ALP 405

cinética optimizada

For alkaline phosphatase determination. DGKC and SSCC

SUMMARY

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

In adults it comes in part from the liver (thermostable fraction) and in part from the bone, RES and vascular system (thermolabile fraction), yielding different isoenzymes.

Serum activity of bone alkaline phosphatase, under normal conditions, reaches maximum levels in children during growth (up to three times adult's values) since this isoenzyme is found in osteoblasts (related to calcification and bone-formation). The increase produced at the end of the first trimester of pregnancy is also physiologic, at the expense of the placental isoenzyme which reaches its highest levels during this period (about two times over normal levels). Pathologies affecting alkaline phosphatase serum activity include: metastatic carcinomas in bone and liver (enzyme producers), biliary cholestasis, osteoblastic phenomena, malabsorption disorders with ulcerous lesions (where vitamin D deficiency produces osteomalacia leading to increase of bone alkaline phosphatase) and even lesions in process of cure such as acute myocardial infarction, lung or kidney infarction.

PRINCIPLE

Alkaline phosphatase (ALP or orthophosphoric monoester phosphohydrolase - EC 3.1.3.1.) hydrolyzes colorless paranitrophenyl phosphate (pNPP) producing phosphate and p-nitrophenol at pH 9.8. The speed at which the p-nitrophenolate anion (yellow) appears, read at 405 nm, is directly proportional to the enzymatic activity of the sample. Diethanolamine (DEA) regulates the reaction's pH and acts as acceptor of the phosphate release by the phosphatase (transphosphorylation), resulting in an activation of the reaction. DEA has the best conditions in terms of activation and buffering capacity when p-NPP is used as substrate. Therefore, DGKC and SSCC have selected it for the development of their optimized methods.

PROVIDED REAGENTS

A. Reagent A: tablets for individual tests. Each tablet has enough p-NPP for obtain 10 mM final concentration.

B. Reagent B: 1 mol/l diethanolamine solution, pH 9.8 (at 25ºC), containing 0,5 mmol/l magnesium salts.

INSTRUCTIONS FOR USE

Reagent B: ready to use. Cap immediately after use.

Reagent A: dissolve one tablet of Reagent A with 2.5 ml Reagent B. Shake until complete dissolution.

INSTABILITY OR DETERIORATION OF REAGENTS

Suspect deterioration of reconstituted Reagent A if absorbance readings are higher than 0.700 O.D. after setting the instrument to zero with distilled water.

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.

STABILITY AND STORAGE INSTRUCTIONS

Provided Reagents: stable in refrigerator (2-10ºC) until the expiration date shown on the box.

Reconstituted Reagent A: stable in refrigerator (2-10ºC) for one week since reconstitution date.

SAMPLE

Serum

a) Collection: obtain sera in the usual way.

b) Additives: not required.

c) Known interfering substances:

- Visible or intense hemolysis may cause slight alterations in results.
- Common anticoagulants (such as disodium EDTA, oxalate, citrate or fluoride) inhibit alkaline phosphatase activity between a 50% and a 90%.
- Some drugs may alter serum alkaline phosphatase levels, therefore it is advisable to ask patients about the kind of therapy they are undergoing.

See Young, D.S. in References for effect of drugs on the present method.

d) Stability and storage instructions: use preferably fresh serum. If assay is not performed within 6 hours after collection, samples should be kept frozen (-20ºC). If serum is left at room temperature or inside refrigerator (2-10ºC) there is a 5 to 30% increase of alkaline phosphatase activity in 24 hours.

REQUIRED MATERIAL (non-provided)

- Spectrophotometer.
- Micropipettes and pipettes to measure stated volumes.
- Spectrophotometric square cuvettes.
- Water bath at selected reaction temperature.
- Stopwatch.

ASSAY CONDITIONS

- Wavelength: 405 nm
- Temperature reaction: 25, 30 or 37ºC. Select temperature

according to instruments.
- Reaction time: 3 minutes
- Sample volume: 20 ul
- Reagent A volume (Substrate): 2.5 ml
- Final Reaction volume: 2.52 ml

Sample and Substrate volumes may be proportionally changed, maintaining a 1:125 ratio.

PROCEDURE

In a cuvette kept at the selected reaction temperature, place:

Reconstituted Reagent A	2.5 ml
Pre-incubate a few minutes. Then, add:	
Sample	20 ul

Mix at once. Read initial Absorbance and start stopwatch immediately. Record absorbance 1, 2 and 3 minutes after first reading. Determine average change of absorbance/min (ΔA/min), subtracting each reading from the previous one and average values. Use this mean for calculations.

CALCULATIONS

Alkaline Phosphatase (U/l) at 405 nm = ΔA/min x 6,812

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 activity.

REFERENCE VALUES

The following was observed in normal adults (aged 20-60):

Temperature	25ºC	30ºC	37ºC
Adults (U/l)	40-190	45-213	65-300

As a result of osteoclastic process, bone isoenzyme increases during childhood and adolescence (up to 18 years approximately), yielding higher alkaline phosphatase levels than in adults.

The following table shows extremes values found under normal circumstances:

Temperature	25ºC	30ºC	37ºC
Children and adolescents (U/l)	up to 400	up to 450	up to 645

IFCC recommends each laboratory to set its own reference values, selecting groups of people based upon established criteria, according to its own population.

SI SYSTEM UNITS CONVERSION

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

PROCEDURE LIMITATIONS

See Known interfering substances under SAMPLE.

PERFORMANCE

a) Reproducibility: when replicates of the same samples were assayed on the same day, the following results were obtained:

Level	S.D.	C.V.
296 U/l	± 9.15 U/l	3.1 %
923 U/l	± 14.6 U/l	1.6 %

b) Linearity: reaction is linear up to 1,360 U/l. For higher values repeat testing, previous to serum dilution 1/5 or 1/10 with saline solution. Correct calculations multiplying by dilution factor used.

c) Detection limit: depends on the photometer used. According to the required sensitivity, in spectrophotometer at 405 nm (with 1 cm optical length square cuvettes, ± 2 nm reproducibility, ≤ 0.5% stray light, ≤ 8 nm pathlength) for ΔA/min of 0.001, the minimum detectable activity change will be of 7 U/l.

WIENER LAB PROVIDES

Kits for 50 determinations (50 x 2,5 ml) (Cat. Nº 1361401).

REFERENCES

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Symbols

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

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

EC REP — Authorized representative in the European Community

IVD — "In vitro" diagnostic medical device

Σ — Conteins sufficient for <n> tests

Use by

Temperature limitation (store at)

Do not freeze

Biological risks

Volume after reconstitution

Cont. — Contents

LOT — Batch code

Manufactured by:

Xn — Harmful

Corrosive / Caustic

Xi — Irritant

Consult instructions for use

Calibr. — Calibrator

CONTROL — Control

CONTROL + — Positive Control

CONTROL − — Negative Control

REF — Catalog number

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