Aspartate aminotransferase (AST/GOT) - Ultimate Single Reagent E.C.2.6.1.1. t

REF: 261 001 (2 x 20 ml) 40 test
REF: 261 002 (6 x 20 ml) 120 test
REF: 261 003 (2 x 100 ml) 200 test
REF: 261 004 (4 x 50 ml) 200 test
REF: 261 005 (2 x 50 ml) 100 test
REF: 261 006 (4 x 100 ml) 400 test
REF: 261 007 (2 x 250 ml) 500 test

Intended Use
Spectrum Diagnostics Ultimate AST reagent is intended for the in-vitro quantitative, diagnostic determination of AST in human serum on both automated and manual systems.

Background
The enzyme aspartate aminotransferase (AST) is widely distributed in erythrocytes and tissues, principally heart, liver, muscle, and kidney. Elevated serum levels are found in diseases involving these tissues such as myocardial infarction, viral hepatitis and muscular dystrophy. Following myocardial infarction, serum AST is elevated and reaches a peak two days after onset. Two isoenzymes of AST have been detected, cytoplasmic and mitochondrial. Only the cytoplasmic isoenzyme occurs in normal serum, while the mitochondrial, together with the cytoplasmic isoenzyme, has been detected in the sera of patients with coronary and hepatobiliary diseases.

Method
Kinetic method according to the International Federation of Clinical Chemistry (IFCC) (9).

Assay Principle
The series of the reaction involved in the assay system is as follows:

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

   \[ \text{L-Aspartate} + \text{AST} \rightarrow \text{Oxaloacetate} + \text{L-Glutamate} \]

2. Oxaloacetate in presence of NADH and malate dehydrogenase (MDH), is reduced to L-malate. In this reaction NADH is oxidized to NAD. The reaction is monitored by measuring the rate of decrease in absorbance at 340 nm due to oxidation of NADH to NAD.

   \[ \text{Oxaloacetate} + \text{MDH} + \text{NADH} + \text{H}^+ \rightarrow \text{L-Malate} + \text{NAD}^+ \]

3. Addition of lactate dehydrogenase (LDH) to the reagent is necessary to achieve rapid and complete reduction of endogenous pyruvate so that it does not interfere with the assay.

   \[ \text{Sample pyruvate} + \text{LDH} + \text{NADH} + \text{H}^+ \rightarrow \text{L-Lactate} + \text{NAD}^+ \]

Reagent (R)
Tris buffer (pH 7.7) 80 mmol/L
L-Aspartate 240 mmol/L
MDH > 450 U/L
LDH > 1200 U/L
Sodium Hydroxide 220 mmol/L
Sodium Azide 8 mmol/L
NADH > 0.18 mmol/L
- Oxoglutarate 18 mmol/L

Irritant (XI): R36/38: Irritating to eyes and skin. S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. S37/39: Wear suitable gloves and eyewash protection.

SYMBOLS IN PRODUCT LABELLING
- Code repenent Representative Use by/Expiration Date
- For in-vitro diagnostic use
- Use CAUTION. Consult instructions
- Batch Code/lot number
- for use
- Manufactured by
- Consult instructions for use
- Temperature Limitation
- (XI) - Irritant

The reagent also contains additives required to maintain NADH in its reduced form.

For further information, refer to the Aspartate 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.

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

Reagent Preparation, Storage and Stability
Spectrum Ultimate AST reagent is supplied ready-to-use and stable up to the expiry date labeled on the bottles. Once opened, the opened vial is stable for 3 months at the specified temperature.

Deterioration
Do not use Spectrum Ultimate AST 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 nonhemolysed serum, Heparin and EDTA are the only acceptable anticoagulants. The biological half-life of AST in serum is 17 hours.

