

Alanine aminotransferase (ALT/GPT)-Liquizyme (4+1) E.C.2.6.1.2.

REF: 292 000	(2 x 20 ml)	40 test
REF: 292 001	(4 x 20 ml)	80 test
REF: 292 002	(10 x 10 ml)	100 test
REF: 292 003	(9 x 20 ml)	180 test
REF: 292 004	(4 x 60 ml)	240 test
REF: 292 005	(5 x 20 ml)	100 test
REF: 292 006	(4 x 50 ml)	200 test
REF: 292 007	(5 x100 ml)	500 test
REF: 292 008	(6 x100 ml)	600 test
REF: 292 009	(4 x100 ml)	400 test

Intended Use

Spectrum Diagnostics liquizyme ALT reagent is intended for the in-vitro quantitative, diagnostic determination of ALT in human serum on both automated and manual systems.

Background

The enzyme alanine aminotransferase ALT is widely distributed with high concentrations in the liver and to a lesser extent in kidneys, heart, skeletal muscles, pancreas and lungs. Elevated serum ALT is found in hepatitis, cirrhosis, obstructive jaundice, carcinoma of the liver, and chronic alcohol abuse. ALT is only slightly elevated in patients who have an uncomplicated myocardial infarction. Although both serum aspartate aminotransferase AST and ALT become elevated whenever disease processes affect liver cell integrity, ALT is the more liver specific enzyme. Moreover, elevations of ALT activity persist longer than elevations of AST activity.

Method

Kinetic method according to the International Federation of Clinical Chemistry (IFCC) ⁽³⁾.

Assay Principle

The series of the reaction involved in the assay system is as follows:

- The amino group is enzymatically transferred by ALT present in the sample from alanine to the carbon atom of 2-oxoglutarate yielding pyruvate and L-glutamate.



- Pyruvate is reduced to lactate by LDH present in the reagent with the simultaneous oxidation of NADH to nicotinamide adenine dinucleotide (NAD). The reaction is monitored by measuring the rate of decrease in absorbance at 340 nm due to the oxidation of NADH.



- Endogenous sample pyruvate is rapidly and completely reduced by LDH during the initial incubation period so that it does not interfere with the assay.



Reagents

Reagent 1 (R1 Buffer / Enzyme)










Tris buffer (pH 7.4)	100	mmol/L
L- Alanine	800	mmol/L
LDH	≥ 2000	U/L
Sodium Azide	8	mmol/L

Reagent 2 (R2 Coenzyme)

NADH	≥ 0.18	mmol/L
2 – Oxoglutarate	18	mmol/L
Sodium Azide	8	mmol/L

For further information, refer to the Alanine aminotransferase reagent material safety data sheet.

SYMBOLS IN PRODUCT LABELLING

 Authorised Representative	 Temperature Limitation
 For in-vitro diagnostic use	 Use by/Expiration Date
 Batch Code/Lot number	 CAUTION. Consult instructions for use
 Catalogue Number	 Manufactured by
 Consult instructions for use	

Reagent preparation

Prepare working solution as following:

REF:292 000	: add 4 ml from R2 to one bottle of R1; mix gently
REF:292 001	: add 4 ml from R2 to one bottle of R1; mix gently.
REF:292 002	: add 2 ml from R2 to one bottle of R1; mix gently.
REF:292 003	: add 4 ml from R2 to one bottle of R1; mix gently.
REF:292 004	: add 12 ml from R2 to one bottle of R1; mix gently.
REF:292 005	: add 4 ml from R2 to one bottle of R1; mix gently.
REF:292 006	: add 10 ml from R2 to one bottle of R1; mix gently.
REF:292 007	: add one bottle of R2 to one bottle of R1;mix gently.
REF:292 008	: add one bottle of R2 to one bottle of R1; mix gently.
REF:292 009	: add one bottle of R2 to one bottle of R1; mix gently.

Or prepare the working solution according to the number of tests required by mixing 4 volumes of reagent 1 (R1) and 1volume of reagent 2 (R2), e.g. 400 µl R1 + 100 µl R2.

Precautions and Warnings

Do not ingest or inhale. In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately. Both reagents (R1) and (R2) contain sodium azide which may react with copper or lead plumbing.

Reagent Storage and Stability

All reagents are stable until expiration date stated on label when stored refrigerated at 2 - 8 °C. Working solution is stable for 4 weeks at 2 – 8 °C or 2 days at 15 - 25 °C.

Deterioration

Do not use liquizyme ALT reagent if it is turbid or if the absorbance of the working reagent is less than 1.0 at 340 nm. Failure to recover control values within the assigned range may be an indication of reagent deterioration.

Specimen Collection and Preservation

Use nonhemolyzed serum or plasma. Heparin and EDTA are the only acceptable anticoagulants; avoid other anticoagulants. The biological half-life of ALT in serum is 47 hours.

