ACID PHOSPHATASE CE

CONTENTS		
REF	1100005 Acid Phosphatase	4 x 10 mL
For *in vitro* diagnostic use only		

ACID PHOSPHATASE

TOTAL AND PROSTATIC
Colorimetric method
KINETIC

OPTIMIZED

PRINCIPLE

The method[1,2] is based on the hydrolysis of α-naphtyl phosphate at pH 5.0 by acid phosphatase (ACP) to produce α-naphtol and inorganic phosphate. The pentanediol acts as a phosphate acceptor increasing the reaction sensitivity.
The α-naphtol reacts with Fast Red TR*, to produce a coloured complex directly proportional to the activity of the ACP in the sample.

$$\alpha\text{-Naphtyl phosphate} + H_2O \xrightarrow{ACP} \alpha\text{-Naphtol} + P_i$$

$$\alpha\text{-Naphtol} + \text{Fast Red TR} \xrightarrow[30\text{-}37^{o}C]{pH\ 5,0} \text{Azo dye}$$

** Diazotized 2- Amino-5-chlorotoluene*

The sample tested in the presence of L-tartrate inhibits the prostatic acid phosphatase of the total ACP activity.

REAGENT COMPOSITION

R1 **Citrate buffer**. Sodium citrate 110 mmol/L, 1,5-pentanediol 220 mmol/L, pH 5.2.

R2 **Citrate/Tartrate buffer.** Sodium citrate 110 mmol/L, 1,5-pentanediol 220 mmol/L, L-tartrate 110 mmol/L, pH 5.2.

R3 **ACP sustrate**. Powder. α-Naphtyl phosphate 12.5 mmol/L, Fast Red TR 1.25 mmol/L, after reconstitution.

R4 **Stabilizer.** Acetate buffer 5 M/L, pH 5.2.

STORAGE AND STABILITY

Store at 2-8ºC.
The reagents are stable until the expiry date stated on the label.

REAGENT PREPARATION

Working reagent. Add 10 mL of **R1** (total ACP) or 10 mL of **R2** (non-prostatic ACP) into a vial of **R3**. Cap and swirl gently until complete solution. Do not shake. The reagent is stable for 10 days at 2-8ºC.
Discard the reagent if presents an absorbance over 0.300 at 405 nm against distilled water or if it fails to recover the declared values of control sera.

SAMPLES

Clear,unhemolyzed serum, separated from the clot, immediately. Do not use plasma. Oxalates and sodium fluoride inhibit ACP while heparin and EDTA cause turbidity in the sample.
To stabilize the enzyme after separation of the serum from the clot, add 50 µL of **R4** to 1 mL of sample. Specimens not preserved in this manner are unsuitable for analysis.
ACP activity in preserved serum is stable for 4-5 days at 2-8ºC.

INTERFERENCES

- Because of the rich content of erythrocytes in ACP activity, hemolytic samples should be avoided.
- High levels of bilirubin also inactivate ACP in a sample.
- Lipemic samples (triglicerides > 5 g/L) do not interfere.[3]

MATERIALS REQUIRED

- Photometer or spectrophotometer with a thermostatted cell compartment set at 30/37ºC, capable to read at 405 nm.
- Stopwatch, strip-chart recorder or printer.
- Cuvettes with 1-cm pathlength.
- Pipettes to measure reagent and samples.

PROCEDURE

1. Preincubate working reagent, samples and controls to reaction temperature.
2. Set the photometer to 0 absorbance with distilled water.
3. For Total acid and/or Non-Prostatic acid tests pipette into labelled cuvettes:

TUBES	Total	Non-Prostatic
Working reagent **R1**	1.0 mL	-
Working reagent R2	-	**1.0 mL**
Sample or control	100 µL	100 µL

4. Mix gently by inversion. Insert cuvette into the cell holder and start stopwatch.
5. Incubate for 5 minutes and record initial absorbance reading.
6. Repeat the absorbance readings exactly after 1, 2 and 3 minutes.
7. Calculate the difference between absorbances.
8. Calculate the mean of the results to obtain the average change in absorbance per minute (ΔA/min).

