**INTENDED USE**
This reagent is intended for the in vitro quantitative determination of AST (Aspartate Aminotransferase EC2.6.1.1) in human serum or plasma.

**CLINICAL SIGNIFICANCE**
AST is widely distributed with high concentrations in the heart, liver, skeletal muscle, kidney and erythrocytes. Damage or disease to any of these tissues such as myocardial infarction, viral hepatitis, liver necrosis, cirrhosis and muscular dystrophy may result in raised serum levels of AST.1

**METODOLOGY**
In 1955, Karmen et al1 described the first kinetic assay of AST for diagnostic purposes. This method was evaluated and improved by many investigators, primarily Henry et al2 and now forms the basis of many national and international recommended procedures. The AST Reagent is based on the recommendations of the IFCC.4

The series of reactions involved in the assay system is as follows:

1. L-Aspartate + 2-Oxoglutarate \(\rightarrow\) Oxaloacetate + L-Glutamate
2. Oxaloacetate + NADH \(\rightarrow\) Malate + NAD
3. Sample Pyruvate + NADH \(\rightarrow\) L-Lactate + NAD

1. AST present in the sample catalyses the transfer of the amino group from L-aspartate to 2-oxoglutarate forming oxaloacetate and L-glutamate.
2. Oxaloacetate in the 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 340nm due to the oxidation of NADH to 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.

**REAGENT COMPOSITION**

<table>
<thead>
<tr>
<th>Active Ingredients</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Oxoglutarate</td>
<td>13.2 mmol/L</td>
</tr>
<tr>
<td>L-Aspartate</td>
<td>220 mmol/L</td>
</tr>
<tr>
<td>MDH (porcine heart)</td>
<td>&gt; 600 U/L</td>
</tr>
<tr>
<td>LDH (microbial)</td>
<td>&gt; 1000 U/L</td>
</tr>
<tr>
<td>NADH</td>
<td>&gt; 0.18 mmol/L</td>
</tr>
<tr>
<td>Tris Buffer</td>
<td>88 mmol/L</td>
</tr>
<tr>
<td>EDTA</td>
<td>5.5 mmol/L</td>
</tr>
</tbody>
</table>

Also contains non-reactive fillers and stabilizers.

**STABILITY AND STORAGE**

**Prior to use:**
When stored refrigerated at 2-8°C the reagent is stable until the expiration date stated on the bottle and kit box label.

**Reconstituted Reagent:**
When stored capped at 2-8°C the reagent is stable for at least 30 days.

**Indications of Reagent Deterioration:**
- Turbidity,
- Absorbance <1.1 at 340 nm (1 cm); and/or
- Failure to recover control values within the assigned range.

**SPECIMEN COLLECTION AND HANDLING**
Serum: Use non-haemolysed serum.
Plasma: Use non-haemolysed plasma.

Storage: AST samples may be stored for at least 7 days at 4°C.

**ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED**
- A clinical chemistry analyser capable of maintaining constant temperature (37°C) and measuring absorbance at 340 nm.
- Assay specific consumables, eg: sample cups.
- Distilled or deionised water for reagent preparation and related equipment eg: pipettes.
- Normal and Abnormal assayed control material.

**ASSAY PROCEDURE**
The following system parameters are recommended. Individual instrument applications are available upon request from the Technical Support Group.

**SYSTEM PARAMETERS**

- Temperature: 37°C
- Wavelength: 340 nm
- Assay Type: Rate/Kinetic
- Direction: Decrease
- Sample : Reagent Ratio 1:10, eg: Sample Vol 30 μL, Reagent Vol 300 μL
- Delay/Lag Time: 60 seconds
- Read Time: 60 seconds
- Reagent Blank: Low 1.1 AU
- (1cm lightpath, 340nm) High 2.0 AU
- Linearity: 0 - 450 U/L
- (refer to Linearity section) Sensitivity 0.57 ΔmA/min per U/L

**CALCULATIONS**
Results are calculated, usually automatically by the instrument, as follows:

**Activity in U/L = \(\frac{\Delta \text{Abs/min}}{\text{Factor}}\)**

Where:
- TV = Total reaction volume in mL
- SV = Sample volume in mL
- 6.3 = millimolar absorption coefficient of NADH at 340nm
- (See note 4)
- P = Cuvette pathlength in cm.

