Alkaline PHOSPHATASE

Kinetic Method – DGKC

R1: 2 x 40 ml + R2: 2 x 10 ml CL32-100
R1: 2 x 80 ml + R2: 2 x 20 ml CL32-200

INTENDED USE

Kit for quantitative determination of alkaline phosphatase in serum and plasma according to S.C.E. method.

CLINICAL MEANING

The enzyme Alkaline phosphatase (ALP) can be found in various tissues, particularly in bones and liver. Its levels can indicate different skeletal pathologies or hepatic diseases.

PRINCIPLE

Alkaline phosphatase hydrolizes p-nitrofenilphosphate in phosphate and p-nitrophenol. The variation of absorbance in the time unit measured at 405 nm is proportional to the activity of the enzyme in the sample.

SAMPLE

Serum, plasma (heparin only). Avoid hemolyzed samples.
Stability: store the samples at 2-8°C and analyse as soon as possible. Samples kept at room temperature show an increase in activity: 1% in 6 hours and 2-6% after 1-4 days.

REAGENTS

Only for in Vitro diagnostics.

Package content	CL32-100	CL32-200
REAGENT 1 Buffer DEA/HCl (pH 10.0) 1 mol/L, magnesium chloride 0.5 mmol/L.	2 x 40 ml	2 x 80 ml
REAGENT 2 Glycine buffer (pH 9.5) 30 mmol/L, p-nitrophenilphosphate 50 mmol/L, sodium azide 15 mmol/L.	2 x 10 ml	2 x 20 ml

STABILITY: store the reagents at 2-8°C away from direct light to keep them stable up to the expiration date on the label. Once opened, the reagents are stable for 2 months at 2-8°C if contaminations are avoided. Keep the bottles closed when not in use. Do not use turbid reagents.

NECESSARY ITEMS – NOT PROVIDED

Usual laboratory equipment: UV/VIS Spectrophotometer with temperature control; automatic micropipettes; Optical glass cuvettes or, alternatively, disposable ones in optical polystyrene; Saline solution.

WORKING REAGENT PREPARATION

(only for monoreagent procedure)

Mix 4 volumes of Reagent 1 with 1 volume of Reagent 2.
STABILITY: 5 days at 20-25°C, 4 weeks at 2-8°C (if kept tightly closed and away from light).

MANUAL ASSAY PROCEDURE

Wavelenght :	405 nm
Optical Path:	1 cm
Reading:	Against blank reagent
Temperature:	25 – 30 - 37°C
Method:	Increasing kinetic
Reaction time:	3 minutes
Sample/Reagent Ratio (Bireagent):	1/100/25
Sample/Reagent Ratio (monoreagent)	1/100

BIREAGENT PROCEDURE

Bring the necessary reagents to the chosen temperature.

Pipette in cuvette:

Sample	20 µl
Reagent 1	1,0 ml

Stir and incubate to the chosen temperature for 1 minute. Add:

Reagent 2	250 µl

Incubate at the chosen temperature for 1 minute. Read initial absorbance, repeat reading every minute for 3 minutes. Calculate the average value of absorbance variations (ΔA/min).

MONOREAGENT PROCEDURE

Bring the reagent to the chosen temperature.

Pipette in cuvette:

Sample	20 µl
Working reagents	1,0 ml

Incubate at the chosen temperature for 1 minute. Read initial absorbance, repeat reading every minute for 3 minutes. Calculate the average value of absorbance variations (ΔA/min).
Reaction volumes (for both procedures) can be proportionally varied without change in calculation.

CALCULATION

Calculate enzymatic activity in the sample multiplying the ΔA/min by the right factor in the following table.

Monoreagent Procedure	Bireagent Procedure
2757	3424

REFERENCE VALUES

	25°C	30°C	37°C
Adults	60 ÷ 180 U/L	80 ÷ 210 U/L	100 ÷ 275 U/L
Children	110 ÷ 172 U/L	145 ÷ 950 U/L	180 ÷ 1200 U/L

Each laboratory should define their own reference values.

QUALITY CONTROL – CALIBRATION

All Clinical Chemistry laboratories should implement a quality control program. Control serums of human origin are available for this purpose on request:
PRE-NORM serums with normal values
PRE-PATH serums with pathological values
If the method requires it, a multiparameter calibrator of human origin is available.

PERFORMANCE CHARACTERISTICS

Sensitivity
The sensitivity of the method is 5 U/L.
Linearity
The method is linear up to 2500 U/L (a 37°C).
For higher values dilute the samples 1:10 with saline solution and multiply the result by 10.
Precision

Within run (n=10)	Average [U/L]	SD	CV %
Sample 1	182,6	4	2,2
Sample 2	510,9	10,2	2

Within run (n=20)	Average [U/L]	SD	CV %
Sample 1	194,2	9,9	5,1
Sample 2	490,7	28,5	5,9

Interference
Bilirubin does not interfere up to 30 mg/dl. The presence of Hemoglobyn (Hemolysis) causes overestimated values. Detergents inhibit the enzyme. Disposable tools are preferable.
Correlation against a reference method
The correlation of FAR method (Y) with reference method (X) highlighted the following equation:

Y = 1,0583X + 2,8975 r = 0,9973

DISPOSAL

The product must be used for professional analysis only. The product must be disposed of according to national/international laws.

WARNINGS AND PRECAUTIONS

WARNING: REAGENT 1 causes severe eye irritation (H319). It also causes skin irritation (H315).
IN CASE OF CONTACT WITH THE EYES OR SKIN: rinse thoroughly with water for several minutes. If the irritation continues, call a doctor.
REAGENT 2 is not classified as dangerous.

REFERENCES

1. Scandinavian Society for Clinical Chemistry, Committee on Enzymes. Recommended methods for the determination of four enzymes in blood. Scan. J. Clin. Lab. Invest. 33, 291, (1974).
2. German Society for Clinical Chemistry, Standard method for determination of Alkaline Phpsphatase (AP) activity. J. Clin. Chem. Clin. Biochem. 10, 290 (1972).
3. NCCLS Document, "Procedures for the collection of arterial blood specimens", Approved Standard, 3rd Ed. (1999).
4. EU-Dir 1999/11 Commission Directive of 8 March 1999 adapting to technical progress the principles of good laboratory practice as specified in Council Directive 87/18/EEC.

MANUFACTURER

FAR
Via Fermi, 12 - 37026 Pescantina - VERONA - ITALY
tel +39 045 6700870
sito web http://www.fardiag.com
e-mail: order@fardiag.com
e-mail: fardiag@fardiag.com

KEY SYMBOLS

IVD	in Vitro diagnostic medical device
LOT	batch number
REF	catalogue number
	temperature limits
	use by
	caution
	consult accompanying documents

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