CALCULATIONS

A. ***Total Acid Phosphatase***

U/L = ΔA/ min x 853

B. ***Non-Prostatic Acid Phosphatase***

U/L = ΔA/ min x 853

C. ***Prostatic Acid Phosphatase***

A (U/L) – B (U/L) = Prostatic Acid Phosphatase

Samples with ΔA/min exceeding 0.170 at 450 nm should be diluted 1:3 with saline and assayed again. Multiply the results by 3.

If results are to be expressed as SI units apply:
U/L x 16.67 = µkat/L

CS/00/034
Joaquim Costa, 18, 2ª planta. 08390 Montgat – Barcelona (Spain). Tel. (+34) 93 4694990 Fax (+34) 93 4693435
Almacén: Sant Antoni Mª Claret, 8 bis. 08390 Montgat – Barcelona (Spain) www.linear.es e-mail: info@linear.es

REFERENCE VALUES [4]

Serum

Reaction temperature	37ºC	30ºC
Total ACP, up to	6.6 U/L (110 nkat/L)	7.0 U/L (278 nkat/L)
Prostatic ACP, up to	3.5 U/L (108 nkat/L)	2.6 U/L (43 nkat/L)

It is recommended that each laboratory establishes its own reference range.

QUALITY CONTROL

To ensure adequate quality control (QC), each run should include a set of controls (normal and abnormal) with assayed values handled as unknowns.

REF **1980005** HUMAN MULTISERA NORMAL
Borderline level of ACP. Assayed.

REF **1985005** HUMAN MULTISERA ABNORMAL
Elevated level of ACP. Assayed.

CLINICAL SIGNIFICANCE

Determination of acid phosphatase activity in serum is directed toward the prostatic enzyme with the intent of detecting carcinoma of the prostate.

Elevations of the activity are found in the sera of about 60% of men with prostatic cancer with metastases.
Slight elevations in total enzyme are observed in cases of thromboembolic phenomena, multiple myeloma, thrombocytopenia and liver disease.

Moderate elevations in total acid phosphatase activity often occur in Paget´s disease, in hyperparathyroidism with skeletal involvement, and in the presence of malignant invasion of the bones by cancers. The serum activity in these cases is not inhibited by tartrate. The only non-bone condition in which elevated activities of tartrate-resistant osteoclast-type acid phosphatase are found in serum is Gaucher´s disease.

ANALYTICAL PERFORMANCE

- ***Linearity.*** Up to 150 U/L
- ***Precision***

U/L	Within-run		Between-run	
Mean	11.2	51.5	8.22	36.2
SD	0.31	0.65	0.42	0.80
CV%	2.76	1.26	5.10	2.21
N	10	10	5	5

Replicates: 10 for each level. Replicates: 5 for each level
Instrument: COBAS MIRA for 4 days.

- ***Sensitivity.*** Using this reagent and method an ΔA/min of 0.100 read at 405 nm is equivalent to 85 mU/mL of phosphatase activity.
- ***Correlation.*** This assay (y) was compared with a similar commercial method (x). The results were:
  N = 28 r = 0.999 y = 0.987x + 0.221

REFERENCES

1. Hillmann, G.V. Clin. Chem. Biochem. 9 : 273 (1971).
2. Fabiny-Byrd, DL and Ertinghausen. Clin.Chem 13:841 (1972).
3. Young, D.S., Pestaner, L.D. and Gibberman, V. Clin. Chem. 21, Vol. 5, 10-432D (1975).
4. Junge, W. et al. Pecs, Hongrie: 3rd Alpe-Adria Congress on Clinical Chemistry and Laboratory Medicine, 7-9 Septembre 1994.

.

B1100-2/0506
R1.ing

Joaquim Costa, 18, 2ª planta. 08390 Montgat – Barcelona (Spain). Tel. (+34) 93 4694990 Fax (+34) 93 4693435
Almacén: Sant Antoni Mª Claret, 8 bis. 08390 Montgat – Barcelona (Spain) www.linear.es e-mail: info@linear.es