

AST Kinetic

1.0 INTENDED USE

This reagent is intended for the quantitative determination of aspartate aminotransferase (AST) in serum.

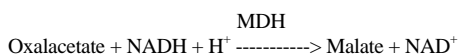
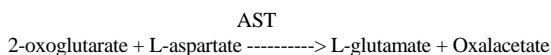
2.0 BACKGROUND

2.1 METHOD AND HISTORY

Karmen (10.1) introduced a method for the determination of aspartate aminotransferase, formerly called glutamate oxalacetic transaminase (GOT). Henry et. al.(10.2) optimized the method. The procedure used in this kit is a modification of the method of Henry. The NADH concentration is increased to extend the linearity and oxamic acid is added to remove interference by serum pyruvate (10.3,10.4,10.5).

2.2 TEST PRINCIPLE

The AST catalyzes the reaction of 2-oxoglutarate and L-aspartate to L-glutamate and oxalacetate. Then malate dehydrogenase (MDH) catalyzes the oxidation of NADH to NAD.



The rate of decrease in absorbance of the reaction mixture at 340 nm, due to the oxidation of NADH is directly proportional to the AST activity.

2.3 CLINICAL SIGNIFICANCE (10.8)

The principle causes of elevated aspartate aminotransferase activity in serum are damage to or disease of heart and liver. Decreased levels of AST activity in serum may occur as a result of pyridoxal phosphate (vitamin B6) deficiency. Low levels of this vitamin can occur in patients undergoing dialysis.

3.0 SPECIMEN COLLECTION AND HANDLING

3.1 PATIENT PREPARATION

No special patient preparation is required.

3.2 SPECIMEN COLLECTION.

Fresh, clear, unhemolyzed serum is the preferred specimen.

Use a standard venipuncture tube to draw patient sample.

The amount of sample required will depend on the analyzer used. The amount of serum required is in the range of 5-200 μl . Call Biotron's technical service department at 1-800-595-8766 for the recommended sample volume for your analyzer.

Record the patient's name, date and time of sample collection and preparation.

3.3 SPECIMEN STORAGE

Serum samples should be kept refrigerated (2° to 8°C) and analyzed within 24 hours. If this is not possible, serum samples may be stored refrigerated (2° to 8°C) or frozen (-20° to 0° C) and are stable for up to 7 days. Frozen samples should be thawed at room temperature and mixed completely before analysis. Thawed samples should not be refrozen.

4.0 MATERIALS

(10 X 10 ml)
(6 X 50 ml)

Reagents necessary for the determination of AST are included in the kit.

4.1 REAGENT

AST REAGENT contains, after reconstitution with deionized water:

NADH	0.21 mM
malate dehydrogenase (pig heart)	≥ 600 U/L
2-oxoglutarate	12 mM
L-aspartic acid	200 mM
tris buffer	100 mM (pH = 7.8)
preservative	

4.2 WARNINGS AND PRECAUTIONS

For In Vitro Diagnostic Use. Not for Internal use in Humans or Animals. In Vitro Diagnostics reagents may be hazardous. Avoid ingestion and skin or eye contact.

4.3 REAGENT PREPARATION

4.3.1

Add 10 ml of the deionized water to each of the required number of vials of AST reagent. Replace the rubber stopper and allow 5 minutes for reconstitution. Swirl gently until the contents of the vial are completely dissolved. Record the date and time of reconstitution.

4.3.2

Add 50 ml of the deionized water to each of the required number of vials of AST reagent. Replace the rubber stopper and allow 5 minutes for reconstitution. Swirl gently until the contents of the vial are completely dissolved. Record the date and time of reconstitution.

4.4 REAGENT STORAGE AND STABILITY

Unopened reagents are stable at 2-8° C (refrigerated) until the expiration date stated on the labels. The reconstituted reagent is stable at 2-8° C (refrigerated) for 14 days or at 18-26° C (room temperature) for 8 hours.

The reconstituted reagent solution should be clear. Cloudiness indicates contamination and the reagent should be discarded. The initial absorbance of the reagent read against distilled water at 340 nm should be 1.100 or greater.

4.5 ADDITIONAL MATERIALS REQUIRED

- 4.5.1 Spectrophotometer capable of reading absorbance at 340 nm.
- 4.5.2 1 cm cuvettes or a flow cell capable of transmitting light at 340 nm.
- 4.5.3 Test tubes capable of holding 4 ml.
- 4.5.4 Pipettes capable of delivering 3.0 ml and 200 µl.
- 4.5.5 Timer with one minute increments.
- 4.5.6 Constant temperature source which can be adjusted to 30° C or 37° C.
- 4.5.7 Normal and abnormal control for quality control.

5.0 TEST PROCEDURE

The following is a general procedure for use on a manual instrument.

