Spectrum For Diagnostic Industries

Alanine aminotransferase (ALT/GPT)- (1+1)

IVD

REF.	Pack size
180 04 025	(4 X 25 ml) 100 tests

Intended Use

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

Introduction

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, liver carcinoma, and chronic alcohol abuse. ALT is only slightly elevated in patients who have an uncomplicated myocardial infarction.
Although both 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.

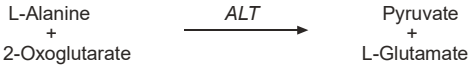
Method

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

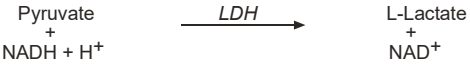
Principle

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

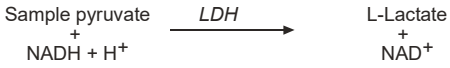
1. The amino group is enzymatically transferred by ALT present in the sample from alanine to the carbon atom of 2-oxoglutarate yielding pyruvate and L-glutamate.



2. 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.



3. 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

Buffer reagent

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

Coenzyme

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

Reagent preparation, storage and stability

All reagents are stable until expiration date stated on label when stored refrigerated at 2 - 8 ^{o}C.Once opened, the reagent is stable for 2 months at the specified temperature.

Prepare working solution as following:

Prepare working solution by adding equal volumes from R1 and R2,Working solution is stable for 4 weeks at 2 – 8 ^{o}C or 2 days at 15 - 25 ^{o}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.

Precautions and Warnings

Do not ingest or inhalate. 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.

Specimen collection and preservation

Use non-hemolyzed 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 ^{o}C or
7 days at either 4- 8 ^{o}C or at -20 ^{o}C

Procedure

Wavelength	340 nm
Optical path	1 cm
Assay type	Kinetic
Direction	decrease
Sample : Reagent Ratio	1 : 10
Temperature	37 ^{o}C or 30 ^{o}C
Delay time	60 seconds.
Read time	1 to 3 minutes
Zero adjustment	Against air
Reagent Blank Limits	Low 1.00 AU
	High 2.5 AU

Working solution	1.0 ml (or add 0.5 ml R1+ 0.5 ml R2)
Specimen	100 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

U/L = 1746 x ΔA 340 nm /min

Quality control

Normal and abnormal control serum of known concentration should be analyzed with each run.

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).

Interference

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)

Performance characteristics

A comparison between Spectrum Diagnostics ALT (1+1) reagent and a commercial reagent of the same methodology was performed on 20 human sera.A correlation of 0.997 was obtained.

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

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. Henry RJ, et al. Am J clin Path 1960 :34:381
2. Zilva JF, pannall PR : plasma enzymes in diagnosis in clinical chemistry in diagnosis and treatment lioydluke london 1979:chap

SYMBOLS IN PRODUCT LABELLING

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

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