Alanine aminotransferase (ALT/GPT) - Colorimetric

REF: 264 001 (2 x 50 ml) 100 test
REF: 264 002 (2 x 100 ml) 200 test

Intended Use

Spectrum Diagnostics ALT reagent is intended for the in-vitro quantitative, diagnostic determination of ALT in human serum.

Background

The enzyme alanine aminotransferase (ALT) is widely distributed with high concentrations in 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

ALT – (colorimetric method).

Assay Principle

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 carbonyl atom of 2-oxoglutarate yielding pyruvate and L-glutamate.

\[
\text{L-Alanine} + 2-\text{Oxoglutarate} \rightarrow \text{ALT} \rightarrow \text{Pyruvate} + \text{L-Glutamate}
\]

ALT activity is measured by monitoring the concentration of pyruvate hydrazine formed with 2,4-dinitrophenylhydrazine.

Reagents

Reagent 1 (R1 Buffer)
Phosphate buffer 100 mmol/L
DL-Alanine 200 mmol/L
2 - Oxoglutarate 6 mmol/L
Sodium Azide 12 mmol/L

Reagent 2 (R2)
2,4-dinitrophenylhydrazine 2.0 mmol/L
(C) Corrosive contains caustic materials.
R35 Causes severe burns.
R41 Risk of serious damage to eyes.
S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S28 After contact with skin, wash immediately with plenty of soap and water.

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 (R1) contains sodium azide which may react with copper or lead plumbing.

Additional Reagent

Sodium hydroxide 0.4 mol/L.

Reagent Preparation, Storage and Stability

The reagents are supplied ready-to-use and stable up to the expiry date labeled on the bottles when stored at 2 – 8 °C.

Deterioration

Do not use the ALT regents if precipitate forms. Failure to recover control values within the assigned range may be an indication of reagent deterioration.

Specimen Collection and Preservation

Use only non haemolysed serum. The biological half-life of ALT in serum is 47 hours. The only acceptable anticoagulants are heparin and EDTA.

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

System Parameters

Wavelength 546 nm (530-550 nm)

Optical path 1 cm

Assay type End-point

Direction Increase

Sample: Reagent Ratio 1:60

Temperature 37 °C and 20 – 25 °C

Zero adjustment Reagent or Sample blank

Sensitivity 4 U/L

Linearity 94 U/L

Procedure

1. Measurement against Reagent Blank

Pipeette into test tubes

<table>
<thead>
<tr>
<th>Reagent blank</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1 (buffer)</td>
<td>0.5 ml</td>
</tr>
<tr>
<td>Sample</td>
<td>------</td>
</tr>
<tr>
<td>Distilled water</td>
<td>100 µl</td>
</tr>
</tbody>
</table>

Mix and incubate for exactly 30 minutes at 37 °C

R2 0.5 ml 0.5 ml

Mix and incubate for exactly 20 minutes at 20 – 25 °C

Sodium hydroxide 5.0 ml 5.0 ml

Mix, measure absorbance of specimen against reagent blank at 546 nm after 5 minutes.

2. Measurement against Sample Blank

Mix and incubate for exactly 30 minutes at 37 °C

Pipeette into test tubes

<table>
<thead>
<tr>
<th>Sample blank</th>
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<tbody>
<tr>
<td>R1 (buffer)</td>
<td>0.5 ml</td>
</tr>
<tr>
<td>Sample</td>
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</tr>
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</table>

R2 0.5 ml 0.5 ml

Mix and incubate for exactly 20 minutes at 20 – 25 °C

Sodium hydroxide 5.0 ml 5.0 ml

Mix, measure absorbance of specimen against sample blank at 546 nm after 5 minutes.
**Calculation**

The ALT activity in the serum can be determined from the following table:

<table>
<thead>
<tr>
<th>Absorbance</th>
<th>U/L</th>
<th>Absorbance</th>
<th>U/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.025</td>
<td>4</td>
<td>0.275</td>
<td>48</td>
</tr>
<tr>
<td>0.050</td>
<td>8</td>
<td>0.300</td>
<td>52</td>
</tr>
<tr>
<td>0.075</td>
<td>12</td>
<td>0.325</td>
<td>57</td>
</tr>
<tr>
<td>0.100</td>
<td>17</td>
<td>0.350</td>
<td>62</td>
</tr>
<tr>
<td>0.125</td>
<td>21</td>
<td>0.375</td>
<td>67</td>
</tr>
<tr>
<td>0.150</td>
<td>25</td>
<td>0.400</td>
<td>72</td>
</tr>
<tr>
<td>0.175</td>
<td>29</td>
<td>0.425</td>
<td>77</td>
</tr>
<tr>
<td>0.200</td>
<td>34</td>
<td>0.450</td>
<td>83</td>
</tr>
<tr>
<td>0.225</td>
<td>39</td>
<td>0.475</td>
<td>88</td>
</tr>
<tr>
<td>0.250</td>
<td>43</td>
<td>0.500</td>
<td>94</td>
</tr>
</tbody>
</table>

**Quality Control**

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

**Sensitivity**

When run as recommended, the sensitivity of this assay is 4 U/L.

**Linearity**

The assay is linear up to 94 U/L. If the absorbance exceeds 0.5 at 546 nm (ALT 94 U/L) samples should be diluted 1 x 9 using sodium chloride and repeat the assay (result x 10).

**Interfering Substances**

Serum, plasma

**Haemolysis**

Erythrocyte contamination may elevate results, since ALT activities in erythrocytes are three to five times than those in normal sera.

**Icterus**

No significant interference.

**Lipemia**

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

**Note**

High concentration of aldehydes, ketones, or oxo-acids in some sera may cause false high transaminase levels. Measurement against a serum blank instead of a reagent blank avoids the risk of finding such artifacts.

**Expected Values**

Serum : up to 12 U/L.

**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**

4 – 94 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.

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**References**


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**ORDERING INFORMATION**

<table>
<thead>
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<th>CATALOG NO.</th>
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