ALKALINE PHOSPHATASE FL DGKC

AL F080 CH	4 x 20 ml
AL F245 CH	12 x 20 ml
AL F400 CH	8 x 50 ml
AL F600 CH	5 x 120 ml
AL 100F CH	5 x 200 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) hydrolizes 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 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.

ALP DGKC 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 F600: 4 x 120 ml (liquid) blue cap 100F: 4 x 200 ml (liquid) blue cap

ALP DGKC 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

F600: 1 x 120 ml (liquid) red cap 100F: 1 x 200 ml (liquid) red cap

Composition in the test: DEA buffer pH 9.8 1 M, MgCl, 0.5 mM, 4-Nitrophenilphosphate 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:

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.

Code F600/100F: mix 1 part of reagent R2 with 4 parts of reagent R1.

Stability of working reagent: preferably within 60 days at 2-8°C, away from light sources.

Reagent as starter procedure:

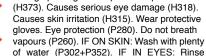
use separate reagents ready to use.

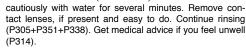
Stability: up to expiration date on labels at 2-8°C;

Stability since first opening of vials: preferably within 60 days at 2-8°C

PRECAUTIONS

ALP DGKC R1: Danger. May cause damage to organs through prolonged or repeated exposure





ALP DGKC 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)

Wavelenght: 405 nm Ligthpath: 1 cm Temperature: 37°C dispense in cuvette working reagent: 1 ml

preincubate at 37°C for 5 minutes.

20 µl add sample:

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)

Wavelenght: 405 nm Ligthpath: 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 $\Delta A/min$.

RESULTS CALCULATION

Perform calculation in units per litre, multiplying the $\Delta A/min$ by the factor as it is indicated.

Calculation in U/I: ΛA/min x 2757

Activity in ukat/l: $U/I \times 0.0167 = \mu kat/I$

EXPECTED VALUES

Men: < 270 U/I(< 4.50 ukat/l)Women: < 240 U/I $(< 4.00 \, \mu kat/l)$

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

with normal or close to normal control values

QUANTIPATH 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/I.

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 7 U/I.

Interferences

no interference was observed by the presence of:

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

Precision

intra-assay (n=10)	mean (U/I)	SD (U/I)	CV%
sample 1	175.70	0.95	0.50
sample 2	426.70	2.41	0.60
inter-assay (n=20)	mean (U/I)	SD (U/I)	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:

> ALP Chema = x ALP competitor = y n = 112

y = 0.96x - 2.17 U/I

 $r^2 = 0.999$

WASTE DISPOSAL

This product is made to be used in professional laboratories

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

REFERENCES

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

MANUFACTURER

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SYMBOLS

IVD in vitro diagnostic medical device

LOT batch code

REF catalogue number X temperature limit

2 use by date ⚠ caution

 \prod i consult instructions for use