

# CONCEPT DIAGNOSTICS

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## AST (SGOT) REAGENT SET (UV-KINETIC METHOD)

### AST (SGOT) REAGENT SET

For the quantitative determination of Aspartate Aminotransferase (AST) in serum.

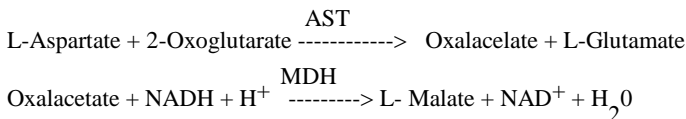
### INTRODUCTION

Serum aspartate aminotransferase (AST) also known as serum glutamic oxalacetic transaminase (SGOT) is a tissue enzyme that catalyzes the exchange of amino and keto groups between alpha-amino acids and alpha-keto acids. AST is widely distributed in tissue principally cardiac, hepatic, muscle and kidney. Injury to these tissues results in the release of the AST (SGOT) enzyme to general circulation. Following a myocardial infarction, serum levels of AST (SGOT) are elevated and reach a peak 48 to 60 hours after onset. Hepatobiliary diseases, such as cirrhosis, metastatic carcinoma, and viral hepatitis also will increase serum AST levels.<sup>1</sup>

The first kinetic assay of AST for diagnostic purposes was described by Karmen et al. in 1955, using a coupled reaction of malate dehydrogenase (MDH) and NADH<sup>2</sup>. This assay system was critically evaluated and optimized in 1960 by Henry et al.<sup>3</sup> In 1977 the International Federation of Clinical Chemistry recommended a reference procedure for the measurement of AST activity based upon Karmen's procedures.<sup>4</sup> The AST reagent applies the formulation recommended by the IFCC.

### PRINCIPLE

The enzymatic reaction sequence employed in the assay of aspartate aminotransferase is as follows:



AST catalyzes the transfer of an amino group between L-aspartate and 2-oxoglutarate. The oxalacetate formed in the first reaction is then reacted with NADH in the presence of malate dehydrogenase (MDH) to form NAD. AST activity is determined by measuring the rate of oxidation of NADH at 340nm. Lactate dehydrogenase is included in the reagent to convert endogenous pyruvate in the sample to lactate during the lag phase prior to measurement.

### REAGENT COMPOSITION

When reconstituted as directed, the reagent for AST contains the following: (Concentrations refer to reconstituted reagent.) 2-Oxoglutarate 12 mM, L-Aspartic Acid 200 mM, NADH 0.19 mM, LDH 800 U/L, MDH 600 U/L, Buffer 100 mM, pH 7.8 ± 0.1. Nonreactive preservatives and fillers.

### WARNINGS AND PRECAUTIONS

- For in vitro diagnostic use.  
**CAUTION:** In vitro diagnostic reagents may be hazardous. Handle in accordance with good laboratory procedures which dictate avoiding ingestion, and eye or skin contact.
- Serum specimens should be considered infectious and handled appropriately.
- Use distilled or deionized water where indicated.

### STORAGE AND STABILITY

- Store dry reagent at 2 - 8°C.
- The reconstituted reagent is stable for eight (8) hours at room temperature and for twenty one (21) days when refrigerate immediately.

### REAGENT DETERIORATION

The reagent should be discarded if:

- The initial absorbance, read against water at 340 nm, is below 0.800.
- The reagent fails to meet stated parameters of performance.

### SPECIMEN COLLECTION

This assay is intended for use with serum. Reports indicate that AST in serum remains stable at 4°C for a minimum of 7 days. Hemolyzed specimens should not be used as erythrocytes contain Fifteen times the AST activity in serum.<sup>5</sup>

### INTERFERING SUBSTANCES

Pyridoxal phosphate can elevate AST values by activating the apoenzyme form of the transaminase. Pyridoxal phosphate may be found in diluent water contaminated with microbial growth.<sup>6</sup> High levels of um pyruvate may also interfere with assay performance. Young et al. give a list of drugs and other substances that interfere with the determination of AST activity.<sup>7</sup> Refer also to N.E. Saris for a list of references.<sup>8</sup>

### MATERIALS REQUIRED BUT NOT PROVIDED

- Pipetting devices.
- Test tubes/rack.
- Timing device.
- Spectrophotometer capable of reading at 340 nm (UV).
- Heating block or bath (37°C).

### GENERAL INSTRUCTIONS

The reagent for AST is intended for use either as an automated procedure on chemistry instruments or as a manual procedure on a suitable spectrophotometer.

### PROCEDURE (AUTOMATED)

Consult the appropriate instrument application guide available from us.

### PROCEDURE (MANUAL)

- Reconstitute Reagent according to instructions on vial label.
- Pipette 1.0 ml of reagent into a 1cm cuvette and allow to equilibrate to 37°C.
- Add 0.10 ml (100 ul) of specimen to reagent and mix gently.
- Maintain the solution at 37°C. After one (1) minute, measure the absorbance at 340 nm.
- Take two additional absorbance readings at 1 minute intervals. Calculate the mean absorbance change per minute. ( $\Delta A/\text{min.}$ )
- Multiply the  $\Delta A/\text{min.}$  by 1768 to calculate IU/L of AST.

### ALTERNATE VOLUMES

If the spectrophotometer being used requires a final volume greater than 1.0 ml for accurate readings, follow the "ALTERNATE PROCEDURE".

