

AST (GOT) Reagent

2 Part Liquid

PRODUCT SUMMARY

Stability	:	Until Expiry at 2-8°C
Linear Range	:	Up to 1500 U/L (25.1 µkat/L)
Specimen Type	:	Serum
Method	:	Kinetic UV
Reagent Preparation	:	Supplied ready to use.

IVD

INTENDED USE

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

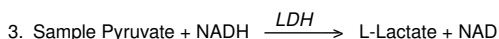
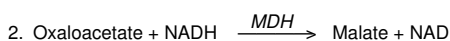
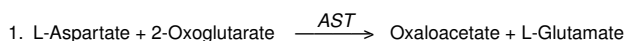
CLINICAL SIGNIFICANCE

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



- AST present in the sample catalyses the transfer of the amino group from L-aspartate to 2-oxoglutarate forming oxaloacetate and L-glutamate.
- 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.
- 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

Reagent 1:	Concentration
NADH	0.42 mmol/L
LDH (microbial)	>1500 U/L
2-Oxoglutarate	15 mmol/L
Tris Buffer	47.5 mmol/L
Reagent 2:	Concentration
L-Aspartate	1000 mmol/L
MDH (microbial)	> 500 U/L
Tris Buffer	250.5 mmol/L

pH 8.1 ± 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) 2 part Liquid reagent Material Safety Data Sheet.

REAGENT PREPARATION

Reagents are supplied ready to use.

STABILITY AND STORAGE

Prior to use:

When stored between 2-8°C the reagents are stable until the expiration date stated on the bottle and kit box label.

Once the Reagent is Opened:

When stored capped at 2-8°C the reagents are stable until the expiration date stated on the bottle and kit box label.

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	REAG 1	Reagent 1 (R1)
REAG 2	Reagent 2 (R2)		

Indications of Reagent Deterioration:

- Turbidity;
- Reagent 1 absorbance < 2.0 AU at 340nm (1 cm light path); and/or
- Failure to recover control values within the assigned range.

SPECIMEN COLLECTION AND HANDLING

Serum: Use non-haemolysed serum.

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.
- Assayed Normal and Abnormal 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
Primary Wavelength	340 nm (334, 365nm)
Secondary Wavelength	380 nm
Assay Type	Rate/Kinetic
Direction	Decrease
Sample : Reagent Ratio	1 : 16 (R1) : 4 (R2)
eg: Sample Vol	15 µL
Reagent 1 Vol	240 µL
Reagent 2 Vol	60 µL
Delay time (sample + R1)	≤5 minutes
Lag time (sample + R1 + R2)	>30 seconds
Read Time	1 - 2 minutes
Reagent Blank (R1 + R2)	Low 1.6 AU
(340nm, 1cm lightpath)	High 2.5 AU
Linearity	Up to 1,500 U/L
(refer to Linearity section)	(25.1 µkat/L)
Analytical Sensitivity	0.30 ΔmA/min per U/L
(340nm, 1cm lightpath)	(18.0 ΔmA/min per µkat/L)

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:

$$\begin{aligned} \Delta\text{Abs}/\text{min} &= 0.08 \\ \text{Factor} &= 3333 \\ \text{AST} &= 0.08 \times 3333 = 267 \text{ U/L} \end{aligned}$$

NOTES

- The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
- If the change in absorbance is greater than 0.45/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.

- Valid results depend on an accurately calibrated instrument, timing, and temperature control.
- The millimolar absorption coefficient for NADH at 334nm = 6.18 and at 365nm = 3.40.
- 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 once per day or as established by the laboratory.
- 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

- Studies to determine the level of interference from haemoglobin, bilirubin and pyruvate were carried out. The following results were obtained:
Haemoglobin: No interference from haemoglobin up to 500 mg/dL.
Free bilirubin: No interference from free bilirubin up to 684 µmol/L (40 mg/dL).
Conjugated Bilirubin: No interference from conjugated bilirubin up to 684 µmol/L (40 mg/dL).
Pyruvate: No interference from pyruvate up to 2.3 mmol/L (20 mg/dL).
- Haemolyzed serum specimens should not be used. AST activity levels in erythrocytes are some 15 times higher, than those in sera.⁵
- Avoid the use of lipaemic samples.
- Young DS⁶ has published a comprehensive list of drugs and substances which may interfere with this assay.

EXPECTED VALUES*

At 37°C 534 U/L (0.084 - 0.568 µkat/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.⁷

PERFORMANCE DATA

The following data was obtained using the AST(GOT) 2 part Liquid reagent on a well maintained automated clinical chemistry analyser. Users should establish product performance on their specific analyser used.

IMPRECISION

Imprecision was evaluated using two levels of commercial control and following the NCCLS EP5-T procedure.⁸

Within Run:	LEVEL I	LEVEL II
Number of data points	80	80
Mean (U/L)	25.4	169.7
Mean (µkat/L)	0.424	2.83
SD (U/L)	0.93	1.80
SD (µkat/L)	0.016	0.030
CV (%)	3.7	1.1

Total:	LEVEL I	LEVEL II
Number of data points	80	80
Mean (U/L)	25.4	169.7
Mean (µkat/L)	0.424	2.83
SD (U/L)	1.36	6.84
SD (µkat/L)	0.023	0.114
CV (%)	5.3	4.0

METHOD COMPARISON

Comparison studies were carried out using a similar commercially available AST(GOT) 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	64
Range of sample results	9 - 204 U/L (0.150 - 3.41 µkat/L)
Mean of reference method results	47 U/L (0.785 µkat/L)
Mean of AST(GOT) results	39 U/L (0.651 µkat/L)
Slope	0.84
Intercept	-0.92 U/L (-0.015 µkat/L)
Correlation coefficient	0.999

LINEARITY


When run as recommended the assay is linear up to 1500 U/L (25.1 µkat/L).

ANALYTICAL SENSITIVITY

When run as recommended the sensitivity of the assay is 0.30 ΔmA/min per U/L (18.0 ΔmA/min per µkat/L).

REFERENCES

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- Young DS, Effects of Drugs on Clinical Laboratory Tests. Third Edition. 1990; 3:45-52.
- Wachtel M et al, Creation and Verification of Reference Intervals. Laboratory Medicine 1995; 26:593-7.
- National Committee for Clinical Laboratory Standards. User evaluation of Precision Performance of Clinical Chemistry devices NCCLS, 1984, NCCLS Publication EP5-T.

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REF

Reorder Information

Catalogue No.	REAG 1	REAG 2
TR17920	1 x 125 mL	1 x 35 mL
TL17901 (iLab 600)	5 x 80 mL	5 x 20 mL
TH17901 (Hitachi)	4 x 80 mL	4 x 20 mL
TY17901 (Hitachi)	4 x 53 mL	4 x 15 mL
7500-106A	4 x 500 mL	
7500-206A		2 x 250 mL