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CE CORMAY

Liquick Cor - ALP

Kit name	(EN) Cat. No
Liquick Cor-ALP mini	1-291
Liquick Cor-ALP 30	1-218
Liquick Cor-ALP 60	1-212
Liquick Cor-ALP 500	1-317

INTENDED USE

Diagnostic kit for determination of alkaline phosphatase activity used both for manual assay (Sample Start and Reagent Start method) and in several automatic analysers.
The reagents must be used only for *in vitro* diagnostic, by suitably qualified laboratory personnel, only for the intended purpose, under appropriate laboratory conditions.

INTRODUCTION

Alkaline phosphatase (ALP) is actually a group of isoenzymes that hydrolyse monophosphate esters in alkaline medium. Optimum pH for these ALP isoforms activities is about 9-10. Alkaline phosphatase level is the highest in liver, bone, intestine, kidney and placenta. Measurement of ALP isoenzymes is useful in diagnosis of these organs diseases.

METHOD PRINCIPLE

Kinetic method recommended by International Federation of Clinical Chemistry (IFCC).

2-amino-2-methyl-1-propanol + p-nitrophenylophosphate + H_2O $\xrightarrow{ALP}$ 4-nitrophenol + 2-amino-2-methyl-1-propanol phosphate

The rate of 4-nitrophenol formation is directly proportional to the ALP activity.

REAGENTS

Package

	Liquick Cor-ALP mini	Liquick Cor- ALP 30	Liquick Cor- ALP 60	Liquick Cor- ALP 500
1-ALP	2 x 24 ml	5 x 24 ml	5 x 48 ml	3 x 400 ml
2-ALP	1 x 12 ml	1 x 30 ml	1 x 60 ml	1 x 300 ml

The reagents when stored at 2-8°C are stable up to expiry date printed on the package. The reagents are stable for 12 weeks (Biolis 24i Premium) on board the analyser at 2-10°C.

Working reagent preparation and stability

Assay can be performed with use of separate 1-ALP and 2-ALP reagents or with use of working reagent. For working reagent preparation mix gently 4 parts of 1-ALP with 1 part of 2-ALP. Avoid foaming.
Stability of working reagent: 4 weeks at 2-8°C
5 days at 15-25°C

Concentrations in the reagents

1- REAGENT	
2-amino-2-methyl-1-propanol (AMP)	≤ 510 mmol/l
Mg^{2+}	≤ 3,0 mmol/l
Zn^{2+}	≤ 1,5 mmol/l
HEDTA	≤ 3,0 mmol/l
preservative	
2-REAGENT	
2-amino-2-methyl-1-propanol (AMP)	≤ 60 mmol/l
p-nitrophenyl	≤ 100 mmol/l
preservative	

Warnings and notes

- Protect from direct sunlight and avoid contamination!
- During the reaction p-nitrophenol is produced. Do not swallow or inhale, avoid contact with skin.
- Slightly yellow colour of working reagent is normal and does not influence the result.
- The reagents are usable when the absorbance of the working reagent is less than 1.300 (read against distilled water, wavelength λ=405 nm, cuvette l=1 cm, at temp. 25°C).
- Please refer to the MSDS for detailed information concerning safe storage and use of the product.

ADDITIONAL EQUIPMENT

- automatic analyzer or photometer able to read at 410 nm (405/412 nm);
- thermostat at 37ºC;
- general laboratory equipment;

SPECIMEN [12]

Serum, heparinized plasma free from hemolysis.
Do not use EDTA, citrate and oxalate as anticoagulants because of ALP activity inhibition!
ALP activity remains stable in specimen up to 4 hours at 15-25ºC. Freezing of sample causes a decrease of enzyme activity. Frozen specimens should be thawed and kept at room temperature for 18 to 24 hours before measurement to achieve full enzyme reactivation.
Nevertheless it is recommended to perform the assay with freshly collected samples!

PROCEDURE

Applications for analysers are available on request.

Manual procedure

wavelength	410 nm (405/412 nm)
temperature	37°C
cuvette	1 cm

Sample Start method

Pipette into the cuvette:

working reagent	1000 µl

Bring up to the temperature of determination. Then add:

sample	18 µl

Mix and incubate at adequate temperature. After about 1 min. read the absorbance against air or water. Repeat the reading after exactly 1, 2 and 3 minutes. Calculate the mean absorbance change per minute (ΔA/min.).

Reagent Start method

The determination can be also performed with use of separate 1-ALP and 2-ALP reagents.