Stability: 1 day at 15 – 25 °C; 7 days at 4 - 8 °C; 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

<table>
<thead>
<tr>
<th>Macro</th>
<th>Semi-Micro</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent (R)</td>
<td>1.0 ml</td>
</tr>
<tr>
<td>Specimen</td>
<td>100 μl</td>
</tr>
<tr>
<td></td>
<td>50 μl</td>
</tr>
</tbody>
</table>

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 AST/GOT activity use the following formulae:

\[ \text{U} = 1780 \times A \times \text{nm} / \text{min} \]
\[ \text{U} = 1746 \times A \times \text{nm} / \text{min} \]
\[ \text{U} = 3235 \times A \times \text{nm} / \text{min} \]

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

Performance Characteristics

<table>
<thead>
<tr>
<th>Precision</th>
<th>Level 1</th>
<th>Level 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Mean (U/L)</td>
<td>32.6</td>
<td>133</td>
</tr>
<tr>
<td>SD</td>
<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
<td>CV%</td>
<td>4.08</td>
<td>0.97</td>
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</tbody>
</table>

Run to run (Reproducibility)

<table>
<thead>
<tr>
<th>Level 1</th>
<th>Level 2</th>
</tr>
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<tbody>
<tr>
<td>n</td>
<td>20</td>
</tr>
<tr>
<td>Mean (U/L)</td>
<td>33.1</td>
</tr>
<tr>
<td>SD</td>
<td>1.5</td>
</tr>
<tr>
<td>CV%</td>
<td>4.25</td>
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</table>

Methods Comparison
A comparison between Spectrum Diagnostics AST reagent and a commercial reagent of the same methodology was performed on 20 human sera. A correlation of 0.991 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 AST concentration of 400 U/L; specimens showing higher concentration should be diluted 1+6 with physiological saline and repeat the assay (result×6).

Interfering Substances

<table>
<thead>
<tr>
<th>Serum, plasma</th>
<th>Hemolysis</th>
<th>Erythrocyte contamination elevates results, since AST activities in erythrocytes are 15 times higher than those in normal sera.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Icterus</td>
<td>No significant interference.</td>
<td></td>
</tr>
<tr>
<td>Lipemia</td>
<td>Lipemic specimens may cause high absorbance flagging. Diluted sample is recommended.</td>
<td></td>
</tr>
<tr>
<td>Anticoagulants</td>
<td>Citrate and fluoride inhibit the enzyme activity.</td>
<td></td>
</tr>
<tr>
<td>Drugs</td>
<td>Calcium dobesilate and doxycycline HCL cause artificially low AST values at the tested drug level.</td>
<td></td>
</tr>
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</table>

Expected values

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Activity</th>
<th>Units</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>37 °C</td>
<td>Females</td>
<td>up to 31 U/L</td>
<td>(up to 0.52 µKat/L)</td>
</tr>
<tr>
<td>Males</td>
<td>up to 37 U/L</td>
<td>(up to 0.62 µKat/L)</td>
<td></td>
</tr>
<tr>
<td>30 °C</td>
<td>Females</td>
<td>up to 21 U/L</td>
<td>(up to 0.35 µKat/L)</td>
</tr>
<tr>
<td>Males</td>
<td>up to 25 U/L</td>
<td>(up to 0.42 µKat/L)</td>
<td></td>
</tr>
</tbody>
</table>

Temperature conversion factor is 1.37 (25 to 30 °C) and 2.04 (25 to 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.

S56: dispose of this material and its container at hazardous or special waste collection point.

S57: use appropriate container to avoid environmental contamination.

S61: available release in environment. Refer to special instructions/safety data sheets.

References

ORDERING INFORMATION

<table>
<thead>
<tr>
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<tr>
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Egyptian Company for Biotechnology (S.A.E)
Obour city industrial area, block 20008 piece 19 A. Cairo. Egypt.
Tel: +202 4665 1848 - Fax: +202 4665 1847
www.spectrum-diagnostics.com
E-mail:info@spectrum-diagnostics.com

MDSS GmbH
Schiffgraben 41
30175 Hannover, Germany

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