in  $\mu$ kat/l:  $U/l \times 0.0167 = \mu$ kat/l

### PROSTATIC ACID PHOSPHATASE (P-ACP)

The value is obtained by subtracting the result of the NP-ACP assay from the Total-ACP.

## EXPECTED VALUES

Total ACP: < 9.0 U/l (< 0.150  $\mu$ kat/l)  
Prostatic ACP: < 3.0 U/l (< 0.050  $\mu$ kat/l)

## QUALITY CONTROL AND CALIBRATION

It is suggested to perform an internal quality control. For this purpose the following human based control sera are available:

**QN 0050 CH QUANTINORM CHEMA 10 x 5 ml**  
with normal or close to normal control values

**QP 0050 CH QUANTIPATH CHEMA 10 x 5 ml**  
with pathological control values.

If required, a multiparametric, human based calibrator is available:

**AT 0030 CH AUTOCAL H 10 x 3 ml**

Please contact Customer Care for further information.

## TEST PERFORMANCE

### Linearity

the method is linear up to 60 U/l.

If a  $\Delta A/min$  of 0.050 is exceeded, it is suggested to dilute sample 1+9 with saline and to repeat the test, multiplying the result by 10.

### Sensitivity/limit of detection (LOD)

the limit of detection is 1 U/l.

### Interferences

High levels of bilirubin (icteric samples) reportedly inhibit acid phosphatase activity determined by this procedure.

A number of drugs and substances affect acid phosphatase activity. Young et al. has published a comprehensive list.

### Precision

intra-assay (n=20)	mean (U/l)	SD (U/l)	CV%
sample 1	8.90	0.70	7.80
sample 2	19.00	1.40	7.50

inter-assay (n=15)	mean (U/l)	SD (U/l)	CV%
sample 1	10.20	1.20	11.70
sample 2	20.20	1.50	7.40

### Methods comparison

a comparison between Chema and a commercially available product gave the following results:

ACP Chema = x  
ACP competitor = y  
n = 22

y = 0.96x + 0.38 r = 0.97

## WASTE DISPOSAL

This product is made to be used in professional laboratories. Please consult local regulations for a correct waste disposal.

S56: dispose of this material and its container at hazardous or special waste collection point.

S57: use appropriate container to avoid environmental contamination.

S61: avoid release in environment. Refer to special instructions/safety data sheets.

## REFERENCES

Bergmeyer, H.V., Methods of Enzymatic analysis, Weinheim, Verlag Chemie, 3rd p.92 (1984).

Tietz, N.W., Fundamentals of Clinical Chemistry, Philadelphia, W.B. Saunders, p.614 (1976).

Babson, A.L., et al, Am. J. Clin. Path. 32:83 (1959).

Amador, E. et al, Am. J. Clin. Path. 51:202 (1969).

Hillman, G.Z., Clin. Chem. Clin. Biochem 3:273 (1971).

Fabiny-Byrd, D.L., Ertingshausen, G. Clin. Chem. 13:841 (1972).

Ellis, G., et al, J. Clin. Path. 24:493 (1971)

Henry, R.J., Clin. Chem. Prin. and Tech., Hoeber, New York (1964).








Shaw, L.M., et al, Am. J. Clin. Path. 68:57 (1977)

Young, D.S., et al, Clin. Chem. 21:No. 5 (1975)

## MANUFACTURER

Chema Diagnostica  
Via Padre Vincenzo Pellegrini 3  
60035 Jesi (AN) - ITALY - EU  
phone +39 0731 213360  
fax +39 0731 213361  
e-mail: mail@chema.com  
website: http://www.chema.com

## SYMBOLS

	for in vitro diagnostic use only
	lot of manufacturing
	code number
	storage at temperature interval
	expiration date (year/month)
	warning, read enclosed documents
	read the directions