

ALKALINE PHOSPHATASE FL

AL F245 CH	12 x 20 ml
AL F400 CH	8 x 50 ml
AL F600 CH	5 x 120 ml

SUMMARY OF TEST

The alkaline phosphatase is present in practically all tissues of the body, and it occurs at particularly high levels in intestinal epithelium, kidney tubules, bone, liver, and placenta. Although the precise metabolic function of the enzyme is not yet understood, it appears that the enzyme is associated with lipid transport in the intestine and with the calcification process in bone.

The form present in the sera of normal adults probably originates mainly in the liver, with up to half the total activity coming from the skeleton. The respective contributions of these two forms to the total activity are markedly age dependent. Serum ALP measurements are of particular interest in the investigation of two groups of conditions: hepatobiliary disease and bone disease associated with increased osteoblastic activity. For many years, it was believed that ALP reaching the liver from other tissues (especially bone) was excreted into the bile and that the elevated serum enzyme activity found in hepatobiliary disease was a result of a failure to excrete the enzyme through the bile. However, it is now known that the response of the liver to any form of biliary tree obstruction is to synthesize more ALP. Intrahepatic obstruction of the bile flow also raises serum ALP, but usually to a lesser extent. Liver diseases that principally affect parenchymal cells, such as infectious hepatitis, typically also show only moderately elevated or even normal serum ALP levels. Among the bone diseases, the highest levels of serum ALP activity are encountered in Paget's disease. Only moderate rises are observed in osteomalacia, the levels slowly declining in response to vitamin D therapy. Primary hyperparathyroidism and secondary hyperparathyroidism are associated with slight to moderate elevations of ALP activity in serum. Very high enzyme levels are present in patients with osteogenic bone cancer. Transient elevations may be found during healing of bone fractures. Physiological bone growth elevates ALP in serum, and this accounts for the fact that in the sera of growing children one finds enzyme activity some 1.5 to 2.5 times that in normal adult serum. An increase of up to two to three times normal may be observed in women in the third trimester of pregnancy, although the interval is very wide and levels may not exceed the upper limit of the reference interval in some cases. The additional enzyme is of placental origin.

Methods of determining ALP activity have a long history, and numerous methods have had a more or less wide clinical use. The most popular of the chromogenic substrates for ALP is 4-nitrophenyl phosphate. This ester is colorless, but the reaction product is yellow at the pH of the reaction; thus the enzyme reaction can be continuously monitored by observing the rate of formation of the 4-nitrophenoxide ion. With improvements in the reaction conditions, this reaction forms the basis of current recommended and standard methods of ALP assay.

A large number of manual, automated, and commercial kit procedures that use these buffers and chromogenic substrates have been introduced. The standard procedure for the assay of ALP proposed by DGKC uses DEA buffer at pH 9.8 and 4-NPP as substrate.

PRINCIPLE OF THE METHOD

The enzyme alkaline phosphatase (EC 3.1.3.1, orthophosphoric-monoester phosphohydrolase) hydrolyzes the 4-nitrophenol to release 4-nitrophenol, under alkaline conditions. The 4-nitrophenol formed is detected spectrophotometrically at 405 nm to give a measurement of alkaline phosphatase activity in the sample. The present method has been made according to DGKC.

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 F245: 12 x 16 ml (liquid) blue cap
F400: 8 x 40 ml (liquid) blue cap
F600: 4 x 120 ml (liquid) blue cap

Reagent B F245: 3 x 16 ml (liquid) red cap
F400: 2 x 40 ml (liquid) red cap
F600: 1 x 120 ml (liquid) red cap

Composition in the test: DEA buffer pH 9.8 1 M, MgCl₂ 0.5 mM, 4-Nitrophenylphosphate 10 mM.

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

Serum as starter procedure:

Code F245: add 4 ml of reagent B to a bottle of reagent A.
Code F400: add 10 ml of reagent B to a bottle of reagent A.
Code F600: mix 1 part of reagent B with 4 parts of reagent A.
Stability of working reagent: ≥ 60 days at 2-8°C, away from light sources.