**Example**
\[\frac{\Delta \text{Abs/min}}{\text{Factor}} = \frac{0.10}{1746} = 0.175 \text{ U/L}\]

**REAGENT PREPARATION**
Reconstitute the reagent with the volume of distilled or deionised water stated on the vial label.
NOTES
1. The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
2. If the change in absorbance is greater than 0.26/min repeat the assay with less sample or dilute with saline. Remember to adjust the factor for the smaller sample volume or to multiply the final result by the dilution factor.
3. Valid results depend on an accurately calibrated instrument, timing, and temperature control.
4. The millimolar absorption coefficient for NADH at 334nm = 6.18 and at 365nm = 3.40.
5. Unit Conversion: U/L x 16.67 x 10^-14 µkat/L

CALIBRATION
Not required. The rate of reaction is converted to U/L of activity by a calculation factor. Refer to the calculation section of this package insert.

QUALITY CONTROL
To ensure adequate quality control, normal and abnormal control with assayed values should be run as unknown samples:-
• At least every eight hours.
• When a new bottle of reagent is used.
• After preventative maintenance is performed or a critical component is replaced.
Control results falling outside the upper or lower limits of the established ranges indicate the assay may be out of control.
The following corrective actions are recommended in such situations:-
• Repeat the same controls.
• If repeated control results are outside the limits, prepare fresh control serum and repeat the test.
• If results on fresh control material still remain outside the limits, then repeat the test with fresh reagent.
• If results are still out of control, contact Technical Services or your local distributor.

LIMITATIONS
1. Studies to determine the level of interference from haemoglobin, bilirubin, pyruvate and lipaemia were carried out. The following results were obtained:
   Haemoglobin: No interference from haemoglobin up to 920 mg/dL.
   Bilirubin: No interference from bilirubin up to 1000 µmol/L (60 mg/dL).
   Pyruvate: No interference from pyruvate up to 0.60 mmol/L.
   Lipaemia: No interference from lipaemia, measured as triglycerides, up to 6.0 mmol/L (530 mg/dL).
2. Haemolized serum specimens should not be used. AST activity levels in erythrocytes are some 15 times higher, than those in sera.9
3. Young DS5 has published a comprehensive list of drugs and substances which may interfere with this assay.

EXPECTED VALUES2
At 37°C 5-34 U/L

Levels approximately twice the adult levels are seen in neonates and infants. These levels decline to normal adult levels after 6 months.
The quoted values are representative of the expected range for this method and should serve as a guide only. It is recommended that each laboratory verify this range or derive a reference interval for the population that it serves.8

PERFORMANCE DATA
The following data was obtained using the AST(GOT) reagent on a well maintained automated clinical chemistry analyser. Users should establish product performance on their specific analyser used.

IMPRECISION
Within Run: LEVEL I LEVEL II
Number of Samples 20 20
Mean (U/L) 33 169
SD (U/L) 0.44 0.88
CV% 1.32 0.52

Between Day: LEVEL I LEVEL II
Number of Samples 20 20
Mean (U/L) 34 308
SD (U/L) 1.57 8.24
CV% 4.69 2.68

ACCURACY
Comparison studies were carried out using a similar commercially available reagent as a reference. Serum samples were assayed in parallel and the results compared by least squares regression. The following statistics were obtained.

Number of sample pairs 50
Range of sample results 7-298 U/L
Mean of reference method results 48 U/L
Mean of AST(GOT) results 45 U/L
Slope 0.96
Intercept -0.78 U/L
Correlation coefficient 0.997

LINEARITY
When run as recommended, the assay is linear up to 450 U/L.

SENSITIVITY
When run as recommended the sensitivity of this assay is 0.57 ΔmA/min per U/L.

REFERENCES

© 2008 Thermo Fisher Scientific Inc. All rights reserved.

Fisher Diagnostics
a division of Fisher Scientific Company, LLC
a subsidiary of Thermo Fisher Scientific Inc.
Middletown, VA 22645-1905 USA
Phone: 800-528-0494
Fax: 540-869-8132

MDCI Ltd.
Arundel House
1 Liverpool Gardens
Worthing, West Sussex BN11 1SL UK

Reorder Information
Catalogue No.     Configuration
1150-200  20 x 10 mL
TR17515  20 x 20 mL
TR17503/1180-500 10 x 50 mL
TR17504  10 x 200 mL

© 2008 Thermo Fisher Scientific Inc. All rights reserved.