Fosfatasas Alcalina

optimizada

For the determination of alkaline phosphatase activity in serum

SUMMARY

Alkaline phosphatase is an enzyme widely distributed in the body. It hydrolyzes the orthophosphoric acid monoesters in alkaline medium.

In adults it comes in part from the liver (thermostable fraction) and in part from the bone, reticuloendothelial and vascular systems (thermolabile fraction), producing different isoenzymes. In normal conditions, bone alkaline phosphatase serum activity reaches its highest activity in children (sometimes being three times the adult levels), as this isoenzyme locates in the osteoblasts (related with calcification and bone formation). The increase that is produced at the end of the third month of pregnancy is also physiological, due to the placental isoenzyme that reaches its highest levels in that period (approximately twofold normal values).

Among the pathologies that affect the alkaline phosphatase serum activity, it can be mentioned: liver and bone (enzyme producers) metastatic carcinoma, bile cholestasis, osteoblast phenomena, malabsorption affections along with ulcer lesions (where a deficiency in vitamin D produces osteomalacia with the concurrent increase of the bone alkaline phosphatase) and even recovering lesions such as acute myocardial infarction and pulmonary or renal infarction.

PRINCIPLE

Alkaline phosphatase breaks the sodium phenyl phosphate in buffered alkaline medium with amino-methyl-propanol (AMP). The freed phenol is determined by reacting with 4-aminoantipyrine and ferricyanide as oxidant agent. The developed color is directly proportional to the enzymatic activity and is measured at 520 nm.

PROVIDED REAGENTS

A. Reagent A: 29 mmol/l 4-aminoantipyrine in 3 mol/l amino-methyl-propanol solution.
B. Reagent B: 1.4 mmol sodium phenyl phosphate.
C. Reagent C: 10 mmol/l potassium ferrocyanide.
S. Standard: phenol solution equivalent to 200 U/l.

NON-PROVIDED REAGENTS

Distilled water.

INSTRUCTIONS FOR USE

Reagent A; preparation: transfer the Reagent B bottle content to the Reagent A bottle, mixing until complete dissolution (14 mM final concentration). Write on the label the preparation date.

Reagent C; preparation: dissolve the contents in 500 ml distilled water. Label and date.
Standard: ready to use.

WARNINGS

Reagents are for “in vitro” diagnostic use.

Use the reagents according to the working procedures for clinical laboratories.

The reagents and samples should be discarded according to the local regulations in force.

Reagent C and Standard: H301 + H311 + H331: Toxic if swallowed, in contact with skin or if inhaled. H314: Causes severe skin burns and eye damage. P262: Do not get in eyes, on skin, or on clothing. P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P302 + P352: IF ON SKIN: Wash with plenty of soap and water. P280: Wear protective gloves/protective clothing/eye protection/face protection.

STABILITY AND STORAGE INSTRUCTIONS

Provided Reagents: stable in refrigerator (2-10ºC) until the expiration date shown on the box.
Reconstituted Reagent A: stable for 5 months in refrigerator (2-10ºC).
Reagent C: once prepared, is stable for 5 months at room temperature and protected from light.

INSTABILITY OR DETERIORATION OF REAGENTS

Blank values above 0.120 O.D. indicate contaminations, discard the reagents.

SAMPLE

Serum
a) Collection: use only fresh serum, not hemolyzed.
b) Additives: not required.
c) Known interfering substances: anticoagulants inhibit the reaction in a 50 to 90%.
See Young, D.S. in References for effect of drugs on the present method.
d) Stability and storage instructions: if the test cannot be performed within 6 hours, the sample should be frozen (-4ºC), since in 24 hours there is a 30 to 50% increase of activity at room temperature or refrigerated (2-10ºC).

REQUIRED MATERIAL (non-provided)
- Spectrophotometer or photocolorimeter.

- Micropipettes and pipettes for measuring the stated volumes
- Test tubes.
- Graduated cylinder.
- Water bath at 37°C.
- Stopwatch.

ASSAY CONDITIONS
- Wavelength: 520 nm in spectrophotometer or 500-550 nm in photocolorimeter with green filter.
- Reaction temperature: 37°C
- Reaction time: 10 minutes
- Sample volume: 50 ul
- Final reaction volume: 3.05 ml

PROCEDURE

In three tubes labeled B (Blank), S (Standard) and U (Unknown), place:

	B	S	U
Reconstituted Reagent A	0.5 ml	0.5 ml	0.5 ml
Pre-incubate in water bath at 37°C a couple of minutes. Then add:			
Serum	-	-	50 ul
Standard	-	50 ul	-
Mix and incubate for exactly 10 minutes. Then, add:			
Reagent C	2.5 ml	2.5 ml	2.5 ml

Immediately mix each tube. Remove the tubes from the bath and read in spectrophotometer at 520 nm or in photocolorimeter with green filter, setting the instrument to zero with distilled water.