Reagent as starter procedure:

use separate reagents ready to use.
Stability: up to expiration date on labels at 2-8°C;
Stability since first opening of vials: ≥ 60 days at 2-8°C.

PRECAUTIONS

Labelling: Xn R48/22-41-38

R48/22: Harmful. Danger of serious damage to health by prolonged exposure if swallowed;
R41: Risk of serious damage to the eyes;
R38: Irritating to skin;
S20/21: When using do not eat, drink or smoke;
S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice;
S36/37/39: Wear suitable protective clothing, gloves and eye/face protection;
S46: If swallowed, seek medical advice immediately and show this container or label.

SPECIMEN

Serum, plasma (heparinate only).

Sera kept at room temperatures usually show a slight but real increase in activity, which varies from 1% over a 6-h period to 3 to 6% over a 1 to 4 days period. Even in sera stored at refrigerator temperature, activity increases slowly. In frozen sera, activity decreases but slowly recovers after thawing the serum.

A similar enhancement of activity, but of greater magnitude, occurs with reconstituted lyophilized preparations, such as those available as control sera or calibrators. In reconstituted material the increases with storage at 4 and 20°C are about 10 and 30%, respectively. Enhancement of activity continues for several days, but at a decreasing rate. The cause of this phenomenon is not known but may be attributed to renaturation of partially denatured enzyme or to dissociation, on warming, of a phosphate-lipoprotein complex or a multimer of the enzyme that was formed in the freeze-drying process.

TEST PROCEDURE (sample as starter)

Wavelength:	405 nm
Lightpath:	1 cm
Temperature:	37°C
dispense in cuvette working reagent:	1 ml
preincubate at 37°C for 5 minutes.	
add sample:	20 µl
Mix, execute a first reading of absorbance after 1 minute, incubating at 37°C. Perform other 3 readings at 60 seconds intervals. Calculate the ΔA/min.	

TEST PROCEDURE (reagent as starter)

Wavelength:	405 nm
Lightpath:	1 cm
Temperature:	37°C
dispense in cuvette reagent A:	1 ml
add sample:	25 µl
incubate at 37°C for 5 minutes.	
dispense in cuvette reagent B:	250 µl
Mix, execute a first reading of absorbance after 1 minute, incubating at 37°C. Perform other 3 readings at 60 seconds intervals. Calculate the ΔA/min.	

RESULTS CALCULATION

Perform calculation in units per litre, multiplying the ΔA/min by the factor as it is indicated.

Calculation in U/l: ΔA/min x 2757

Activity in µkat/l: U/l x 0.0167 = µkat/l

EXPECTED VALUES

Men: < 270 U/l (< 4.50 µkat/l)
Women: < 240 U/l (< 4.00 µkat/l)

Each laboratory should establish appropriate reference intervals related to its population.

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 2800 U/L.

If a ΔA/min of 0.500 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

no interference was observed by the presence of:

hemoglobin	≤ 400 mg/dl
bilirubin	≤ 27 mg/dl
lipids	≤ 1000 mg/dl

Precision

intra-assay (n=10)	mean (U/L)	SD (U/L)	CV%
sample 1	175.70	0.95	0.50
sample 2	426.70	2.41	0.60

inter-assay (n=20)	mean (U/L)	SD (U/L)	CV%
sample 1	167.26	3.99	2.40
sample 2	408.28	8.61	2.10

Methods comparison

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

$$\text{ALP Chema} = x \\ \text{ALP competitor} = y \\ n = 112$$

$$y = 0.96x - 2.17 \text{ U/L} \quad r = 0.999$$

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

J. Clin.Chem.Clin.Biochem 8 (1970) 658; 10 (1972) 182
Tietz Textbook of Clinical Chemistry, Second Edition, Bur-tis-Ashwood (1994).

MANUFACTURER

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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

