**Intended Use**

For the quantitative determination of Aspartate Aminotransferase (AST) in human serum. For in vitro diagnostic use only.

**Clinical Significance**

AST is widely distributed in tissues with the highest concentrations found in the liver, heart, skeletal muscle and kidneys. Diseases involving any of these tissues can lead to elevated levels of AST in serum. Following myocardial infarction, AST levels are elevated and reach a peak after 48 to 60 hours. Hepatobiliary diseases such as cirrhosis, metastatic carcinoma and viral hepatitis can show increased levels of AST. Other disorders which can lead to an elevated level of AST are muscular dystrophy, dermatomyositis, acute pancreatitis and infectious mononucleosis.¹

**Method History**

Karmen² developed a kinetic assay procedure in 1955 which was based upon the use of malate dehydrogenase and NADH. Optimized procedures were presented by Henry³ in 1960 and Amador and Wacker⁴ in 1962. These modifications increased accuracy and lowered the effect of interfering substances. The Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology⁵ published a recommended method based on optimized modifications in 1974. In 1976, the Expert Panel on Enzymes of the International Federation of Clinical Chemistry (IFCC)⁶ proposed the addition of pyridoxal-5-phosphate to the reaction mixture to ensure maximum activity. The IFCC⁷ published a recommended method that included P-5-P in 1978. The present method is based on IFCC recommendations but does not contain P-5-P since most specimens contain adequate amounts of this cofactor for full recovery of AST activity.⁸³⁹¹⁰

**Principle**

AST catalyzes the transfer of the amino group from L-α-ketoglutaric acid to L-glutamate to yield oxaloacetate and L-glutamate. The oxaloacetate undergoes reduction with simultaneous oxidation of NADH in the malate dehydrogenase (MDH) catalyzed indicator reaction. The resulting rate of decrease in absorbance at 340nm is directly proportional to the AST activity. Lactate dehydrogenase (LDH) is added to prevent interference from endogenous pyruvate which is normally present in serum.

**Reagents**

After combining R1 and R2 the reagent contains: L-aspartic acid >200mM, α-ketoglutaric acid 12mM, LDH (microbial) >1000U/L, MDH (microbial) >800U/L, NADH >0.18mM, buffer, pH 7.8±0.1, Sodium Azide 0.25%, Stabilizers.

**Reagent Preparation**

The reagents are provided as "ready to use" Liquids.

**Reagent Storage**

Store reagents at 2-8°C.

**Reagent Deterioration**

Do not use reagent if:

1. The initial absorbance at 340nm is below 0.800.
2. The reagent fails to meet stated parameters of performance.

---

**Precautions**

1. This reagent set is for in vitro diagnostic use only.
2. The reagent contains sodium azide (0.25%) as a preservative. Do not ingest. May react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with a large volume of water to prevent azide build up.

**Specimen Collection and Storage¹¹**

1. Non-hemolyzed serum is recommended. Red cells contain AST which can give falsely elevated results.
2. AST in serum is reported stable for ten days when refrigerated (2-8°C), two weeks when frozen (-20°C), and four days when stored at room temperature (15-30°C).

**Interferences**

1. A number of drugs and substances affect AST activity. See Young, et al.¹²
2. Patients with severe vitamin B6 deficiency could have a decreased recovery of AST, presumably due to a lack of pyridoxal phosphate.¹³
3. Bilirubin to at least 18 mg/dl, and hemoglobin to at least 300 mg/dl, have been found to have a negligible effect on this procedure.

**Materials Provided**

AST (SGOT) Reagents R1 and R2.