STABILITY OF FINAL REACTION

The reaction color is stable for 30 minutes, thus the absorbance should be read within that period of time.

CALCULATIONS

Alkaline phosphatase (IU/l) = factor x (U-B)

where:

$$f = \frac{200 \text{ IU/l}}{(U-B)}$$

QUALITY CONTROL METHOD

Each time the test is performed, analyze two levels of a quality control material (**Standatrol S-E 2 niveles**) with known alkaline phosphatase activities.

REFERENCE VALUES

Adults: 68-240 IU/l
Children: 100-400 IU/l

Note: due to the osteoblast process, the bone isoenzyme is increased in childhood and adolescence (until approximately 18 years old), giving alkaline phosphatase values higher than in adults. It has been observed values up to 700 IU/l values in children with no pathologies that would justified an extra-bone origin of the enzyme.

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

SI SYSTEM UNITS CONVERSION

ALP (U/l) x 0.017 = ALP (ukat/l)

PROCEDURE LIMITATIONS
- See Known interfering substances under SAMPLE.
- Reagent time and temperature are critical. A minute or a centigrade in excess or in deficiency may produce a ± 10% error.
- Phenol contamination: it may come from the glass material, other reagents that contains it, or from the PVC tubing used for transferring distilled water. Containers that have contain phenol, should not be used (Reagent 1 of Uremia Wiener lab., Kunkel phenol, etc.) to prepare the Reagent C.
- A Reagent Blank should be read with each lot of tests.

PERFORMANCE

a) Reproducibility: processing replicates of one sample on the same day, the following results were obtained:

Level	S.D.	C.V.
35 IU/l	± 1.75 IU/l	5.0 %
235 IU/l	± 5.40 IU/l	2.4 %
500 IU/l	± 6.00 IU/l	1.2 %

b) Linearity: the reaction is linear up to 800 IU/l. For higher values, repeat the determination diluting the sample 1:2 or 1:5 with saline solution, so that the obtained values fall within the linearity range. The result should be multiplied by the dilution performed.

c) Detection limit: depends on the photometer used. In spectrophotometers (at 520 nm, with 1 cm optical length square cuvettes, ± 2 nm reproducibility, ≤ 0.5% stray light and ≤ 8 nm pathlength) for a 0.001 O.D. the minimum detectable change of activity will be 1 IU/l.

WIENER LAB. PROVIDES

Kit for 200 tests (Cat. 1361003).

REFERENCES

- Mc Comb, R.B.; Bowers, G.N. - Clin. Chem. 18/2:97 (1972).
- Bowers, G.N.; Mc Comb, R.B. - Clin. Chem. 21/13:1988 (1975).
- Pric, C.P.; Woodmn, D.D. - Clin. Chim. Acta 35/2:265 (1971).
- Conyers, R.A.; Birkett, D.J.; Neale, F.C.; Posen, S. and Brudenell-Woods, J - Biochim. Biophys. Acta 139:363 (1967).
- Skillen, A.W.; Harrison, J. - Clin. Chim. Acta 45:287 (1973).
- Kind, P.R..; King, E.J. - J. Clin. Path. 7:322 (1954).
- Demaría, L.; Setta, F.; Lorenzo, L.E. - VIII Congreso Argentino de Bioquímica, Rev. Asoc. Bioq. Arg. 54/3 (1990).
- Young, D.S. - "Effects of Drugs on Clinical Laboratory Tests", AACC Press, 4th ed., 2001.

Symbols

The following symbols are used in the packaging for Wiener lab. diagnostic reagent kits.

This product fulfills the requirements of the European Directive 98/79 EC for "in vitro" diagnostic medical devices

Authorized representative in the European Community

"In vitro" diagnostic medical device

Contains sufficient for <n> tests

Use by

Temperature limitation (store at)

Do not freeze

Biological risks

Volume after reconstitution

Contents

Batch code

Manufactured by:

Harmful

Corrosive / Caustic

Irritant

Consult instructions for use

Calibrator

Control

Positive Control

Negative Control

Catalog number

Wiener Laboratorios S.A.I.C.
Riobamba 2944
2000 - Rosario - Argentina
http://www.wiener-lab.com.ar
Dir. Téc.: Viviana E. Cétola
Biochemist
A.N.M.A.T. Registered product
Disp. Nº: 1287/77 - 216/00

Wiener lab.

2000 Rosario - Argentina

UR170512