Liquick Cor-ASAT

DIAGNOSTIC KIT FOR DETERMINATION OF ASPARTATE AMINOTRANSFERASE ACTIVITY

Kit name	Cat. No
Liquick Cor-ASAT 500	1-313
Liquick Cor-ASAT "bulk"	1-282

INTRODUCTION

Aspartate aminotransferase (ASAT, AST, GOT) is an enzyme participated in amino acids metabolism. ASAT is found in all tissues but particularly high level of ASAT is observed in heart muscle, skeletal muscle, liver and kidney. This is why elevated ASAT serum level is marker of myocardial infarction and kidney, liver or skeletal muscle injury.

METHOD PRINCIPLE

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

L-aspartate + 2-oxoglutarate $\xleftrightarrow{\text{ASAT}}$ oxalacetate + L-glutamate

oxalacetate + NADH + H^+ $\xleftrightarrow{\text{MDH}}$ malate + NAD^+

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

REAGENTS

Package

	Liquick Cor-ASAT 500	Liquick Cor-ASAT "bulk"
1-ASAT	3 x 400 ml	--*
2-ASAT	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 12 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-ASAT and 2-ASAT reagents or with use of working reagent. For working reagent preparation mix gently 4 parts of 1-ASAT with 1 part of 2-ASAT. Avoid foaming.

Stability of working reagent: 4 weeks at 2-8°C
5 days at 15-25°C

Protect from light and avoid contamination!

Concentrations in the test

Tris (pH 7.8)	80 mmol/l
L-aspartate	240 mmol/l
MDH	> 10 µkat/l
LDH	> 20 µkat/l
2-oxoglutarate	15 mmol/l
NADH	0.18 mmol/l
sodium hydroxide	< 1 %

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.
- 1-ASAT is classified as an irritant!

Ingredients: Contains sodium hydroxide.

Xi – Irritating.
R 36/38: Irritating to eyes and skin.
S 26-28-45: In case of contact with eyes, rinse immediately with plenty of water and see medical advice. After contact with skin, wash immediately with plenty of water. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

- 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 334nm, 365nm);
- thermostat at 37ºC;
- general laboratory equipment;

SPECIMEN

Serum, heparinized or EDTA plasma free from hemolysis.
Do not use heparine ammonium salt.
Hemolysis should be avoided, since ASAT activity in erythrocytes is 10 times higher than in normal serum.
Do not freeze the samples. ASAT activity remains stable in specimen up to 1 day at 15-25ºC or up to 4 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

ASAT 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-ASAT and 2-ASAT reagents.

Pipette into the cuvette:

1-ASAT	1000 µl

Bring up to the temperature of determination. Then add:

sample	100 µl

Mix well, incubate for 5 min. Then add:

2-ASAT	250 µl

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

Calculation

ASAT 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	
female	up to 31 U/l	up to 0.518 µkat/l
male	up to 37 U/l	up to 0.618 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) is recommended.

The calibration curve should be prepared every 12 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 an automatic analyser Biolis 24i Premium. Results may vary if a different instrument or a manual procedure is used.

- **Sensitivity:** 9.1 U/l (0.152 µkat/l).

- **Linearity:** up to 500 U/l (8.35 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	43.40	1.30	2.98
level 2	189.42	1.21	0.64

Reproducibility (day to day) n = 80	Mean [U/l]	SD [U/l]	CV [%]
level 1	42.76	0.98	2.29
level 2	191.24	3.26	1.70

- **Method comparison**

A comparison between ASAT values determined at Biolis 24i Premium (y) and at ADVIA 1650 (x) using 49 samples gave following results:

y = 1.0729 x - 4.3771 U/l;

R = 0.9979 (R – correlation coefficient)

WASTE MANAGEMENT

Please refer to local legal requirements.

LITERATURE

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Date of issue: 05. 2012.

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