5.1 PROCEDURE CONDITIONS

Wavelength	340 nm
Temperature	30° C or 37° C
Pathlength	1.0 cm
Mode	kinetic
Reaction time	2 - 4 min
Sample volume	200 µl
Reagent volume	3.0 ml
Total volume	3.2 ml
Sample to reagent ratio	1/15

5.2 INSTRUMENT

Any instrument capable of reading absorbance accurately with a sensitivity of 0.001 absorbance at 340 nm may be used. The band width should be 10 nm or less, stray light 0.5% or less, and the wavelength accuracy within 2 nm.

5.3 CALIBRATION

No reagent calibration is necessary as this procedure is standardized based on the millimolar absorptivity of NADH which is taken as 6.22 at 340 nm under the test conditions described.

5.4 PROCEDURE

- 5.4.1 Prepare the required volume of AST working reagent. (See 4.3 Reagent Preparation section.)
- 5.4.2 Into separate test tubes pipette 200 µl of serum to be assayed.
- 5.4.3 Add 3.0 ml of reagent, mix, and incubate for one to three minutes at 30° C or 37° C. The lag time will be decreased if the reagent is prewarmed to the incubation temperature.
- 5.4.4 Record the decrease in absorbance at 340 nm at one minute intervals until the absorbance change is constant.

5.5 CALCULATION AND RESULTS

$$\text{AST (U/L)} = \frac{\Delta A/\text{min} \times \text{assay volume (ml)} \times 1000}{6.22 \times \text{light path (cm)} \times \text{sample volume (ml)}} = \Delta A/\text{min} \times 2572$$

ΔA/min = change in absorbance per minute
 Assay volume = total reaction volume expressed in ml
 1000 = converts U/ml to U/L
 6.22 = absorbance coefficient of NADH at 340 nm
 Light path = length of the light path expressed in cm (usually 1)
 Sample volume = sample volume expressed in ml
 2572 = factor derived from constants in the equation
 Example: AST (U/L) =
 .015 X 3.2 X 1000
 ----- = .015 X 2572 = 39 U/L
 6.22 X 1 X 0.2
 0.015 = change in absorbance per minute
 3.2 = assay volume in ml
 1 = light path in cm
 0.2 = sample volume in ml

6.0 INTERPRETATION OF RESULTS

6.1 EXPECTED VALUES (10.7)

The range of expected values is:
 8 - 20 U/L (30° C)
 12 - 31 U/L (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.

6.2 MEDICAL ALERT VALUES (10.9)

Each laboratory should establish low and high values beyond which the patient would require immediate attention by a physician. If a "medical alert value" is reached, always repeat the test to confirm the result and notify a physician if the result is confirmed.

6.3 LIMITATIONS OF PROCEDURE

This procedure measures total AST. Red blood cells contain high concentrations of AST, therefore hemolysis can elevate results. A summary of the influence of drugs on clinical laboratory tests may be found by consulting Young, D.S., et. al, (10.6).

7.0 QUALITY CONTROL

Standard practice for quality control should be applied to this system. Commercially available lyophilized controls can be used to monitor the daily acceptable variations. Normal and abnormal controls should be assayed at the beginning of each run of patient samples, whenever a new reagent or a different lot number is being used, and following any system maintenance. A satisfactory level of performance is achieved when the analyte values obtained are within the "acceptable range" established by the laboratory.

8.0 CALIBRATION PROCEDURES

No reagent calibration is necessary as this procedure is standardized based on the millimolar absorptivity of NADH which is taken as 6.22 at 340 nm under the test conditions described.

9.0 PERFORMANCE CHARACTERISTICS

9.1 PRECISION

The estimates of precision shown below were obtained from assays of human control serum.
Within-Run

In this study, 30 replicates of 2 control sera were run.

Mean (U/L)	SD (U/L)	CV (%)
33.3	± 1.1	3.3
96.0	± 3.3	3.4

Between-Run

In this study, 10 runs were made on 2 control sera.

<u>Mean (U/L)</u>	<u>SD (U/L)</u>	<u>CV (%)</u>
25.4	± 2.4	9.3
73.5	± 4.0	5.4

9.2 CORRELATION

A correlation study was done comparing this method and a similar AST method. The samples range between 7 and 103 U/L.

<u>Number of Samples</u>	<u>Regression Equation</u>	<u>Correlation Coefficient</u>
42	$y = .999x - 1.314$.984

9.3 LINEARITY

This procedure is linear to 350 U/L. Procedures on automated instruments which use greater than a one to fifteen dilution factor will have an extended linearity.

A sample with AST activity exceeding the linearity limit should be diluted with 0.9% saline and reassayed incorporating the dilution factor in the calculation of the result.

9.4 SENSITIVITY

An absorbance change of 0.001 ΔA/min corresponds to approximately 2.6 U/L AST activity.

10.0 REFERENCES

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- 10.8 Henry, R.J., Cannon, D.C. and Winkelman, J.W. (Editors), Clinical Chemistry Principles and Technics, 2nd ed., Harper and Row.
- 10.9 G.J. Kost, "Critical Limits for Urgent Clinician Notification at U.S. Medical Centers"; JAMA, Feb. 2, 1990; Vol 263, No.5, p.704

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