Cat. No 4-512

DIAGNOSTIC KIT FOR DETERMINATION OF ALKALINE PHOSPHATASE ACTIVITY

HC – ALP

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

1-Reagent	6 x 76 ml
2-Reagent	6 x 19.5 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 on board the analyser at 2-10°C. Protect from light and avoid contamination!

Concentrations in the test

2-amino-2-methyl-1-propanol (AMP)	350 mmol/l
Mg^{2+}	2.0 mmol/l
Zn^{2+}	1.0 mmol/l
HEDTA	2.0 mmol/l
p-nitrophenylphosphate	16.0 mmol/l

Warnings and notes
- Product for in vitro diagnostic use only.
- The reagents contain sodium azide (< 0.1%) as a preservative. Avoid contact with skin and mucous membranes.
- During the reaction p-nitrophenol is produced. Do not swallow or inhale, avoid contact with skin.

SPECIMEN
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 loss 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
The reagents are ready to use.
These reagents may be used in automatic analyser Hitachi 911/912.
Application should be entered using handheld barcode scanner and attached barcodes sheet, according to procedure described below:
1. Delete previous version of application and calibrators assigned to it and restart the analyser.
2. Enter codes of calibrators according to the attached list.
3. Enter barcoded application and assign proper values to calibrators.
4. To activate entered application go to the tab UTILITY | APPLICATION | RANGE and change value of field DATA MODE from INACTIVE to ON BOARD. Confirm the change using UPDATE button.
5. Put reagents on board the analyser – they will be assigned to relevant tests automatically. Perform also measurement of level of reagents inside the bottles.
6. After calibration analyser is ready to use.

REFERENCE VALUES [9 10]

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
	20 – 50 years	42 – 98	0.71 – 1.67
	≥ 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
	20 – 50 years	53 – 128	0.90 – 2.18
	≥ 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
For internal quality control it is recommended to use the CORMAY SERUM HN (Cat. No 5-172) and CORMAY SERUM HP (Cat. No 5-173) with each batch of samples.
For the calibration of automatic analysers systems the CORMAY MULTICALIBRATOR LEVEL 1 (Cat. No 5-174; 5-176) is recommended. **Calibrator and 0.9% NaCl** should be used for calibration.
The calibration curve should be prepared every 10 days, with change of reagent lot number or as required e.g. quality control findings outside the specified range.

PERFORMANCE CHARACTERISTICS
These metrological characteristics have been obtained using the automatic analyser Hitachi 912. Results may vary if a different instrument or a manual procedure is used.

- **Sensitivity:** 6.95 U/l (0.12 µkat/l).
- **Linearity:** up to 660 U/l (11.02 µkat/l).
- **Specificity / Interferences**
  Haemoglobin up to 2.5 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) n = 20	Mean [U/l]	SD [U/l]	CV [%]
level 1	104.55	0.92	0.88
level 2	419.55	8.67	2.07

Reproducibility (day to day) n = 80	Mean [U/l]	SD [U/l]	CV [%]
level 1	101.52	5.94	5.85
level 2	448.28	13.07	2.92

- **Method comparison**

A comparison between ALP values determined at Hitachi 912 (y) and at COBAS INTEGRA 400 PLUS (x) using 93 samples gave following results:
y = 0.9458 x + 4.0644 U/l;
R = 0.9971 (R – correlation coefficient)

WASTE MANAGEMENT
Please refer to local legal requirements.

LITERATURE

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MANUFACTURER

PZ CORMAY S.A.
22 Wiosenna Street,
05-092 Łomianki, POLAND
tel.: +48 (0) 22 751 79 10
fax: +48 (0) 22 751 79 14
http://www.cormay.pl

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