Alanine aminotransferase (ALT/GPT) - Colorimetric

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

\[
\begin{align*}
\text{L-Alanine} & \quad \text{ALT} \quad \text{Pyruvate} \\
2\text{-Oxoglutarate} & \quad \text{ALT} \quad \text{L-Glutamate}
\end{align*}
\]

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

Reagents
Reagent 1 (R1 Buffer)
- Phosphate buffer
- DL-Alanine
- 2-Oxoglutarate
- Sodium Azide

Reagent 2 (R2)
- 2,4-dinitrophenylhydrazine

(C)-Corrosive contains caustic material.

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

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

<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>
<tr>
<td>Mix and incubate for exactly 30 minutes at 37 °C</td>
<td></td>
</tr>
</tbody>
</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 reagent blank at 546 nm after 5 minutes.

2. Measurement against Sample Blank

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

<table>
<thead>
<tr>
<th>Sample 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>Mix and incubate for exactly 30 minutes at 37 °C</td>
<td></td>
</tr>
</tbody>
</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
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 + 9 using sodium chloride and repeat the assay (result × 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.

References

ORDERING INFORMATION

<table>
<thead>
<tr>
<th>CATALOG NO.</th>
<th>QUANTITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>264 001</td>
<td>2 x 50 ml</td>
</tr>
<tr>
<td>264 002</td>
<td>2 x 100 ml</td>
</tr>
</tbody>
</table>

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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