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, $MgCl_2$ 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 (H373). Causes serious eye damage (H318). Causes skin irritation (H315). Wear protective gloves. Eye protection (P280). Do not breath vapours (P260). IF ON SKIN: Wash with plenty of water (P302+P352). IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing (P305+P351+P338). Get medical advice if you feel unwell (P314).

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.	
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)

Wavelenght:	405 nm
Ligthpath:	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:	< 270 U/l	(< 4.50 µkat/l)
Women:	< 240 U/l	(< 4.00 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
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/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 7 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:

ALP Chema = x
ALP competitor = y
n = 112

y = 0.96x - 2.17 U/l 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
	temperature limit
	use by date
	caution
	consult instructions for use

CE