**Materials Required but not Provided**

1. Controls
2. Beckman Coulter AU™ analyzer
3. Application and operation manual.

**Procedure (Beckman Coulter AU™400 application)**

---

**SPECIFIC TEST PARAMETERS**

<table>
<thead>
<tr>
<th>TEST NUMBER</th>
<th>TEST NAME</th>
<th>TYPE</th>
<th>OPERATIONAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1 VOLUME</td>
<td>R2 VOLUME</td>
<td>150</td>
<td>30</td>
</tr>
<tr>
<td>DIL. VOL.</td>
<td>DIL. VOL.</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MIN. OD</td>
<td>MAX. OD</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>OD MAX. OD</td>
<td>0.300</td>
<td>2.500</td>
<td></td>
</tr>
<tr>
<td>REAGENT OD LIMIT</td>
<td>WAVELENGTH</td>
<td>PRI. 340</td>
<td>SEC. 380</td>
</tr>
<tr>
<td>METHOD RATE</td>
<td>DYNAMIC RANGE</td>
<td>V</td>
<td>V</td>
</tr>
<tr>
<td>REACTION SLOPE</td>
<td>MEASURING POINT 1</td>
<td>FIRST</td>
<td>LAST</td>
</tr>
<tr>
<td>LINEARITY</td>
<td>MEASURING POINT 2</td>
<td>FIRST</td>
<td>LAST</td>
</tr>
<tr>
<td>NO LAG TIME</td>
<td>ON BOARD STABILITY PERIOD</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

**SPECIFIC TEST PARAMETERS**

<table>
<thead>
<tr>
<th>VALUE FLAG</th>
<th>LEVEL</th>
<th>AGE L</th>
<th>MONTH</th>
<th>AGE H</th>
<th>MONTH</th>
<th>L</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>#</td>
</tr>
<tr>
<td>2.</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>#</td>
</tr>
<tr>
<td>3.</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>#</td>
</tr>
<tr>
<td>4.</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>#</td>
</tr>
<tr>
<td>5.</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>#</td>
</tr>
<tr>
<td>6.</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>#</td>
</tr>
<tr>
<td>NONE SELECTED</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>#</td>
</tr>
<tr>
<td>OUT OF RANGE</td>
<td>L</td>
<td>H</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>#</td>
</tr>
<tr>
<td>PANIC VALUE</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>#</td>
</tr>
</tbody>
</table>
### CALIBRATION SPECIFIC PARAMETERS

<table>
<thead>
<tr>
<th>CAL TYPE</th>
<th>MB</th>
<th>FORMULA: Y=AX+B</th>
<th>V</th>
<th>COUNTS: 4</th>
<th>PROCESS: V</th>
</tr>
</thead>
<tbody>
<tr>
<td>POINT 1.</td>
<td>#</td>
<td>OD CONC. FAC/OD-L FAC/OD-H</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>POINT 2.</td>
<td>#</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>POINT 3.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>POINT 4.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>POINT 5.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>POINT 6.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>POINT 7.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-POINT CAL. POINT:</td>
<td>o</td>
<td>WITH CONC-0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MB TYPE FACTOR:</td>
<td>6007</td>
<td>CALIBRATION STABILITY PERIOD: #</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Quality Control

The validity of the reaction should be monitored using control sera with known normal and abnormal AST (SGOT) values. These controls should be run at least every shift in which AST (SGOT) assays are performed. It is recommended that each laboratory establish their own frequency of control determination.

### Expected Values

1. 8 to 22 IU/L (30°C)
2. 5 to 34 IU/L (37°C)

Since the expected values are affected by age, sex, diet, and geographical location, each laboratory is strongly urged to establish its own reference range for this procedure.

### Procedure Notes

1. Turbid or highly icteric samples may give readings whose initial absorbance exceeds the capabilities of the spectrophotometer. More accurate results may be obtained by using 0.05ml (50 ul) of sample and multiplying the final answer by two.

### Limitations

1. Samples with values above 500 IU/L should be diluted 1:1 with saline, re-assayed and the results multiplied by two.
2. Patients with severe vitamin B6 deficiency could have a decreased recovery of AST, presumably due to a lack of pyridoxal phosphate.

### Calibration

The procedure is standardized by means of the millimolar absorbptivity of NADH taken as 6.22 at 340nm under the test conditions described.

### Calculation

One international Unit (IU/L) is defined as the amount of enzyme that catalyzes the transformation of one micromol of substrate per minute under specified conditions.

\[
\text{AST (IU/L)} = \frac{\Delta \text{Abs./Min.} \times 1.1 \times 1000}{1.1} = \frac{\Delta \text{Abs./min.} \times 1768}{6.22 \times 0.10 \times 1.0}
\]

Where \(\Delta \text{Abs./Min.}\) = Average absorbance change per minute

1000 = Conversion of IU/ml to IU/L

1.1 = Total reaction volume (ml)

6.22 = Millimolar absorbptivity of NADH

0.10 = Sample Volume (ml)

1.0 = Light path in cm

Example: If the average absorbance change per minute = 0.12 then 0.12 x 1768 = 212 IU/L

### References