

ALKALINE PHOSPHATASE FL IFCC

AF F080 CH	4 x 20 ml
AF F245 CH	12 x 20 ml
AF F400 CH	8 x 50 ml

INTENDED USE

Reagent for quantitative in vitro determination of alkaline phosphatase in biological fluids.

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.

PRINCIPLE OF THE METHOD

The enzyme alkaline phosphatase (EC 3.1.3.1, orthophosphoric-monoester phosphohydrolase) hydrolyzes the 4-NPP 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 IFCC.

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.

ALP IFCC R1 F080: 4 x 16 ml (liquid) blue cap
F245: 12 x 16 ml (liquid) blue cap
F400: 8 x 40 ml (liquid) blue cap

ALP IFCC R2 F080: 1 x 16 ml (liquid) red cap
F245: 3 x 16 ml (liquid) red cap
F400: 2 x 40 ml (liquid) red cap

Composition in the test: 2-amino-2-methyl-1-propanol buffer 0.35 M pH 10.40 (30°C), magnesium acetate 2 mM, zinc sulfate 1 mM, HEDTA 2 mM, 4-NPP 16 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:

Codes F080/F245: add 4 ml of reagent R2 to a bottle of reagent R1.

Code F400: add 10 ml of reagent R2 to a bottle of reagent R1.

Stability of working reagent: preferably within 30 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: preferably within 60 days at 2-8°C.

PRECAUTIONS

ALP IFCC R1: Danger. Causes serious eye damage (H318). Causes skin irritation (H315). IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. (P305+P351+P338). Wear protective gloves / eye protection / face protection (P280). Immediately call a POISON CENTER / doctor (P310). Wash with water thoroughly after handling (P264).

ALP IFCC R2: It is not classified as hazardous.

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 R1:	1 ml
add sample:	25 µl
incubate at 37°C for 5 minutes.	
dispense in cuvette reagent R2:	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: 40 - 129 U/l (0.67 - 2.15 µkat/l)
Women: 35 - 104 U/l (0.58 - 1.74 µkat/l)

QUALITY CONTROL AND CALIBRATION

It is suggested to perform an internal quality control. For this purpose the following human based control sera are available:

QUANTINORM CHEMA - MULTINORM CHEMA

with normal or close to normal control values

QUANTIPATH CHEMA - MULTIPATH CHEMA

with pathological control values.

If required, a multiparametric, human based calibrator is available:

AUTOCAL H

Please contact Customer Care for further informations.

TEST PERFORMANCE

Linearity

the method is linear up to 3000 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 5.2 U/l.

Interferences

no interference was observed by the presence of:

hemoglobin ≤ 400 mg/dl
bilirubin ≤ 40 mg/dl
lipids ≤ 900 mg/dl

Precision

intra-assay (n=10)	mean (U/l)	SD (U/l)	CV%
sample 1	84.40	2.41	2.86
sample 2	222.40	5.74	2.58

inter-assay (n=20)	mean (U/l)	SD (U/l)	CV%
sample 1	86.66	2.66	3.07
sample 2	210.39	6.08	2.89

Methods comparison

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

ALP Chema = x
ALP competitor = y
n = 150

y = 1.03x - 2.57 U/l r² = 0.998

WASTE DISPOSAL

This product is made to be used in professional laboratories.

P501: Dispose of contents according to national/international regulations.








REFERENCES

Clin. Chim. Acta, (1983) 339F - 367F
Tietz Textbook of Clinical Chemistry, Second Edition, Burtis-Ashwood (1994).

MANUFACTURER

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SYMBOLS

	in vitro diagnostic medical device
	batch code
	catalogue number
	temperature limit
	use-by date
	caution
	consult instructions for use