

AST (GOT) Reagent

Aspartate Aminotransferase

PRODUCT SUMMARY

Stability	:	30 days at 2-8 °C
Linear Range	:	Up to 450 U/L
Specimen Type	:	Serum or plasma
Method	:	Kinetic UV
Reagent Preparation	:	Add specified volume of distilled or deionised water.

IVD

SYMBOLS IN PRODUCT LABELLING

EC REP	Authorised Representative		Temperature Limitation
IVD	For in vitro diagnostic use		Use by/Expiration Date
LOT	Batch code/Lot number		CAUTION: CONSULT INSTRUCTIONS FOR USE.
REF	Catalogue number		Manufactured by
	Consult instructions for use		Xn - Harmful

INTENDED USE

This reagent is intended for the in vitro quantitative determination of AST (Aspartate Aminotransferase EC2.6.1.1) in human serum or plasma.

CLINICAL SIGNIFICANCE

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.¹

METHODOLOGY

In 1955, Karmen et al² described the first kinetic assay of AST for diagnostic purposes. This method was evaluated and improved by many investigators primarily Henry et al³ and now forms the basis of many national and international recommended procedures. The AST Reagent is based on the recommendations of the IFCC.⁴

The series of reactions involved in the assay system is as follows:

1. L-Aspartate + 2-Oxoglutarate \xrightarrow{AST} Oxaloacetate + L-Glutamate
2. Oxaloacetate + NADH \xrightarrow{MDH} Malate + NAD
3. Sample Pyruvate + NADH \xrightarrow{LDH} L-Lactate + NAD

1. AST present in the sample catalyses the transfer of the amino group from L-aspartate to 2-oxoglutarate forming oxaloacetate and L-glutamate.
2. Oxaloacetate in the presence of NADH and Malate dehydrogenase (MDH), is reduced to L-malate. In this reaction NADH is oxidized to NAD. The reaction is monitored by measuring the rate of decrease in absorbance at 340nm due to the oxidation of NADH to NAD.
3. Addition of Lactate dehydrogenase (LDH) to the reagent is necessary to achieve rapid and complete reduction of endogenous pyruvate so that it does not interfere with the assay.

REAGENT COMPOSITION

Active Ingredients	Concentration
2-Oxoglutarate	13.2 mmol/L
L-Aspartate	220 mmol/L
MDH (porcine heart)	> 600 U/L
LDH (microbial)	> 1000 U/L
NADH	> 0.18 mmol/L
Tris Buffer	88 mmol/L
EDTA	5.5 mmol/L

Also contains non-reactive fillers and stabilizers
pH 8.0 ± 0.1 at 20 °C.

WARNING: Do not ingest. Avoid contact with skin and eyes. If spilt, thoroughly wash affected areas with water. Reagent contains Sodium Azide which may react with copper or lead plumbing. Flush with plenty of water when disposing. For further information consult the AST(GOT) Reagent Material Safety Data Sheet. **The Packaging of This Product Contains Dry Natural Rubber.** Exercise precaution when handling crimps and broken glass vials, as sharp edges can injure the user.

R22 Harmful if swallowed
S28 After contact with skin, wash immediately with plenty of soap and water.

REAGENT PREPARATION

Reconstitute the reagent with the volume of distilled or deionised water stated on the vial label.

STABILITY AND STORAGE

Prior to use:

When stored refrigerated at 2-8 °C the reagent is stable until the expiration date stated on the bottle and kit box label.

Reconstituted Reagent:

When stored capped at 2-8 °C the reagent is stable for at least 30 days.

Indications of Reagent Deterioration:

- Turbidity,
- Absorbance <1.1 at 340 nm (1 cm);and/or
- Failure to recover control values within the assigned range.

SPECIMEN COLLECTION AND HANDLING

Serum: Use non-haemolysed serum.

Plasma: Use non-haemolysed plasma.

Storage: AST samples may be stored for at least 7 days at 4 °C⁴

ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED

- A clinical chemistry analyser capable of maintaining constant temperature (37 °C) and measuring absorbance at 340 nm.
- Analyser specific consumables, eg: sample cups.
- Distilled or deionised water for reagent preparation and related equipment eg: pipettes.
- Normal and Abnormal assayed control material.

ASSAY PROCEDURE

The following system parameters are recommended. Individual instrument applications are available upon request from the Technical Support Group.

SYSTEM PARAMETERS

Temperature	37 °C
Wavelength	340 nm
Assay Type	Rate/Kinetic
Direction	Decrease
Sample : Reagent Ratio	1:10
eg: Sample Vol	30 µL
Reagent Vol	300 µL
Delay/Lag Time	60 seconds
Read Time	60 seconds
Reagent Blank	Low 1.1 AU
(1cm lightpath, 340nm)	High 2.0 AU
Linearity	0 - 450 U/L
(refer to Linearity section)	
Sensitivity	0.57 ΔmA/min per U/L
(1cm lightpath, 340nm)	

CALCULATIONS

Results are calculated, usually automatically by the instrument, as follows:

Activity in U/L = ΔAbs/min x Factor

$$\text{Factor} = \frac{\text{TV} \times 1000}{6.3 \times \text{SV} \times \text{P}}$$

Where:

- TV = Total reaction volume in mL
SV = Sample volume in mL
6.3 = millimolar absorption coefficient of NADH at 340nm (See note 4).
P = Cuvette pathlength in cm.

