Aspartate aminotransferase (AST/GOT)-Colorimetric

REF: 260 001 (2 x 50 ml) 100 test
REF: 260 002 (2 x 100 ml) 200 test

**Intended Use**
Spectrum Diagnostics colorimetric AST reagent is intended for the in-vitro quantitative, diagnostic determination of AST in human serum.

**Background**
The enzyme aspartate aminotransferase (AST) is widely distributed in erythrocytes and tissues, principally heart, liver, muscles, and kidneys. 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**
AST – (Colorimetric method).

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

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:

\[
\begin{align*}
\text{L-Aspartate} & \rightarrow \text{Oxaloacetate} + \\
& \rightarrow \text{2-Oxoglutarate} + \text{L-Glutamate}
\end{align*}
\]

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

**Reagents**

**Reagent 1 (R1 Buffer)**
- Phosphate buffer: 100 mmol/L
- L-aspartate: 100 mmol/L
- 2-Oxoglutarate: 5 mmol/L
- Sodium Hydroxide: 140 mmol/L
- Sodium Azide: 12 mmol/L

**Reagent 2 (R2)**
- 2,4-dinitrophenylhydrzone: 2 mmol/L
- HCl: 8.4 %

**Additional Reagent**
Sodium hydroxide: 0.4 mol/L.

**Precautions and Warnings**
Do not ingest or inhale. In case of contact with eyes or skin; rinse immediately with plenty of water and seek medical advice immediately.

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

**SYMBOLS IN PRODUCT LABELLING**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>Reagent 1</td>
</tr>
<tr>
<td>R2</td>
<td>Reagent 2</td>
</tr>
<tr>
<td>E</td>
<td>Extinction</td>
</tr>
<tr>
<td>A</td>
<td>Absorbance</td>
</tr>
<tr>
<td>C</td>
<td>Concentration</td>
</tr>
</tbody>
</table>

**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 AST reagents 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 only acceptable anticoagulants are heparin and EDTA. 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:** 546 nm (530-550 nm)
- **Optical path:** 1 cm
- **Assay type:** Endpoint
- **Direction:** Increase
- **Sample : Reagent Ratio:** 1: 60
- **Temperature:** 37 °C and 20 – 25 °C
- **Zero adjustment:** Reagent or Sample blank
- **Sensitivity:** 7 U/L
- **Linearity:** 89 U/L

**Procedure**

1. **Measurement against Reagent Blank**

**Pipette 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>0.5 ml</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>

<table>
<thead>
<tr>
<th>R2</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 ml</td>
</tr>
<tr>
<td>Mix and incubate for exactly 20 minutes at 20 – 25 °C</td>
</tr>
</tbody>
</table>

**Sodium hydroxide**
- 5.0 ml
- Mix, measure absorbance of specimen against reagent blank at 546 nm after 5 minutes.

2. **Measurement against Sample Blank**

<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>100 µl</td>
</tr>
<tr>
<td>Mix and incubate for exactly 30 minutes at 37 °C</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>R2</th>
</tr>
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<tbody>
<tr>
<td>0.5 ml</td>
</tr>
<tr>
<td>Mix and incubate for exactly 20 minutes. at 20 – 25 °C</td>
</tr>
</tbody>
</table>

**Sodium hydroxide**
- 5.0 ml
- Mix, measure absorbance of specimen against sample blank at 546 nm after 5 minutes.
Calculation
Obtain the AST activity 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>0.020</td>
<td>7</td>
<td>0.100</td>
<td>36</td>
</tr>
<tr>
<td>0.030</td>
<td>10</td>
<td>0.110</td>
<td>41</td>
</tr>
<tr>
<td>0.040</td>
<td>13</td>
<td>0.120</td>
<td>47</td>
</tr>
<tr>
<td>0.050</td>
<td>16</td>
<td>0.130</td>
<td>52</td>
</tr>
<tr>
<td>0.060</td>
<td>19</td>
<td>0.140</td>
<td>59</td>
</tr>
<tr>
<td>0.070</td>
<td>23</td>
<td>0.150</td>
<td>67</td>
</tr>
<tr>
<td>0.080</td>
<td>27</td>
<td>0.160</td>
<td>74</td>
</tr>
<tr>
<td>0.090</td>
<td>31</td>
<td>0.170</td>
<td>89</td>
</tr>
</tbody>
</table>

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

Sensitivity
If run as recommended, the minimum detection level is 7 U/L.

Linearity
The assay is linear up to 89 U/L. If the absorbance exceeds 0.170 at 546 nm (89 U/L), samples should be diluted 1 + 9 using sodium chloride and repeat the assay (result × 10).

Interfering Substances
Serum, plasma

Haemolysis
Erythrocyte contamination elevates results, since AST activities in erythrocytes are 15 times higher 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 transaminases levels. Measurement against a serum blank instead of a reagent blank avoids the risk of finding such artifacts.

Expected values
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
7 – 89 U/L.

Waste Disposal
This product is made to be used in professional laboratories. Please consult local regulations for a correct waste disposal.

SS6: dispose of this material and its container at hazardous or special waste collection point.

SS7: use appropriate container to avoid environmental contamination.

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

References

<table>
<thead>
<tr>
<th>ORDERING INFORMATION</th>
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<tbody>
<tr>
<td>CATALOG NO.</td>
</tr>
<tr>
<td>260 001</td>
</tr>
<tr>
<td>260 002</td>
</tr>
</tbody>
</table>

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