

# 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

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

#### INTENDED USE

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

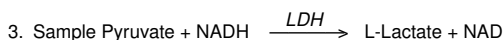
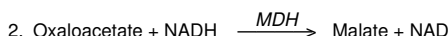
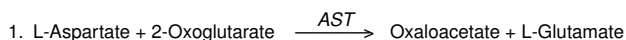
#### CLINICAL SIGNIFICANCE

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.<sup>1</sup>

#### METHODOLOGY

In 1955, Karmen et al<sup>2</sup> described the first kinetic assay of AST for diagnostic purposes. This method was evaluated and improved by many investigators primarily Henry et al<sup>3</sup> and now forms the basis of many national and international recommended procedures. This AST Reagent is based on the recommendations of the IFCC.<sup>4</sup>

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	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<sup>5</sup>

#### ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED

- If required, pipettes for accurately dispensing measured volumes.
- A clinical chemistry analyser capable of maintaining constant temperature (37°C) and measuring absorbance at 340 nm.
- Analyser 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 coefficient of NADH at 340nm (See note 4).
- P = Cuvette pathlength in cm.

**Example:**

$\Delta$ Abs/min = 0.08  
 Factor = 1746  
 AST = 0.08 x 1746 = 140 U/L

**NOTES**

- The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
- 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.
- 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<sup>-3</sup> =  $\mu$ kat/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**

- 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  $\mu$ mol/L (15 mg/dL).  
**Conjugated bilirubin:** No interference from conjugated bilirubin up to a level of 116  $\mu$ mol/L (6.8 mg/dL).  
**Lipaemia:** No interference from lipaemia, measured as an absorbance at 630nm, up to 1.68 AU.
- Haemolyzed serum specimens should not be used. AST activity levels in erythrocytes are some 15 times higher, than those in sera.<sup>6</sup>
- Young DS<sup>7</sup> has published a comprehensive list of drugs and substances which may interfere with this assay.

**EXPECTED VALUES<sup>5</sup>**

At 37°C 5 - 34 U/L (0.084 - 0.568  $\mu$ kat/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.<sup>8</sup>

**PERFORMANCE DATA**

The following data was obtained using the Infinity AST(GOT) Liquid Stable 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<sup>9</sup>.

	LEVEL I	LEVEL II
Number of data points	80	80
Mean (U/L / $\mu$ kat/L)	45 / 0.752	194 / 3.24
<b>Within Run:</b> SD (U/L / $\mu$ kat/L)	0.7 / 0.012	1.4 / 0.023
CV (%)	1.6	0.7
<b>Total:</b> SD(U/L / $\mu$ kat/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 $\mu$ kat/L)
Mean of reference method results	37 U/L (0.618 $\mu$ kat/L)
Mean of Infinity AST (GOT) results	38 U/L (0.635 $\mu$ kat/L)
Slope	0.98
Intercept	2.1 U/L (0.035 $\mu$ kat/L)
Correlation coefficient	0.997

**LINEARITY**

When run as recommended, the assay is linear up to 450 U/L (7.52  $\mu$ kat/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  $\Delta$ mA /min per U/L (34.31  $\Delta$ mA /min per  $\mu$ kat/L).

**REFERENCES**

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840374 (R0)

**REF**

**Reorder Information**

Catalogue No.	Configuration
TR70121	2 x 125 mL
TR70198	2 x 500 mL
1184-200H	4 x 50 mL

\*\*Patented: 7,105,52 - Australia; 5,802,402 - United States, 0817841 - Europe