N BIO SGPT (ASAT)

Mod. IFCC Method

KIT NAME	KIT SIZE	CAT. NO
N BIO - SGPT	2 x 50 ml	DGPT02050M

INTRODUCTION

Alanine aminotransferase (ALAT, ALT, GPT) is an enzyme participated in amino acids metabolism. ALAT is present in alltissues 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 $<\underline{ALAT}$ > pyruvate + L-glutamate pyruvate + NADH + H+ <LDH > lactate + NAD+

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

KIT CONTENTS

Reagent Name	DGPT02050M
R1 SGPT reagent	2 x 40 ml
R2 SGPT reagent	2 x 10 ml

The reagents when stored at $2-8^{\circ}$ C are stable up to expiry date printed on the package. The reagents are stable for 4 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 R1-ALAT and R2-ALAT reagents or with use of working reagent. For working reagent preparation mix gently 4 parts of R1-ALAT with 1 part of R2-ALAT. 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 7.5)	80 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 0.09% 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 2=340 nm, cuvette l=1 cm, at temp. 25°C).

ADDITIONAL EQUIPMEN

- Automatic analyzer or photometer able to read at 340 nm (Hg 334 nm, 365 nm)
- Thermostat at 25°C, 30°C or 37°C
- 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.

ALAT activity remains stable in specimen up to 3 days at 15-25°C or up to 7 days at 2-8°C. Although samples frozen at -20°C can be stored longer than 7 days, 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.

Wavelength	340 nm
Temperature	37°C
Cuvette	1 cm

Pipette into the cuvette:

Reagent	Test (T)
R1 SGPT reagent	800 µl
R2 SGPT reagent	200 µl
Bring to assay temperature, 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/I] =∆A/min. x 2250 (Factor)

REFERENCE VALUES

Female	upto 32 U/L
Male	upto 42 U/L

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

QUALITY CONTROL

To ensure adequate quality control, each run should include assayed normal and abnormal controls. If commercial controls are not available it is recommended that known value samples be aliquoted, frozen and used as controls.

PERFORMANCE CHARACTERISTICS

- Sensitivity / Limit of Quantitation: 5 U/l.
- Linearity: up to 500 U/I.
- Specificity / Interferences: Haemoglobin up to 2.5 g/dl, ascorbate up to 7.75 mg/l, bilirubin up to 0.625 mg/dl and triglycerides up to 500 mg/dl do not interfere with the test.
- The working reagent should not be used, if the absorbance is less than 0.900 at 340 nm. against distilled water

WASTE MANAGEMENT

Please refer to local legal requirements.

LITERATURE

- 1. Wallhofer H., Schmidt E., Schmidt U.F. W.: Synopsis Der Leberkrankheiten. G. Thieme Verlag, Stuttgart (1974).
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- Henry R.J. Cannon D.C. Winkerman J. W.: Clinical Chemistry Principles and Technics, 2nd ed. Hagerstown MD: Harper and Row, 815, 888 (1974).
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SYSTEM PARAMETERS

Method	Kinetic
Wavelength	340 nm
Zero Setting	distilled water
Temperature Setting	37° C
Incubation Temperature	37° C
Incubation Time	
Delay Time	60 secs
Read Time	180 secs
No. of Reading	3
Interval Time	60 secsc
Sample Volume	0.1 ml (100 ul)
Reagent Volume	1.0 ml (1000 ul)
Calibrator Concentration	
Units	U/L
Factor	2250
Reaction Slope	Decreasing
Linearity	500 U/L





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