CATACHEM

ASPARTATE AMINOTRANSFERASE (AST)
MANUAL APPLICATION
C154-0A, C154-0B, V154-0A

Intended Use
For **IN VITRO** quantitative determination of AST (GOT) in serum or plasma using manual or automated applications.

Clinical Significance
Measurements of AST activity are used for diagnosing liver and heart diseases, as well as, for monitoring the causes and treatment.[1]

Method History
Aspartate Aminotransferase (EC 2.6.1.1) enzyme activity in human blood was first reported by Karmen, et al.[2] In 1977, the International Federation for Clinical Chemistry (IFCC) published recommendations on optimum assay conditions for aspartate aminotransferase (AST) assay.[3,4] Catachem's Aspartate Aminotransferase for manual or automated applications is based upon the work reported by the IFCC.

Method Principle
The Aspartate Aminotransferase enzyme catalyzes the conversion of alpha-ketoglutarate and L-aspartate to L-glutamate and oxalacetate. The oxalacetate produced is then quantitatively determined by the MDH-NADH reaction. The decrease in absorbance due to the oxidation of NADH to NAD^+ is monitored at 340nm. The rate of decrease in absorbance of the reaction mixture is directly proportional to the AST enzyme activity in the serum sample. The reaction scheme illustrates the reactions that occur in this method.

$$\text{L-Aspartate + Alpha-KG} \xrightarrow{\text{AST}} \text{L-Glutamate + Oxalacetate}$$

$$\text{Oxalacetate + NADH + H} \xrightarrow{\text{MDH}} \text{L-Malate + } NAD^+$$

Reagent Content
The reagents contain approximately:

AST Substrate Reagent
Each liter contains:
Buffer

L. Aspartic Acid	288 mmol/L
Lactate Dehydrogenase	≥1200 U/L
Malate Dehydrogenase	≥700 U/L
Alpha-ketoglutarate	13.2 mmol

Non-reactive ingredients and stabilizers

AST Activator Reagent
Each liter contains:
Buffer

NADH	2.5 mmol/L

Non-reactive ingredients and stabilizers

Preparation of Working Reagents
The Catachem AST Substrate Reagent (R1) and the AST Activator Reagent (R2) are liquid, ready for use and require no preparation.

Reagent Storage and Stability
Store the AST Reagents at 2-8˚C. When unopened and stored as directed, these reagents are stable until the expiration date stated on the label. Once opened, the Catachem AST Reagents stable for at least 30 days when stored as directed, refrigerated at 2-8˚C and capped when not in use.

Specimen Collection and Preparation
Test sera should be fresh, clear, and unhemolyzed. When blood is drawn, it should be processed as soon as possible and the serum should be isolated from the clot without delay. In separated unhemolyzed serum the AST concentration is stable for 1-3 days at 2-8˚C and minimal loss of activity occurs if stored frozen.[1] Plasma can also be used if collected using ammonium, lithium or sodium heparin. Anticoagulants that should not be used include potassium oxalate/sodium fluoride and sodium citrate.

Precautions
Avoid contact of reagents or specimens with skin and eyes. Should contact occur, wash affected area with plenty of cold water. **DO NOT PIPETTE REAGENTS BY MOUTH.**

Quality Control
To monitor the performance of the Catachem AST Reagents and the procedure used, the regular use of a normal and abnormal control serum is recommended.

Interfering Substances
A comprehensive discussion has been reported on the effects of interfering substances on AST assays.[5] A summary of the influence of drugs on clinical laboratory procedures may be found by consulting D.S. Young, et al.[5]

Expected Values
The range of expected human values determined for this method is 5-40 U/L at 37˚C. These values are suggested guidelines. It is recommended that each laboratory establish the normal range for the area in which it is located.

Procedure
Important: Read the entire procedure instructions before proceeding with the assay.

