

AST (SGOT) Reagent

Catalog #: 43705

for use with the

SDI CA480 Clinical Chemistry System

INTENDED USE

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

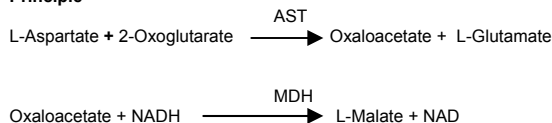
SUMMARY AND EXPLANATION¹

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.

METHODOLOGY

In 1955 Karmen² developed a kinetic assay procedure for AST which was based upon the use of malate dehydrogenase and NADH. Henry³ in 1960 and Amador and Wacker⁴ in 1962 later presented optimized procedures. These modifications increased accuracy and lowered the effect of interfering substances. The IFCC⁵ published a recommended method that included P-5-P in 1986. The present method is based on IFCC recommendations but does not contain P-5-P.

Principle



Aspartate aminotransferase (AST) catalyzes the transfer of the amino group from L-aspartate to 2-oxoglutarate to yield oxaloacetate and L-glutamate. The oxaloacetate undergoes reduction with simultaneous oxidation of NADH to NAD in the malate dehydrogenase (MDH) catalyzed indicator reaction. The resulting rate of decrease in absorbance at 340nm is directly proportional to the AST activity. Lactate dehydrogenase (LDH) is added to prevent interference from endogenous pyruvate which is normally present in serum.

REAGENT COMPOSITION

Active Ingredients	Concentrations
2-Oxoglutarate	13 mM
L-Aspartate	220 mM
MDH (microbial)	>100 U/L
LDH (microbial)	>1500 U/L
NADH	>0.12 mM
pH	7.9 ± 0.1.

Precautions:

This reagent is for *in vitro* diagnostic use only.

REAGENT PREPARATION

Reagent is supplied ready to use.

REAGENT STORAGE

1. Store the reagent at 2-8°C
2. The reagent is stable until the expiration date when stored at 2-8°C.

REAGENT DETERIORATION

DO NOT USE REAGENT IF:

1. The initial absorbance, read against water at 340nm, is below 1.000
2. The reagent fails to meet stated parameters of performance.

SPECIMEN COLLECTION AND HANDLING⁶

1. Non-hemolyzed serum is recommended. Red blood cells contain AST.
2. AST in serum is reported stable for seven days when refrigerated, one month frozen, and three days when stored at room temperature.

INTERFERENCE

Studies to determine the level of interference for hemoglobin, bilirubin, and lipemia were carried out, the following results were obtained:

Hemoglobin:

Use non-hemolyzed serum, as red blood cells contain AST

Bilirubin:

No significant interference (± 10%) from bilirubin up to 25.8 mg/dL

Lipemia:

No significant interference (± 10%) from lipemia up to 1086 mg/dL measured as triglycerides

See Young, et al.⁷ or other interfering substances.

ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED

1. A SDI CA480 Clinical Chemistry System
2. Deionized water and related equipment, e.g.: pipettes
3. Analyzer specific consumables, e.g.: sample cups
4. Control material such as those provided by SDI Biomed

ASSAY PROCEDURE

System Parameters

AST (Liquid)	
TEMPERATURE:	37°C
WAVELENGTH:	340 nm
ASSAY TYPE:	Rate/Kinetic
DIRECTION:	Decrease
SAMPLE / RGT RATIO:	1 : 10
e.g. Sample Vol.	0.10mL (100mL)
Reagent Vol.	1.0 mL
DELAY/LAG TIME:	30 Sec
READ TIME:	1-3 Min

Procedure Notes:

Samples with values above 600 U/L should be diluted 1:1 with saline, re-assayed and the results multiplied by two.

Calculation

One Unit (U/L) is defined as the amount of enzyme that catalyzes the transformation of one micromole of substrate per minute under specified conditions. For example:

$$\text{AST (U/L)} = \frac{\text{DAbs./min} \times 1.10 \times 1000}{6.22 \times 0.10 \times 1.0} = \text{DAbs./min} \times 1768$$

Where

DAbs./min.	= Average absorbance change per minute
1.10	= Total reaction volume (ml)
1000	= Conversion of U/mL to U/L
6.22	= Millimolar absorptivity of NADH
0.10	= Sample Volume (mL)
1.0	= Light path in cm

Example:

If the average absorbance change per minute = 0.12 then $0.12 \times 1768 = 212$ U/L

NOTES:

1. To convert to nkat/L multiply U/L by 16.67
2. If any of the test parameters are altered, a new factor must be calculated using the above formula

CALIBRATION

The procedure is standardized by means of the millimolar absorptivity of NADH taken as 6.22 at 340nm under the test conditions described.

QUALITY CONTROL

The integrity of the reaction should be monitored by use of a two level control with known AST values.

EXPECTED RANGE⁸

5-34 U/L (37°C)

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

PERFORMANCE

Linearity:

When run as recommended the assay is linear from 0 to 600 U/L.

Method Comparison:

Studies performed between this procedure and a similar methodology yielded the following results:

Number of samples pairs:	45
Range of samples:	0 – 449 (U/L)
Correlation Coefficient:	0.9997
Slope:	0.951
Intercept:	1.6 (U/L)

Precision:

Within Run	Level 1	Level 2	Level 3
n=40			
Mean (U/L)	22.9	44.4	179.8
S.D. (U/L)	0.7	0.7	1.2
C.V. (%)	2.8	1.5	0.7

Total

n=40 (10 days / 2 runs per day / 2 replicates per run)

Mean (U/L)	22.9	44.4	179.8
S.D. (U/L)	1.1	0.9	3.2
C.V. (%)	5.0	2.1	1.8

REFERENCES

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Manufactured for:



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