Spectrum For Diagnostic Industries

Alanine aminotransferase
(ALT/GPT) - *Ultimate Single Reagent*

IVD

REF.	Pack size
181 02 020	40 T
181 06 020	120 T

Intended Use

SDI 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 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).

Assay Principle

The series of the reaction 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.

L-Alanine + 2-Oxoglutarate —*ALT*→ Pyruvate + 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.

Pyruvate + NADH + H^+ —*LDH*→ L-Lactate + NAD^+

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.

Sample pyruvate + NADH + H^+ —*LDH*→ L-Lactate + NAD^+

Reagent (R)

Tris buffer (pH 7.4)	100	mmol/L
L- Alanine	800	mmol/L
LDH	≥ 2000	U/L
Sodium Azide	8	mmol/L
NADH	≥ 0.18	mmol/L
2 – Oxoglutarate	18	mmol/L

The reagent also contains additives required to maintain NADH in its reduced form.
For further information, refer to the Alanine aminotransferase reagent material safety data sheet.

Reagent Preparation, Storage and Stability

SDI ALT reagent is supplied ready-to-use and stable up to the expiry date labelled on the bottles.
Once opened, the reagent is stable for 1 month at the specified temperature.

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.

The reagent (R) contain sodium azide which may react with copper or lead plumbing.

Deterioration

Do not use SDI ALT reagent if it is turbid or if the absorbance of the working reagent is less than 0.9 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 , 7 days at 4- 8 °C
or 12 weeks 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	180 seconds
Zero adjustment	Against air
Reagent Blank Limits	Low 0.9 AU
	High 2.5 AU
Sensitivity	5 U/L
Linearity	400 U/L

Procedure

	Macro	Semi-Micro
Reagent (R)	1.0 ml	500 µl
Specimen	100 µl	50 µl

Mix, read initial absorbance after 60 seconds and start timer simultaneously. Read again after 60, 120 and 180 seconds. Determine the mean absorbance change per minute (ΔA/min).

Calculation

To calculate the ALT/GPT activity use the following formula

U/l = 1780 x ΔA 334 nm /min
U/l = 1746 x ΔA 340 nm /min
U/l = 3235 x ΔA 365 nm /min

Quality Control

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

Performance Characterstics

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 ALT 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

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.

Analytical Range

5 – 400 U/L.

Expected values

37 ^{O}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 ^{O}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 ^{O}C) and 1.85 (25 - 37 ^{O}C)

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.
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6. Young DS. Effects of drugs on clinical laboratory tests. Third edition. 1990 :3:6-12.
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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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