Liquick Cor-ALAT

DIAGNOSTIC KIT FOR DETERMINATION OF ALANINE AMINOTRANSFERASE ACTIVITY

Kit name	Cat. No
Liquick Cor-ALAT 500	1-312
Liquick Cor-ALAT "bulk"	1-281

INTRODUCTION

Alanine aminotransferase (ALAT, ALT, GPT) is an enzyme participated in amino acids metabolism. ALAT is present in all tissues but the highest level is found in liver and kidney cells. Damage of hepatocytes or kidney cells causes significant release of ALAT into the circulation. Measurement of ALT activity in serum is valuable in the diagnosis of liver diseases: jaundice, mononucleosis or hepatic cirrhosis.

METHOD PRINCIPLE

Optimized, modified method according to International Federation of Clinical Chemistry (IFCC), without pyridoxal phosphate.

L-alanine + 2-oxoglutarate $\xleftrightarrow{ALAT}$ pyruvate + L-glutamate

pyruvate + NADH + H^+ $\xleftrightarrow{LDH}$ lactate + NAD^+

The rate of absorbance changing at λ=340 nm is directly proportional to alanine aminotransferase activity.

REAGENTS

Package

	Liquick Cor-ALAT 500	Liquick Cor-ALAT "bulk"
1-ALAT	3 x 400 ml	--*
2-ALAT	1 x 300 ml	--*

*reagent volume is printed on the label.

The reagents when stored at 2-8°C are stable up to expiry date printed on the package. The reagents are stable for 8 weeks on board the analyser at 2-10°C. Protect from light and avoid contamination!

Working reagent preparation and stability

Assay can be performed with use of separate 1-ALAT and 2-ALAT reagents or with use of working reagent. For working reagent preparation mix gently 4 parts of 1-ALAT with 1 part of 2-ALAT. Avoid foaming.

Stability of working reagent: 2 weeks at 2-8°C
5 days at 15-25°C

Protect from light and avoid contamination!

Concentrations in the test

Tris (pH 7.5)	100 mmol/l
L-alanine	500 mmol/l
LDH	> 36.7 µkat/l
2-oxoglutarate	15 mmol/l
NADH	0.18 mmol/l

Warnings and notes

- Product for in vitro diagnostic use only.
- The reagents contain (< 0.1%) sodium azide as a preservative. Avoid contact with skin and mucous membranes.
- The reagents are usable when the absorbance of the working reagent is higher than 1.400 (read against distilled water, wavelength λ=340 nm, cuvette l = 1cm, at temp. 25°C).

ADDITIONAL EQUIPMENT

- automatic analyzer or photometer able to read at 340 nm (Hg 334 nm, 365 nm);
- thermostat at 37ºC;
- general laboratory equipment;

SPECIMEN

Serum, heparinized or EDTA plasma free from hemolysis.
Hemolysis should be avoided, since ALAT activity in erythrocytes is 3 to 5 times higher than in normal serum.
Do not freeze the samples. ALAT activity remains stable in specimen up to 3 days at 15-25ºC or up to 7 days at 2-8ºC.
Nevertheless it is recommended to perform the assay with freshly collected samples!

PROCEDURE

These reagents may be used both for manual assay (Sample Start and Reagent Start method) and in several automatic analysers. Applications for them are available on request.

Manual procedure

wavelength	340 nm (Hg 334 nm, 365 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	100 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.).

Calculation

ALAT activity [U/l] = ΔA/min. x F

F value depends on the used wavelength:

λ	334 nm	340 nm	365 nm
F	1780	1746	3235

Reagent Start method

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

Pipette into the cuvette:

1-ALAT	1000 µl

Bring up to the temperature of determination. Then add:

sample	100 µl

Mix well, incubate for 5 min. Then add:

2-ALAT	250 µl

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

Calculation

ALAT activity [U/l] = ΔA/min. x F

F value depends on the used wavelength:

λ	334 nm	340 nm	365 nm
F	2184	2143	3971

REFERENCE VALUES [6]

serum / plasma	37ºC	
women	up to 31 U/l	up to 0.517 µkat/l
men	up to 41 U/l	up to 0.683 µkat/l

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) or LEVEL 2 (Cat. No 5-175; 5-177) are recommended.
The calibration curve should be prepared every 8 weeks, 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 automatic analyser Biolis 24i Premium. Results may vary if a different instrument or a manual procedure is used.

- **Sensitivity:** 4.7 U/l (0.078 µkat/l).
- **Linearity:** up to 500 U/l (8.33 µkat/l).
- **Specificity / Interferences**
  Haemoglobin up to 0.16 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	33.75	1.42	4.20
level 2	103.57	0.89	0.86

Reproducibility (day to day) n = 80	Mean [U/l]	SD [U/l]	CV [%]
level 1	33.15	0.86	2.59
level 2	103.26	2.55	2.47

- **Method comparison**

A comparison between ALAT values determined at Biolis 24i Premium (y) and at COBAS INTEGRA 400 (x) using 58 samples gave following results:
y = 0.9949 x + 0.0216 U/l;
R = 0.9887 (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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