Pipette into the cuvette:

1-ALP	1000 µl

Bring up to the temperature of determination. Then add:

sample	17 µl

Mix well, incubate for 1 min. Then add:

2-ALP	250 µl

Mix well; perform measurement as described for Sample Start method.

Calculation

ALP activity [U/l] = ΔA/min. x F

Sample Start method	Reagent Start method
F = 3038	F = 3442

REFERENCE VALUES [9-11]

Gender	Age	U/l (37°C)	µkat/l (37°C)
female	1 – 30 days	48 – 406	0.80 – 6.77
	31 days – 1 year	124 – 341	2.07 – 5.68
	1 year – 3 years	108 – 317	1.80 – 5.28
	4 – 15 years	54 – 369	0.91 – 6.23
	16 – 18 years	35 – 124	0.58 – 2.07
	19 – 20 years	39 – 118	0.65 – 1.97
	20 – 50 years	42 – 98	0.71 – 1.67
	50 – 60 years	39 – 118	0.65 – 1.97
	≥ 60 years	53 – 141	0.90 – 2.40
male	1 – 30 days	75 – 316	1.25 – 5.27
	31 days – 1 year	82 – 383	1.37 – 6.38
	1 year – 3 years	104 – 345	1.73 – 5.75
	4 – 15 years	54 – 369	0.91 – 6.23
	16 – 18 years	58 – 331	0.97 – 5.52
	19 – 20 years	41 – 137	0.68 – 2.28
	20 – 50 years	53 – 128	0.90 – 2.18
	50 – 60 years	41 – 137	0.68 – 2.28
	≥ 60 years	56 – 119	0.95 – 2.02

It is recommended for each laboratory to establish its own reference ranges for local population.

QUALITY CONTROL

Pay attention to preparation of calibrator and control before ALP determination.
For internal quality control it is recommended to use with each batch of samples the CORMAY SERUM HN (Cat. No 5-172) and CORMAY SERUM HP (Cat. No 5-173).
For the calibration of manual assay the CORMAY MULTICALIBRATOR LEVEL 1 (Cat. No 5-174; 5-176) or LEVEL 2 (Cat. No 5-175; 5-177) is recommended.
For the calibration of automatic analysers systems the CORMAY MULTICALIBRATOR LEVEL 1 (Cat. No 5-174; 5-176) and LEVEL 2 (Cat. No 5-175; 5-177) is recommended. Deionised water should be used as a calibrator 0.
The calibration curve should be prepared every week (Biolis 24i Premium) with change of reagent lot number or as required e.g. quality control findings outside the specified range.

PERFORMANCE CHARACTERISTICS

The following results have been obtained using Multi+ for manual assay and automatic analyser Biolis 24i Premium. Results may vary if a different instrument is used.

- **LOQ:** 6.3 U/l (0.105 µkat/l) - Biolis 24i Premium

- **Sensitivity:** 27.8 U/l (0.46 µkat/l) - Multi+

- **Linearity:**

up to 620 U/l (10.33 µkat/l) - Multi+
up to 760 U/l (12.67 µkat/l) - Biolis 24i Premium

For higher activity dilute sample with 0.9% NaCl and repeat the assay. Multiply the result by the dilution factor.

- **Specificity / Interferences**

Haemoglobin up to 0.625 g/dl, ascorbate up to 62 mg/l, bilirubin up to 20 mg/dl and triglycerides up to 1000 mg/dl do not interfere with the test.

- **Precision**

Repeatability (run to run)		Mean [U/l]	SD [U/l]	CV [%]
Multi + n=7	level 1	98.11	2.79	2.85
	level 2	415.29	3.98	0.96
Biolis 24i Premium n=20	level 1	104.5	0.73	0.70
	level 2	440.5	6.1	1.4
Reproducibility (day to day)		**Mean [U/l]**	**SD [U/l]**	**CV [%]**
Multi + n=10	level 1	101.23	4.33	4.27
	level 2	459.85	11.08	2.41
Biolis 24i Premium n=80	level 1	98.6	4.0	4.1
	level 2	437.4	18.9	4.3

- **Method comparison**

A comparison between ALP values for samples obtained on **Multi+** (y) and **ADVIA 1650** (x) using 32 samples gave following results:
y = 0.9703 x + 8.3352 U/l;
R = 0.996 (R – correlation coefficient)

A comparison between ALP values for samples obtained on **Biolis 24i Premium** (y) and **ADVIA 1800** (x) using 43 serum samples gave following results:
y = 0.9976 x – 1.6528 U/l;
R = 0.994 (R – correlation coefficient)

WASTE MANAGEMENT

Please refer to local legal requirements.

LITERATURE

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