CATACHEM

ASPARTATE AMINOTRANSFERASE (AST)
C154-0B

Contents	Product No.	Package
AST KIT	**C154-0B**	
AST Sample Diluent	C154-05	6 x 45.0 mL
AST Activator	C154-06	6 x 5.0 mL

REAGENT PREPARATION

The Catachem AST reagents are packaged ready for use.
No preparation of either reagent is required.

REAGENT STORAGE AND STABILITY

Store the unopened reagent kit at 2-8°C.
When stored as directed, both unopened reagents are stable until the expiration date stated on the label.

NOT FOR USE IN UNPROFESSIONAL SETTINGS

FOR TECHNICAL ASSISTANCE:
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ASPARTATE AMINOTRANSFERASE (AST) C154-0B MANUAL/AUTOMATED APPLICATION

Intended Use
For **IN VITRO quantitative** determination of AST (SGOT) 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.

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 the 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.

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

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

Reagent Content
When mixed according to the directions, the concentrations of the active ingredients in the reagents will be approximately as follows:

AST (SGOT) 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 (SGOT) Activator Reagent
Each liter contains:
Buffer

NADH	2.5 mmol/L

Non-reactive ingredients and stabilizers

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

Preparation of Working Reagents
If running as a 2-component system, reagents are packaged ready to use. If desired, a working reagent can be prepared by completing the following steps: Prepare the Catachem AST Working Reagent by adding the contents of the AST Activator Reagent vial into the AST Sample diluent bottle. Mix well by inverting the bottle several times.

AST (SGOT) Reagent Storage and Stability
Store the Catachem AST (SGOT) Reagents at 2-8˚C. When stored as directed, these reagents are stable until the expiration date stated on the label. If a single working reagent is prepared store the Catachem AST (SGOT) working Reagent at 2-8˚C. When prepared and stored as directed, the Catachem AST Working Reagent is stable for 30 days. The unopened Catachem AST (SGOT) Reagents have been tested to reflect shipping conditions and are stable for the lifespan of the product if frozen up to 5 times or upon reaching temperatures of up to 40°C for up to one week.

Specimen Collection and Preparation
Test sera should be fresh, clear, and non-hemolyzed. 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 non-hemolyzed serum the AST concentration is stable for 1-3 days at 2-8˚C. 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.

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 normal range of this assay using human samples as performed below is 5 U/L to 40 U/L at 37°C. These values serve as suggested reference points only. For veterinary samples, ranges will be dependent on the species under test. It is recommended that each laboratory establish the normal ranges for the species under study and for the geographic area in which the laboratory is located.

Quality Control
Assay performance should be monitored by running normal/abnormal controls concomitantly with samples. Catachem has optimized this assay using Catatrol Level I (C1200-11) and Catatrol Level II (C1200-12) and recommends their use for daily QC.

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

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

Matching cuvettes	1 cm light path
Timer	to time incubation
Pipette	1.0 mL for reagent

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Pipette	0.05mL for sample
Graduated Cylinders	25 mL and 50 mL

Material Provided

Catachem AST Substrate Reagent (Liquid)
Catachem AST Activator Reagent (Liquid)

Analytical Parameters

Wavelength	340nm
Temperature	37˚C
Path length	1 cm
Reaction Mode	Rate: zero order
Reaction Time:	3 minutes
Reagent Volume:	1.0 mL
Sample Volume:	0.05 mL
Total Volume:	1.05 mL
Sample-to-reagent ratio	1:21

Assay Procedure

1. Prepare the required volume of AST (SGOT) single Working Reagent.
2. Set spectrophotometer wavelength at 340nm and zero the instrument with a cuvette containing water.
3. Pipette 1.0 ml of Working Reagent into each of two cuvettes marked “Sample” and “Control”.
4. Incubate both cuvettes for 3 minutes at 37°C.
4. Pipette 0.05 ml of Control or Sample into their respective cuvettes. Mix both cuvettes well.
5. Replace cuvettes into the spectrophotometer and continuously monitor the change in absorbance at 37°C for at least 3 minutes.
6. Determine the absorbance per minute (ΔOD/min) of both the “Control” and “Sample”.
7. Calculate the AST (SGOT) 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 ml	= total volume in cuvette
SV ml	= volume of sample being assayed
6.22	= mmol absorptivity of NADH at 340nm
L	= cuvette path length in cm.
1000	= converts µmol/ml to U/L

Example: ΔOD/min = 0.01

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

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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