Infinity™
AST (GOT) Liquid Stable Reagent

PRODUCT SUMMARY

Stability	:	Until Expiry at 2-8°C
Linear Range	:	Up to 450 U/L (7.52 µkat/L)
Specimen Type	:	Serum
Method	:	Kinetic
Reagent Preparation	:	Supplied ready to use.

IVD

SYMBOLS IN PRODUCT LABELLING

EC REP	Authorized 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		
	Consult instructions for use		Manufactured by

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 al[2] described the first kinetic assay of AST for diagnostic purposes. This method was evaluated and improved by many investigators primarily Henry et al[3] and now forms the basis of many national and international recommended procedures. This 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 mmol/L
L-Aspartate	220 mmol/L
MDH (microbial)	> 100 U/L
LDH (microbial)	> 1500 U/L
NADH	> 0.12 mmol/L
Tris Buffer	88 mmol/L
EDTA	5.0 mmol/L

pH 8.10 ± 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 Infinity AST(GOT) Liquid Stable Reagent Material Safety Data Sheet.

REAGENT PREPARATION
Reagent is supplied ready to use.

STABILITY AND STORAGE
When stored at 2-8°C reagent is stable until the expiration date stated on the bottle and kit box label. It is recommended that when the reagent is not in use for prolonged periods of time (eg: overnight) the reagent be capped and stored at 2-8°C.
Indications of Reagent Deterioration:
- Turbidity,
- Absorbance < 1.0 at 340 nm (1 cm),
- 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°C.[5]

ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED
- If required, pipettes for accurately dispensing measured volumes.
- A clinical chemistry analyzer capable of maintaining constant temperature (37°C) and measuring absorbance at 340 nm.
- Analyzer specific consumables, eg: sample cups.
- 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		405 nm
Assay Type		Rate/Kinetic
Direction		Decrease
Sample : Reagent Ratio		1 : 10
eg:	Sample Vol	30 µL
	Reagent Vol	300 µL
Delay/Lag		30 seconds
Read Time		1 to 3 minutes
Reagent Blank		Low 1.0 AU
(1cm lightpath, 340nm)		High 2.5 AU
Linearity		450 U/L (7.52 µkat/L)
(refer to Linearity section)		
Analytical Sensitivity		0.573 ΔmA/min per U/L
(1cm lightpath, 340nm)		(34.31 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 coefficent of NADH at 340nm (See note 4).
P = Cuvette pathlength in cm.

Thermo SCIENTIFIC

Example:
ΔAbs/min = 0.08
Factor = 1746
AST = 0.08 x 1746 = 140 U/L

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 coefficent 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 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 the local distributor.

LIMITATIONS
1. Studies to determine the level of interference from bilirubin (free & conjugated), haemoglobin and lipaemia were carried out using commercially available interference check products. The following results were obtained:
   Haemoglobin: No interference from haemoglobin up to a level of 150 mg/dL.
   Free bilirubin: No interference from free bilirubin up to a level of 260 µmol/L (15 mg/dL).
   Conjugated bilirubin: No interference from conjugated bilirubin up to a level of 116 µmol/L (6.8 mg/dL).
   Lipaemia: No interference from lipaemia, measured as an absorbance at 630nm, up to 1.68 AU.
2. Haemolyzed serum specimens should not be used. AST activity levels in erythrocytes are some 15 times higher, than those in sera.[6]
3. Young DS[7] has published a comprehensive list of drugs and substances which may interfere with this assay.

EXPECTED VALUES[5]
At 37°C 5 - 34 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 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 Infinity AST(GOT) Liquid Stable Reagent on a well maintained automated clinical chemistry analyzer. Users should establish product performance on their specific analyzer used.

IMPRECISION
Imprecision was evaluated using two levels of commercial control and following the NCCLS EP5-T procedure[9].

		LEVEL I	LEVEL II
Number of data points		80	80
Mean (U/L / µkat/L)		45 / 0.752	194 / 3.24
Within Run:	SD (U/L / µkat/L)	0.7 / 0.012	1.4 / 0.023
	CV (%)	1.6	0.7
Total:	SD(U/L / µkat/L)	1.2 / 0.020	2.3 / 0.038
	CV (%)	2.7	1.2

METHOD COMPARISON
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	84
Range of sample results	8 - 276 U/L(0.134 - 4.61 µkat/L)
Mean of reference method results	37 U/L (0.618 µkat/L)
Mean of Infinity AST (GOT) results	38 U/L (0.635 µkat/L)
Slope	0.98
Intercept	2.1 U/L (0.035 µkat/L)
Correlation coefficient	0.997

LINEARITY
When run as recommended, the assay is linear up to 450 U/L (7.52 µkat/L).
Linearity on automated instruments will be dependent upon the ratio of sample volume to reagent volume used and the timing of measurements. The specific instrument application should be consulted.

ANALYTICAL SENSITIVITY
When run as recommended the sensitivity of this assay is 0.573 ΔmA /min per U/L (34.31 ΔmA /min per µkat/L).

REFERENCES

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4. IFCC Method for L-Aspartate aminotransferase. J. Clin. Chem. Clin. Biochem. 1986; 24:497-510.
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7. Young DS, Effects of Drugs on Clinical Laboratory Tests. Third Edition. 1990: 3:45-52.
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9. 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.	Configuration
TR70121	2 x 125 mL