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 in high concentrations in the liver and to a lesser extent in kidney, 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) [1].

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.

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
Reagent 1 (R1 Buffer / Enzyme)
- Tris buffer (pH 7.4)
- L-Alanine
- LDH
- Sodium Azide

Reagent 2 (R2 Coenzyme)
- NADH
- 2-Oxoglutarate
- Sodium Azide

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

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.

Reagent Preparation, Storage and Stability
All reagents are stable until expiration date stated on label when stored refrigerated at 2 - 8°C. Working solution can be prepared by adding equal volumes from R1 and R2. Stability: 2 days 2 – 8°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 Handling
Use nonhaemolyzed 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
- e.g.: Reagent volume
- Sample volume
- Temperature
- Equilibration time
- Read time
- Zero adjustment
- Reagent Blank Limits
- Sensitivity
- Linearity

Procedure

Mix, read initial absorbance after 30 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

\[
UI = \frac{1780 \times \Delta A}{334 \text{ nm/ min}}
\]

\[
UI = \frac{1746 \times \Delta A}{340 \text{ nm/ min}}
\]

\[
UI = \frac{3235 \times \Delta A}{385 \text{ nm/ min}}
\]
Quality Control
Normal & abnormal commercial control serum of known concentrations should be analyzed with each run.

Performance Characteristics

### Precision
Within run (Repeatability)

<table>
<thead>
<tr>
<th></th>
<th>Level 1</th>
<th>Level 2</th>
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<tbody>
<tr>
<td>n</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Mean (U/L)</td>
<td>103</td>
<td>190</td>
</tr>
<tr>
<td>SD</td>
<td>6.1</td>
<td>13</td>
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<tr>
<td>CV%</td>
<td>6</td>
<td>7.4</td>
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### Run to run (Reproducibility)

<table>
<thead>
<tr>
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<td>Mean (U/L)</td>
<td>103</td>
<td>190</td>
</tr>
<tr>
<td>SD</td>
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<td>16</td>
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<tr>
<td>CV%</td>
<td>14.3</td>
<td>8</td>
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</table>

Methods Comparison
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.

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 x 6).

Interfering Substances
Serum, plasma

Haemolysis
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

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Females</th>
<th>Males</th>
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<tbody>
<tr>
<td>37 °C</td>
<td>up to 31 U/L</td>
<td>up to 41 U/L</td>
</tr>
<tr>
<td>30 °C</td>
<td>up to 22 U/L</td>
<td>up to 29 U/L</td>
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</tbody>
</table>

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.

References

ORDERING INFORMATION

<table>
<thead>
<tr>
<th>CATALOG NO.</th>
<th>QUANTITY</th>
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<tr>
<td>263 001</td>
<td>2 x 25 ml</td>
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<tr>
<td>263 002</td>
<td>4 x 25 ml</td>
</tr>
<tr>
<td>263 003</td>
<td>2 x 100 ml</td>
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</tbody>
</table>

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EC REP

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