

# Infinity™

## ALT (GPT) Reagent\*\*

### PRODUCT SUMMARY

Stability	:	12 months at 2 - 8°C
Linear Range	:	Up to 450 U/L
Specimen Type	:	Serum
Method	:	Kinetic UV
Reagent Preparation	:	Add specified volume of distilled or deionized water.

**IVD**

#### INTENDED USE

This reagent is intended for the in vitro quantitative determination of ALT (L-Alanine:2-Oxoglutarate Aminotransferase EC2.6.1.2) in human serum.

#### CLINICAL SIGNIFICANCE

ALT is present in high concentrations in the liver and to a lesser extent in kidney, heart and skeletal muscle, pancreas, spleen and lung. Increased levels of ALT however are generally a result of liver disease associated with some degree of hepatic necrosis such as cirrhosis, carcinoma, viral or toxic hepatitis and obstructive jaundice. Characteristically ALT is generally higher than AST in acute viral or toxic hepatitis, whereas for most patients with chronic hepatic disease, ALT levels are generally lower than AST levels. Elevated ALT levels have also been found in extensive trauma and muscle disease, circulatory failure with shock, hypoxia, myocardial infarction and haemolytic disease.<sup>1</sup>

#### METHODOLOGY

Wroblewski and LaDue<sup>2</sup> first described a method for determining ALT using LDH and NADH. This method was later modified by Henry<sup>3</sup> and Bergmeyer<sup>4</sup> to optimize substrate conditions and eliminate side reactions. This method now forms the basis of many national and international recommended procedures. The Infinity ALT reagent is based on the recommendations of the IFCC<sup>5</sup>.

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

1. L-Alanine + 2-Oxoglutarate  $\xrightarrow{\text{ALT}}$  Pyruvate + L-Glutamate
  2. Pyruvate + NADH  $\xrightarrow{\text{LDH}}$  L-Lactate + NAD
  3. Sample Pyruvate + NADH  $\xrightarrow{\text{LDH}}$  L-Lactate + NAD
1. The amino group is enzymatically transferred by ALT present in the sample from alanine to the carbon atom of 2-oxoglutarate yielding pyruvate and L-glutamate.
  2. Pyruvate is reduced to lactate by LDH present in the reagent with the simultaneous oxidation of NADH to NAD. The reaction is monitored by measuring the rate of decrease in absorbance at 340nm due to the oxidation of NADH.
  3. Endogenous sample pyruvate is rapidly and completely reduced by Lactate dehydrogenase (LDH) during the initial incubation period so that it does not interfere with the assay.

#### REAGENT COMPOSITION (prior to reconstitution)

Active Ingredients	Concentration
2-Oxoglutarate	13 mmol/L
L-Alanine	440 mmol/L
NADH	0.26 mmol/L
LDH (microbial)	>3000 U/L
Tris Buffer	97 mmol/L
EDTA	5.0 mmol/L











pH 7.80 ± 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 ALT(GPT) Material Safety Data Sheet.

R22 Harmful if swallowed

S28 After contact with skin, wash immediately with plenty of soap and water.

### SYMBOLS IN PRODUCT LABELLING

	Authorized Representative		Temperature Limitation
	For in vitro diagnostic use		Use by/Expiration Date
	Batch code/Lot number		CAUTION. CONSULT INSTRUCTIONS FOR USE.
	Catalog number		Manufactured by
	Consult instructions for use		Xn - Harmful

#### REAGENT PREPARATION

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

#### STABILITY AND STORAGE

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 12 months or the stated expiry date which ever is the sooner. It is recommended that when the reagent is not in use for prolonged periods of time (eg: over night) the reagent be capped and stored at 2-8°C.

#### Indications of Reagent Deterioration:

- Turbidity,
- Absorbance <1.0 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.

**Storage:** Serum samples may be stored for at least 3 days at room temperature (18-25°C) and for at least 1 week at 4°C.<sup>6</sup>

#### 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 deionized 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
Primary Wavelength	340 nm (334, 365nm)
Secondary Wavelength	405 nm
Assay Type	Rate/Kinetic
Direction	Decrease
Sample : Reagent Ratio	1:10 - 1:20
eg: Sample Vol	30 µL
Reagent Vol	300 µL
Delay/Lag	30 seconds
Read Time	1 to 3 minutes
Reagent Blank	Low 1.00 AU
(1cm lightpath, 340nm)	High 2.50 AU
Linearity	450 U/L
(refer to Linearity section)	
Sensitivity	0.573 ΔmA 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:**

$\Delta$ Abs/min = 0.08  
 Factor = 1746  
 ALT = 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 334 nm = 6.18 and at 365 nm = 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 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**

- The reagent contains LDH to rapidly reduce endogenous sample pyruvate during the initial incubation time. Abnormally high levels of pyruvate may cause falsely high results (The normal level of serum pyruvate is 0.03 to 0.10 mmol/L<sup>7</sup>).
- 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:
 

<b>Haemoglobin:</b>	No interference from haemoglobin up to a level of 500 mg/dL.
<b>Free bilirubin:</b>	No interference from free bilirubin up to a level of 260 mmol/L (15 mg/dL).
<b>Conjugated bilirubin:</b>	No interference from conjugated bilirubin up to a level of 116 mmol/L (6.8 mg/dL).
<b>Lipaemia:</b>	No interference from lipaemia, measured as an absorbance at 630 nm, up to 1.68AU.
- Young DS<sup>9</sup> has published a comprehensive list of drugs and substances which may interfere with this assay.

**EXPECTED VALUES<sup>7</sup>**

At 37°C  
 Adults: 10 - 35 U/L  
 Newborn/Infants: 7 - 40 U/L

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.<sup>9</sup>

**PERFORMANCE DATA**

The following data was obtained using the Infinity ALT(GPT) 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 controls and following the NCCLS EP5-T procedure.<sup>10</sup>

	LEVEL I	LEVEL II
Mean (U/L)	35	121
CV (%) Within run	2.0	0.7
CV (%) Total	2.3	1.0

**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	78
Range of sample results	4 - 238 U/L
Mean of reference method results	28 U/L
Mean of Infinity ALT (GOT) results	26 U/L
Slope	0.95
Intercept	0.00 U/L
Correlation coefficient	0.999

**LINEARITY**

When run as recommended, the assay is linear up to 450 U/L using a SV:RV ratio of 1:20.

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.

**SENSITIVITY**

When run as recommended the sensitivity of this assay is 0.573 $\Delta$ mA/min per U/L.

**REFERENCES**

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REF

**Reorder Information**

Catalogue No.	Configuration
TR71021	2 x 125 mL