Stability: 3 days at 15 - 25 °C or 7 days at either 4- 8 °C or at -20 °C

System Parameters

Wavelength	340 nm (334 – 365 nm)
Optical path	1 cm
Assay type	Kinetic
Direction	decrease
Sample : Reagent Ratio	1 : 10
e.g. : Reagent volume	1 ml
Sample volume	100 µl
Temperature	37 °C or 30 °C
Equilibration time	60 seconds.
Read time	1 to 3 minutes
Zero adjustment	Against air
Reagent Blank Limits	Low 1.00 AU High 2.5 AU
Sensitivity	5 U/L
Linearity	400 U/L

Procedure

	Macro	Semi-Micro
Working solution	1.0 ml	500 µl
Specimen	100 µl	50 µl

Mix, read initial absorbance after 60 seconds. and start timer simultaneously. Read again after 1, 2 and 3 minutes. Determine the mean absorbance change per minute (ΔA/min).

Calculation

To calculate the ALT/GPT activity use the following formula

$$\begin{aligned}U/l &= 1780 \times \Delta A 334 \text{ nm /min} \\U/l &= 1746 \times \Delta A 340 \text{ nm /min} \\U/l &= 3235 \times \Delta A 365 \text{ nm /min}\end{aligned}$$

Quality Control

Normal & abnormal control serum of known concentrations should be analyzed with each run.

Performance Characteristics

Precision

Within run (Repeatability)

	Level 1	Level 2
n	20	20
Mean (U/L)	24.6	105.9
SD	0.93	0.94
CV%	3.78	0.89

Run to run (Reproducibility)

	Level 1	Level 2
n	20	20
Mean (U/L)	25.2	106
SD	1.1	1.05
CV%	3.9	0.95

Methods Comparison

A comparison between Spectrum Diagnostics ALT (4+1) reagent and a commercial reagent of the same methodology was performed on 20 human sera. A correlation of 0.983 was obtained.

Sensitivity

When run as recommended, the minimum detection limit of this assay is 5.0 U/L.

Linearity

The reaction is linear up to ALT concentration of 400 U/L; specimens showing higher concentration should be diluted 1+5 with physiological saline and repeat the assay (result×6).

Interfering Substances

Serum, plasma

Hemolysis

Erythrocyte contamination elevates results, since ALT activities in erythrocytes are 3 to 5 times higher than those in normal sera.

Icterus

No significant interference.

Lipemia

Lipemic specimens may cause high absorbance flagging. Diluted sample is recommended.

Anticoagulants

Citrate and fluoride inhibit the enzyme activity.

Drugs

Calcium dobesilate and doxycycline HCL cause artificially low ALT values at the tested drug level.

Expected values

37 °C Females up to 31 U/l (up to 0.52 µKat/L)
males up to 41 U/l (up to 0.68 µKat/L)

30 °C Females up to 22 U/l (up to 0.37 µKat/L)
males up to 29 U/l (up to 0.48 µKat/L)

Temperature conversion factor is 1.32 (25 → 30 °C) and 1.85 (25 → 37 °C)

Spectrum Diagnostics does not interpret the results of a clinical laboratory procedure; interpretation of the results is considered the responsibility of qualified medical personnel. All indications of clinical significance are supported by literature references.

Analytical Range

5 – 400 U/L.

Waste Disposal

This product is made to be used in professional laboratories. Please consult local regulations for a correct waste disposal.

S56: dispose of this material and its container at hazardous or special waste collection point.

S57: use appropriate container to avoid environmental contamination.

S61: avoid release in environment. refer to special instructions/safety data sheets.

References

1. Breuer J, report on the symposium "drug effects in clinical chemistry methods". Eur J Clin Chem Clin Biochem. 1996;34:385-386.
2. ECCLS. Determination of the catalytic activity concentration in serum on L- alanine aminotransferase (EC 2.6.1.2,ALAT) Clin chem. 1989;20:204-211.
3. IFCC expert panel on enzymes part 3. J Clin Chem Clin Biochem 1986;24:481-95 .
4. Henry RJ, et al. Am J clin Path 1960 :34:381
5. Sherwin JE. Liver function. In:kaplan LA, PESCE AJ, eds. Clinical chemistry, theory, analysis, and correlation. St louis:mosby;1984:420-438.
6. Young DS. Effects of drugs on clinical laboratory tests. Third edition. 1990 :3:6-12.
7. Zilva JF, pannall PR : plasma enzymes in diagnosis in clinical chemistry in diagnosis and treatment lioyd luke london 1979:chap 17 : 338.

ORDERING INFORMATION

CATALOG NO.	QUANTITY
292 000	2 x 20 ml
292 001	4 x 20 ml
292 002	10 x 10 ml
292 003	9 x 20 ml
292 004	4 x 60 ml
292 005	5 x 20 ml
292 006	4 x 50 ml
292 007	5 x 100 ml
292 008	6 x 100 ml
292 009	4 x 100 ml



Egyptian Company for Biotechnology (S.A.E)

Obour city industrial area. block 20008 piece 19 A. Cairo. Egypt.

Tel: +202 4665 1848 - Fax: +202 4665 1847

www.spectrum-diagnostics.com

E-mail:info@spectrum-diagnostics.com



MDSS GmbH
Schiffgraben 41
30175 Hannover, Germany



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