Materials Required (But Not Provided)
Spectrophotometer with a 340 nm wavelength

Matching cuvettes	1 cm light path
Timer	to time incubation period
Pipettes	0.9 mL & 0.1 mL for reagents
Pipette	0.05mL for sample

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Materials Provided
Catachem AST Substrate Reagent (R1)
Catachem AST Activator Reagent (R2)

Analytical Parameters

Wavelength	340nm
Temperature	37˚C
Path Length	1 cm
Reaction Mode	Rate: Zero Order
Reaction Time:	3 minutes
Substrate Reagent (R1) Volume:	0.9 mL
Activator Reagent (R2) Volume:	0.1 mL
Sample Volume:	0.05 mL
Total Volume:	1.05 mL
Sample-to-reagent ratio	1:21

Assay Procedure
1. Set spectrophotometer wavelength at 340nm and zero the instrument with the cuvette containing water.
2. Pipette 0.9 mL of AST Substrate Reagent (R1) into each of two cuvettes marked “Sample” and “Control”.
3. Pipette 0.1 mL of AST Activator Reagent (R2) into the cuvettes marked “Sample” and “Control”.
4. Incubate both cuvettes for 3 minutes at 37˚C.
5. Pipette 0.05 mL of Control or Sample into their respective cuvettes. Mix well.
6. Replace cuvettes into spectrophotometer and continuously monitor the change in absorbance for at least 3 minutes.
7. Read the absorbance of both the “Control” and “Sample”.
8. Calculate the AST concentration (U/L) in the sample(s), as shown in calculations and results.

Results and Calculations

$$\text{AST activity U/L} = \frac{\Delta \text{ OD}}{\text{min}} \times \frac{\text{TV} \times 1000}{6.22 \times \text{L} \times \text{SV}}$$

Where:
Δ OD/min = change in absorbance/minute
TV in mL = total volume in cuvette
SV in mL = volume of sample being assayed
6.22 = mmol absorptivity of NADH at 340nm
1 cm = cuvette path length in cm.
1000 = converts µmol/ml to U/L

Example: ΔOD/min = 0.01

$$\text{AST U/L} = \frac{0.01 \times 1.05 \times 1000}{6.22 \times 1.0 \times 0.05} = 34 \text{ U/L}$$

Method Performance Characteristics

Sensitivity: The sensitivity of this method is 0.0002 absorbance units per U/L.
Linear Range: In this method there is no significant nonlinearity over the range of 0-1000 U/L.
Precision: Within-run and day-to-day precision is summarized below.

Precision Study

AST	Within-Run Precision		Total Precision	
Mean	SD	CV	SD	CV
U/L	U/L	%	U/L	%
32	0.80	2.70	1.80	5.40
385	4.90	1.30	8.20	2.10
732	3.30	0.50	15.00	2.10

Correlation
A comparison of this method using a discrete random access analyzer and a reference procedure based upon the recommendations of IFCC resulted in the following regression statistics:

Range	=	15 - 440 u/L
N	=	126
Y	=	0.994x + 0.95
r	=	0.999
Sy.x	=	3.4

References

1. Fundamentals of Clinical Chemistry. Edited by Norbert Tietz. W.V. Saunders Company, Philadelphia.
2. Karmen A., Wroblewski F., Ladue J. Transaminase activity in human blood.J Clin Inves 34,126 (1955).
3. Expert Panel on Enzymes, Committee on Standards (IFCC).Provisional recommendations (1974) on IFCC methods for the measurement of catalytic concentration of enzymes. Clin Chem Acta 61, following p238, F11 to F24.
(1975). Clin Chem 22, 384 (1976).
4. Expert Panel on Enzymes, Committee on Standards (IFCC). Provisional recommendations on IFCC methods for the measurement of catalytic concentrations of enzymes, part 2. IFCC method for Aspartate Aminotransferase. Clin Chem Acta 70, following p336, F19 to F42 (1976). Clin Chem 23, 887 (1977).
5. Young D.S., Pestaner L.C., Gibberman V. Effect of Drugs on clinical laboratory tests. Clin Chem 21 (5): 1D-432D (1975).

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