LIQUID LINE

ALP 405

AA

Optimized kinetic method at 405 nm (DGKC and SSCC) for the determination of alkaline phosphatase

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 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 p-nitrophenylphosphate (pNPP) producing phosphate and p-nitrophenol at alkaline pH. The speed at which the p-nitrophenolate anion (yellow) appears, read at 405 nm, is directly proportional to the enzymatic activity of the sample.

PROVIDED REAGENTS

A. Reagent A: DEA (diethanolamine) buffer solution, containing magnesium salts.

B. Reagent B: solution containing p-nitrophenyl phosphate (p-NPP).

Final concentrations

DEA	1.0 mol/l
Mg	0.5 mmol/l
p-NPP	10 mmol/l

INSTRUCTIONS FOR USE

Provided Reagent: ready to use.

They may be used separately or as Monoreagent, mixing 4 parts of Reagent A with 1 part of Reagent B (e.g. 4 ml Reagent A + 1 ml Reagent B).

INSTABILITY OR DETERIORATION OF REAGENTS

Suspect deterioration of Monoreagent (premixed) if absorbance readings are higher than 0.900 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 (2-10°C) until the expiration date shown on the box. Once opened, they should not remain uncapped and outside the refrigerator for long periods. Avoid contamination.

Monoreagent (premixed): stable for 1 month in refrigerator (2-10°C) from preparation date.

SAMPLE

Serum or plasma

a) Collection: obtain serum in the usual way.

b) Additives: if plasma is used as sample, use heparin as anticoagulant.

c) Known interfering substances:

- No interferences are observed by: bilirubin up to 16 mg/dl, lipid up to 1000 mg/dl triglycerides, nor heparin up to 50 U/l.
- Mild hemolysis (up to 200 mg/dl) do not interfere, but strong hemolysis produce erroneous results.

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.

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
- Reaction temperature: 25, 30 or 37°C. See the REFERENCE VALUES corresponding to each temperature.
- Reaction time: 3 minutes and 20 seconds.

- Sample volume: 10 ul

Sample and Reagent volumes may be proportionally changed, without altering calculation factors.

PROCEDURE I

MONOREAGENT TECHNIQUE

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

Monoreagent	1.0 ml
Pre-incubate a few minutes. Then, add:	
Sample	10 ul

Mix immediately and simultaneously start stopwatch. Read initial absorbance after 20 seconds. 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 averaging values. Use this mean for calculations.

CALCULATIONS

Alkaline Phosphatase (U/l) at 405 nm = ΔA/min x 5,460

PROCEDURE II

SEPARATE REAGENTS TECHNIQUE

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

Reagent A	1.0 ml
Sample	10 ul
Pre-incubate a few minutes. Then, add:	
Reagent B	0.25 ml

Mix immediately and simultaneously start stopwatch. Read initial absorbance after 20 seconds. 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 averaging 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 range of values was observed in normal adults (aged 20-60):

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

As a result of the 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	25°C	30°C	37°C
Children and adolescents (U/l)	up to 400	up to 450	up to 645

IFCC recommends that each laboratory 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.

Common anticoagulants (such as disodium EDTA, oxalate, citrate or fluoride) inhibit alkaline phosphatase activity.

The reagent may be colored in presence of cleaning solution traces composed of hypochlorite. When using the automatic technique, make sure to rinse all the material that may be in contact with hypochlorite with plenty of demineralized water, including needles and autoanalyzer connectors.

PERFORMANCE

The assays were performed in an Express Plus analyzer(*).

a) Reproducibility: precision studies were performed following the guidelines contained in CLSI (EX-NCCLS) document EP5-A:

Intra-assay precision

Level	S.D.	C.V.
119 U/l	± 2.6 U/l	2.2 %
347 U/l	± 2.6 U/l	0.7 %

Total precision

Level	S.D.	C.V.
119 U/l	± 2.9 U/l	2.4 %
347 U/l	± 3.2 U/l	0.9 %

b) Linearity: reaction is linear up to 1,500 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: the minimum detectable change of ALP activity will be 18 U/l.

PARAMETERS FOR AUTOANALYZERS

For programming instructions check the user's manual of the autoanalyzer in use.

WIENER LAB PROVIDES

Kit for 100 ml (Cat. Nr. 1361402) containing:

- 4 x 20 ml Reagent A
- 1 x 20 ml Reagent B

Kit for 100 ml (Cat. Nr. 1009241) containing:
- 4 x 20 ml Reagent A
- 1 x 20 ml Reagent B

Kit for 125 ml (Cat. Nr. 1009301) containing:
- 5 x 20 ml Reagent A
- 2 x 12,5 ml Reagent B

Kit for 200 ml (Cat. Nr. 1361403) containing:
- 4 x 40 ml Reagent A
- 1 x 40 ml Reagent B

Kit for 200 ml (Cat. Nr. 1009602) containing:
- 8 x 20 ml Reagent A
- 2 x 20 ml Reagent B

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SYMBOLS

The following symbols are used in packaging for Wiener lab. diagnostic reagents 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
	Contains 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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