ALKALINE PHOSPHATASE SL

Kinetic Method - DGKC

Liquid Reagent ready to use

REF. 4096 2x 50 ml
REF. 4097 2x100 ml
REF. 4097/4 4x100 ml

CE
IVD

Azienda certificata DNV

GIESSE DIAGNOSTICS

INTENDED USE

Quantitative determination of alkaline phosphatase (ALP) in serum and plasma in accordance with DGKC recommendations.

PRINCIPLE

The enzyme alkaline phosphatase hydrolizes the p-nitrophenylphosphate (4-NPP) to releasing the p-nitrophenol (4-NP) whose formation rate can be measured spectrophotometrically a 405 nm to quantify the activity of the enzyme present in the sample.

SAMPLE

Serum, plasma with heparin. Do not use other anticoagulants such as EDTA, oxalate and citrate by inhibiting the enzyme. Avoid hemolyzed samples.

Sera kept at room temperature usually show a slight increase in activity, which varies from 1 % over a 6-h period to 3-6 % 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.

KIT COMPONENTS

Reagent (A) ALP Volume = 40/80 ml	Buffer DEA Magnesium chloride	1 mmol/l 0.5 mmol/l
Reagent (B) ALP Volume = 10 ml	p-nitrophenylphosfate	10 mmol/l

The reagents are stable until the expiration date indicated on the label if stored at 2-8°C and protected from light. Do not freeze. Once opened reagents are stable for 2 months at 2-8°C if contamination is avoided.

Keep bottles closed when not in use.

REAGENTS PREPARATION

Liquid Reagents, bring Reagent to room temperature (15-25°C) before use.

For **use as monoreagent**: add a part of Reagent (B) to 4 parts of Reagent (A).

The working solution (A+B) is stable 3 days at 15-25°C and 3 weeks at 2-8°C.

PRECAUTIONS AND WARNINGS

The Reagent (A) contains **Diethanolamine** and according to current regulation is classified as: **Xn-Harmful**. **R41**- Risk of serious damage to eyes. **R48/22**-Harmful: danger of serious damage to health by prolomged exposure if swallowed. **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.

Reagents may contain some non-reactive and preservative components. It is suggested to handle carefully it, avoiding contact with skin and swallow.

Use the normal precautions required in the laboratory.

Dispose of waste according to local laws.

PROCEDURE

Wavelength:	405 nm (410 nm)
Lightpath :	1 cm
Temperature:	37°C
Reading:	against distilled water
Method:	increasing kinetic

Use as monoreagent:

pipette:	
Working solution (A+B)	1000 µl
sample	20 µl

Mix, incubate at 37°C for 1 minute, read the initial absorbance against water. Make 3 readings at a distance of 60 seconds. Calculate the average value of the absorbance variations per minute. (ΔA/min).

Use as bireagent:

pipette:	
Reagent (A)	800 µl
sample	20 µl
mix and after 1 minute add:	
Reagent (B)	200 µl

Mix, incubate at 37°C for 1 minute, read the initial absorbance against water. Make 3 readings at a distance of 60 seconds. Calculate the average value of the absorbance variations per minute. (ΔA/min).

This method describes the manual procedure to use the kit.

For automated procedure, ask for specific applications.

RESULTS CALCULATIONS

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

Activity in U/L : ΔA/min x 2480 (*)

(*) Factor calculated in our laboratories. We recommend the use of Clinical Chemistry Calibrator (Ref. 6002/8 - 8x3 ml) to verify that this factor is correct for your test system.

EXPECTED VALUES

Adults: 98 – 279 U/L
Children: 250 – 775 U/L

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

QUALITY CONTROL

You must perform the controls at each kit's use and verify that the values obtained are within the reference range reported in the operating instructions. For this purpose we recommend the use of control sera: PRECISENORM (REF.6000) and PRECISEPATH (REF.6001).

PERFORMANCE

Sensitivity: the sensitivity of the method is: 15 U/L.

Linearity: the method is linear up to 1600 U/L. For higher values, dilute the sample 1:10 and multiply the result by 10.

Precision intra-assay:

	Level 1	Level 2	Level 3
Mean (U/l)	174.6	316.7	596.2
DS	2.547	1.767	9.223
CV %	1.46	0.56	1.55

Precision inter-assay:

	Level1	Level 2	Level 3
Mean (U/l)	183.5	328.8	591.8
DS	1.958	4.517	6.015
CV %	1.07	1.37	1.02

Interferences: bilirubin does not interfere up to 20 mg/dl. Triglycerides do not interfere up to 1000 mg/dl. Hemoglobin does not interfere up to 400 mg/dl. Glucose up to 500 mg/dl does not interfere.

Correlation against a reference method: Y = 0.96x - 2.7 r = 0.999

REFERENCES

1. J.Clin. Chem. Clin. Biochem 8 658 (1970).
2. Vassault, A. et al. Ann. Biol. Clin., 44,686 (1986).

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