## CALCULATIONS

One International Unit (IU) is defined as the amount of enzyme that catalyzes the transformation of one micromole of substrate per minute under specified conditions.

$$\frac{\Delta \text{ Abs./min.} \times \text{TV} \times 1000}{\epsilon \times \text{SV} \times \text{LD}} = \frac{\Delta \text{ Abs./min.} \times 1.1 \times 1000}{6.22 \times 0.1 \times 1}$$
$$= \Delta \text{ Abs./min.} \times 176$$

Where:  $\Delta \text{ Abs./min.}$  = Average absorbance change per minute  
TV = Total reaction volume (ml)  
1000 = Conversion of IU/ml to IU/L  
 $\epsilon$  = Millimolar absorptivity of NADH  
SV = Sample volume in ml  
LP = Light path in cm

Example: If the average absorbance change per minute = 0.15 then  $0.15 \times 1768 = 265 \text{ IU/L}$

**SI UNIT:** To convert to SI Units (nkat/L) multiply IU/L by 16.67.

**NOTE:** If any of the test parameters are altered a new factor must be calculated using the above formula.

## ALTERNATE PROCEDURE

1. Reconstitute reagent according to instructions.
2. Pipette 3.0 ml of reagent into a 1 cm cuvette and allow to equilibrate to 37°C.
3. Add 0.20 ml (200  $\mu$ l) of specimen and mix gently.
4. Maintain the solution at 37°C. After one (1) minute, measure the absorbance ( $A_1$ ) at 340 nm
5. After exactly five (5) minutes, read and record absorbance ( $A_2$ )
6. The difference in absorbance between readings ( $A_1 - A_2$ ) multiplied by the factor 514 (see "ALTERNATE PROCEDURE CALCULATIONS") will yield results in IU/L.
7. Sample with values above 500 IU/L should be diluted 1:1 with saline, re-assayed and the results multiplied by two (2).

## PROCEDURE NOTES

Turbid or high icteric samples may give readings whose initial absorbance exceeds the capabilities of the spectrophotometer used. Run this kind of sample using 0.10 ml (100  $\mu$ l), sample volume to 3.0 ml reagent and multiply result by two (2).

## ALTERNATE PROCEDURE CALCULATION

One International Unit (IU) is defined as the amount of enzyme that catalyzes the transformation of one micromole of substrate per minute under specified conditions.

$$\text{AST (IU/L)} = \frac{(A_1 - A_2) \times \text{TV} \times 1000}{\Delta T \times \epsilon \times \text{SV} \times \text{LD}} = \frac{(A_1 - A_2) \times 3.2 \times 1000}{5 \times 6.22 \times 0.2 \times 1}$$
$$= (A_1 - A_2) \times 514$$

Where: ( $A_1 - A_2$ ) = Absorbance change  
TV = Total reaction volume (ml)  
1000 = Conversion of IU/ml to IU/L  
AT = Time interval between readings  
LP = Light path in cm  
 $\epsilon$  = Millimolar absorptivity of NADH  
SV = Sample volume in ml

Example: If  $A_1 = 1.45$  and  $A_2 = 1.28$   
then  $(1.45 - 1.28) \times 514 = 87 \text{ IU/L}$

**SI UNIT:** To convert to SI Units (nkat/L) multiply IU/L by 16.67

**NOTE:** If any of the test parameters are altered a new factor must be calculated using the above formula.

## QUALITY CONTROL

It is recommended that control be included in each set of assays. Commercially available control material with established AST values may be used for quality control. The assigned value of the control

material must be confirmed by the chosen application. Failure to obtain the proper range of values in the assay of control material may indicate either reagent deterioration, instrument malfunction, or procedural errors.

## EXPECTED VALUES

Up to 28 IU/L (30°C)      Up to 40 IU/L (37°C)

It is strongly recommended that each laboratory establish its own normal range.

## PERFORMANCE CHARACTERISTICS

1. Linearity: 500 IU/L
2. Comparison: A comparison study between the present method with available commercial product using the same method on 22 fresh serum samples from 12 IU/L to 84 IU/L yielded a coefficient of 0.98 and a regression equation of  $Y = 1.04x - 1.25$ .
3. Sensitivity: Based on an instrument resolution of  $A = 0.001$ , this procedure has a sensitivity of 2 IU/L
4. Precision studies:  
Within Run precision: Two commercial serum controls were assayed twenty times and the following Within Run precision was obtained.

Mean (IU/L)	S.D.	C.V.
22.3	1.1	4.8%
88.3	5.1	5.7%

Run to Run Run precision: Two commercial serum controls were assayed for five consecutive days (triplicate for each level), the following Run to Run precision was obtained.

Mean IU/L	S.D.	C.V.
21.9	2.3	10.5%
83.8	3.6	4.4%

## TEMPERATURE CONVERSION FACTOR (Tf)<sup>3</sup>

Assay Mixture	Tf 25°C	Tf 30°C	Tf 32°C	Tf 37°C
25°C	1.00	1.37	1.57	1.96
30°C	0.78	1.00	1.13	1.43
32°C	0.65	0.89	1.00	1.27
37°C	0.51	0.70	0.73	1.00

Example: If the reaction is performed at 30°C but is to be reported at 37°C, simply multiply the result obtained at 30°C by the factor 1.43 to obtain a correct value.

**NOTE:** Since temperature factors give only an approximate conversion, it is suggested that values be reported at the temperature of the measurement.

## REFERENCES

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