



ALKALINE PHOPHATASE LR liquid reagent

REF C3000620/C3000620A 456 Test

CE C3000650/C3000650A 1152 Test

IVD For in vitro medical device

Use

Kit for measurement of alkaline phosphatase in serum or plasma.
Colorimetric optimized method DEA DGKC.

Summary

Alkaline phosphatase measurements are used in the diagnosis and treatment of hepatobiliary and bone disease.

Principle

Kinetic analysis. Alkaline phosphatase (ALP) catalyzes the hydrolysis, in alkaline environment, of p-nitrophenylphosphate into p-nitrophenol and phosphate. The rate of increase in absorbance due to the formation of p-nitrophenol is directly proportional to sample ALP activity.

Reagents

R1	Diethanolamine buffer	pH 9.8	3.5 mol/l
	magnesium chloride	1.0	mmol/l
R2	P-nitrophenylphosphate	45.0	mmol/l

Reagents Preparation

Reagents are liquid and ready to use. About use as monoreagent ("sample-starter" procedure) add the entire content of one bottle of Alkaline phosphatase R2 in the alkaline phosphatase R1 bottle and mix gently. For minor use add for every 4 ml of R1 reagent, 1 ml of R2 reagent. Keep out the reagents from refrigerator only for the use and recap them immediately.

Storage and stability

- Store the kit at 2-8°C.
- After opening, the vials R1 and R2 are stable 90 days if recapped immediately and protected from contamination, evaporation, direct light, and stored at the correct temperature.
- Working solution stability (R1+ R2): 20 days at 2-8°C.

Precaution

The product is not classified as dangerous (DLg. N. 285 art. 28 l. n. 128/1998).

However the reagent should be handled with care, according to good laboratory practice. Caution: the reagents contain Sodium Azide (0.095%) as preservative. Avoid swallowing and contact with skin, eyes and mucous membranes.

The reagent R1 contains diethanolamine; risk of serious damage for eyes; dangerous for ingestion (R41- R48/22).

Waste Management

Please refer to the local legal requirements.

Specimen Collection and Preparation

- Serum or heparinized plasma.
- Do not use samples with haemolysis. The withdrawal must be made, preferably, after 8 hours of fasting. The anticoagulants as EDTA, citrate and oxalate must not be used because they inhibit the enzyme activity.
- The ALP is stable in the samples up to 7 days at 2-8°C.

Note

- The kit, according to this method, must be used in manual procedures. About automatic use follow specific applications.
- Avoid direct light, contamination and evaporation.
- The volumes in the procedure can be changed proportionally.
- In case of complaint or quality control request, refer to the lot number on the package or the lot number on the singles vials.

Procedures

Wavelength	λ: 405 (400- 410) nm
Working temperature	37°C
Optical path	1 cm
Reaction	"kinetic" (increasing)

Bring the reagents at 15-25°C before use.

Monoreagent Procedure "sample starter"

	BLANK	STD	SAMPLE
Working reagent	1000 µl	1000 µl	1000 µl
Distilled Water	20 µl	--	--
Sample	--	--	20 µl
Standard	--	20 µl	--

Mix, then incubate for 1' at 37°C. Measure the absorbance of sample (EC) against distilled water decrease per minute during 3 minutes. Calculate the absorbance variation ΔE/min from performed readings.

Bireagent Procedure "substrate starter"

	BLANK	STD	SAMPLE
Reagent R1	800 µl	800 µl	800 µl
Distilled Water	20 µl	--	--
Sample	--	--	20 µl
Standard	--	20 µl	--

Mix, incubate at 37°C for 1' and then add:

	BLANK	STD	SAMPLE
Reagent R2	200 µl	200 µl	200 µl

Mix, then incubate for 1' at 37°C. Measure the absorbance of sample (EC) against distilled water decrease per minute during 3 minutes. Calculate the absorbance variation ΔE/min from performed readings.

Calculation

$$ALP [U/l] = \Delta E/min \times 2757$$

The factor and the reagent performances are related to 37°C, 1 cm and 405 nm.

Reference Values to 37°C

	Serum - plasma
Adults	100 -290 [U/l]
Children	180 - 1200 [U/l]

Reference values are considered indicative since each laboratory should establish reference ranges for its own patient population. The analytical results should be evaluated with other information coming from patient's clinical history.

ANALYTICAL PERFORMANCES

Linearity

Reaction is linear up to a concentration of 1600 U/l. Samples with values exceeding this range must be diluted with saline solution. Then multiply the result for diluting factor.

Analytical Sensitivity

The test sensitivity in terms of detection limit is 9.80 U/l.

"Intra-Assay" precision (within-Run)

Determined on 20 samples for each control (N-H) (Normal-High). Results:

MEAN (U/l)	N = 179.35	H = 460.95
S.D.	N = 1.56	H = 3.07
C.V.%	N = 0.87	H = 0.67

"Inter-Assay" precision (between-run)

Determined on 20 samples for each control (N-H). Results:

MEAN (U/l)	N = 173.23	H = 461.50
S.D.	N = 2.17	H = 3.36
C.V.%	N = 1.27	H = 0.73

Correlation

A study based comparing this method with a similar method on 20 samples has given a correlating factor $r = 0.99$

$$y = 1.016x + 8.299$$

Interferences

No interferences was observed by the presence of :

Bilirubin	≤ 25 mg/dl
Triglycerides	≤ 800 mg/dl
Ascorbate acid	≤ 20 mg/dl
Haemoglobin	≤ 100 mg/dl

For a comprehensive review of interfering substances, refer to the publication by Young.

Quality controls

It's necessary, each time the kit is used, to perform quality controls and to check that values obtained are within the acceptance range provided in the insert. Each laboratory should establish its own mean and standard deviation and adopt a quality control program to monitor laboratory testing.

Bibliography

Deutsche Gesellschaft für Klinische Chemie (DGKC). Recommendation of the German Society of Clinical Chemistry. Standardization of methods for measurement of enzymatic activities in biological fluids. Z. Klin. Chem. Klin. Biochem., 10, 182 (1972). Kubler W: Symp. D. Deutsch Ges. für Lab. Med., 8 (1973). Fischbach F, Zawta B: Klin. Lab., 38, 555 (1992). Szasz G. et al.: Z. Kindrheilk, 111, 233 (1971). Kaplan LA, Pesce AJ: "Clinical Chemistry", Mosby Ed. (1996). Young D.S., Effects of Drugs on Clinical Laboratory Tests, AACC Press, Washington, DC 5th ed.2000.

Symbols

CE CE Mark (98/79 CE regulation)

IVD in vitro medical device

LOT Batch Code

Hourglass icon Use by

Thermometer icon Storage temperature limits

Open book icon Read instruction for use

Factory icon Producer

Alkaline Phosphatase LR
Mod. 7.3.5 Rev. 0 of 2006-01

Gesan Production s.r.l.

Sede legale: Via Einaudi, 19 - 91021 TRE FONTANE - Campobello di Mazara (TP) Ufficio e Magazzino: Via Fiera dell'Eremita, 71 - Campobello di Mazara (TP) - Part. IVA 01928730819
Tel +39 0924 912396 - Fax +39 0924 912534 // INTERNET: WWW.GESANPRODUCTION.IT - e-mail: gesan@gesanproduction.it