Example:

$$\begin{aligned} \Delta\text{Abs}/\text{min} &= 0.10 \\ \text{Factor} &= 1746 \\ \text{AST} &= 0.10 \times 1746 = 175 \text{ U/L} \end{aligned}$$

NOTES

1. The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
2. If the change in absorbance is greater than 0.26/min repeat the assay with less sample or dilute with saline. Remember to adjust the factor for the smaller sample volume or to multiply the final result by the dilution factor.
3. Valid results depend on an accurately calibrated instrument, timing, and temperature control.
4. The millimolar absorption coefficient for NADH at 334nm = 6.18 and at 365nm = 3.40.
5. Unit Conversion: U/L x 16.67 x 10⁻³ = µkat/L

CALIBRATION

Not required. The rate of reaction is converted to U/L of activity by a calculation factor. Refer to the calculation section of this package insert.

QUALITY CONTROL

To ensure adequate quality control, normal and abnormal control with assayed values should be run as unknown samples:-

- At least every eight hours.
- When a new bottle of reagent is used.
- After preventative maintenance is performed or a critical component is replaced.

Control results falling outside the upper or lower limits of the established ranges indicate the assay may be out of control.

The following corrective actions are recommended in such situations:-

- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control serum and repeat the test.
- If results on fresh control material still remain outside the limits, then repeat the test with fresh reagent.
- If results are still out of control, contact Technical Services or your local distributor.

LIMITATIONS

1. Studies to determine the level of interference from haemoglobin, bilirubin, pyruvate and lipaemia were carried out. The following results were obtained:

Haemoglobin: No interference from haemoglobin up to 920 mg/dL.

Bilirubin: No interference from bilirubin up to 1000 µmol/L (60 mg/dL).

Pyruvate: No interference from pyruvate up to 0.60 mmol/L.

Lipaemia: No interference from lipaemia, measured as triglycerides, up to 6.0 mmol/L (530 mg/dL).

2. Haemolyzed serum specimens should not be used. AST activity levels in erythrocytes are some 15 times higher, than those in sera.⁵
3. Young DS⁶ has published a comprehensive list of drugs and substances which may interfere with this assay.

EXPECTED VALUES⁷

At 37°C 5-34 U/L

Levels approximately twice the adult levels are seen in neonates and infants. These levels decline to normal adult levels after 6 months.

The quoted values are representative of the expected range for this method and should serve as a guide only. It is recommended that each laboratory verify this range or derives a reference interval for the population that it serves.⁸

PERFORMANCE DATA

The following data was obtained using the AST(GOT) reagent on a well maintained automated clinical chemistry analyser. Users should establish product performance on their specific analyser used.

IMPRECISION

Within Run:	LEVEL I	LEVEL II
Number of Samples	20	20
Mean (U/L)	33	169
SD (U/L)	0.44	0.88
CV%	1.32	0.52

Between Day:	LEVEL I	LEVEL II
Number of Samples	20	20
Mean (U/L)	34	308
SD (U/L)	1.57	8.24
CV%	4.69	2.68

ACCURACY

Comparison studies were carried out using a similar commercially available reagent as a reference. Serum samples were assayed in parallel and the results compared by least squares regression. The following statistics were obtained.

Number of sample pairs	50
Range of sample results	7 - 298 U/L
Mean of reference method results	48 U/L
Mean of AST(GOT) results	45 U/L
Slope	0.96
Intercept	-0.78 U/L
Correlation coefficient	0.997

LINEARITY

When run as recommended, the assay is linear up to 450 U/L.


SENSITIVITY

When run as recommended the sensitivity of this assay is 0.57 ΔmA/min per U/L.

REFERENCES

1. Zilva JF, Pannall PR. "Plasma Enzymes in Diagnosis" in Clinical Chemistry in Diagnosis and Treatment. Lloyd-Luke London. 1979; Chap 15:338-9.
2. Karmen A. J Clin Investigation 1955; 43:131.
3. Henry RJ, et al. Am J Clin Path 1960; 34:381.
4. IFCC Method for L-Aspartate aminotransferase. J. Clin. Chem. Clin. Biochem. 1986; 24:497-510.
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7. Murray RL. "Aspartate aminotransferase" in Clinical Chemistry. Theory, analysis and correlation. Kaplan LA, Pesce AJ (Ed), CV Mosby Company 1984; 1105-8.
8. Wachtel M et al. Creation and Verification of Reference Intervals. Laboratory Medicine 1995; 26:593-7.

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840360 (R0)

REF

Reorder Information

Catalogue No.	Configuration
1180-200	20 x 10 mL
TR17515	20 x 20 mL
TR17503/1180-500	10 x 50 mL
TR17504	10 x 200 mL