

Infinity™










ALT (GPT) 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 UV
Reagent Preparation	: Supplied ready to use.

IVD

SYMBOLS IN PRODUCT LABELLING

	Authorised Representative		Temperature Limitation
	For in vitro diagnostic use		Use by/Expiration Date
	Batch code/Lot number		CAUTION. CONSULT INSTRUCTIONS FOR USE.
	Catalogue number		Manufactured by
	Consult instructions for use		

INTENDED USE

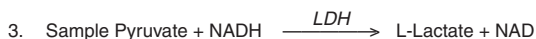
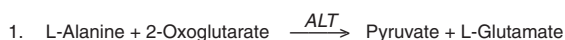
This reagent is intended for the in vitro quantitative determination of ALT (L-Alanine:2-Oxoglutarate Aminotransferase EC2.6.1.2) in human serum.

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.¹

METHODOLOGY

Wroblewski and LaDue² first described a method for determining ALT using LDH and NADH. This method was later modified by Henry³ and Bergmeyer⁴ to optimize substrate conditions and eliminate side reactions. This method now forms the basis of many national and international recommended procedures. This reagent is based on the recommendations of the IFCC⁵. The series of reactions involved in the assay system is as follows:



- 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.
- 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.
- 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

Active Ingredients

	<u>Concentration</u>
2-Oxoglutarate	13 mmol/L
L-Alanine	440 mmol/L
NADH	> 0.12 mmol/L
LDH (microbial)	> 2000 U/L
Tris Buffer	97 mmol/L
EDTA	5.0 mmol/L

pH 7.80 ± 0.10 at 20°C.

WARNING: Do not ingest. Avoid contact with skin and eyes. 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. Flush with plenty of water when disposing. For further information consult the Infinity ALT(GPT) Liquid Stable Reagent Material Safety Data Sheet.

REAGENT PREPARATION

Reagent is supplied ready to use.

STABILITY AND STORAGE

When stored at 2-8°C the 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: over night) the reagent be capped and stored at 2-8°C.

Indications of Reagent Deterioration:

- Turbidity,
- Absorbance < 1.0 at 340 nm (1cm); 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.⁶

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 1.00 AU
(1cm lightpath, 340nm)	High 2.50 AU
Linearity	450 U/L (7.52 µkat/L)
(refer to Linearity section)	
Sensitivity	0.573 ΔmA/min per U/L
(1cm lightpath, 340nm)	(34.3 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:

ΔAbs/min	=	0.08
Factor	=	1746
ALT	=	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 coefficient for NADH at 334nm = 6.18 and at 365nm = 3.40.
5. Unit Conversion: $U/L \times 16.67 \times 10^{-3} = \mu\text{kat/L}$

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

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 500 mg/dL.

Free bilirubin: No interference from free bilirubin up to a level of 260 $\mu\text{mol/L}$ (15 mg/dL).

Conjugated bilirubin: No interference from conjugated bilirubin up to a level of 116 $\mu\text{mol/L}$ (6.8 mg/dL).

Lipaemia: No interference from lipaemia, measured as an absorbance at 630 nm, up to 1.68 AU.

2. Haemolyzed serum specimens should not be used. ALT activity levels in erythrocytes are some 7 times higher, than those in sera.⁷
3. Young DS⁹ has published a comprehensive list of drugs and substances which may interfere with this assay.

EXPECTED VALUES⁹

At 37°C Adults: 10 - 35 U/L (0.167 - 0.585 $\mu\text{kat/L}$)
 Newborn/Infants*: 7 - 40 U/L (0.117 - 0.668 $\mu\text{kat/L}$)

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.¹⁰

* These values have not been validated with this reagent.

PERFORMANCE DATA

The following data was obtained using the Infinity ALT(GPT) 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.¹¹

	LEVEL I	LEVEL II
Number of data points	40	40
Mean (U/L / $\mu\text{kat/L}$)	35 / 0.585	121 / 2.02
Within run SD (U/L / $\mu\text{kat/L}$)	0.7 / 0.012	0.8 / 0.013
CV (%)	2.0	0.7
Total SD (U/L / $\mu\text{kat/L}$)	0.8 / 0.013	1.2 / 0.020
CV (%)	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 (0.067 - 3.97 $\mu\text{kat/L}$)
Mean of reference method results	28 U/L (0.468 $\mu\text{kat/L}$)
Mean of Infinity ALT (GPT) results	26 U/L (0.434 $\mu\text{kat/L}$)
Slope	0.95
Intercept	0.00 U/L (0.000 $\mu\text{kat/L}$)
Correlation coefficient	0.999

LINEARITY

When run as recommended, the assay is linear up to 450 U/L (7.52 $\mu\text{kat/L}$). 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.

SENSITIVITY

When run as recommended the sensitivity of this assay is 0.573 $\Delta\text{mA/min}$ per U/L (34.3 $\Delta\text{mA/min}$ per $\mu\text{kat/L}$).

REFERENCES

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11. 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 and Technical Support

Catalogue No.

Configuration

TR71121	2 x 125 mL
TR71198	2 x 500 mL
1164-200H	4 x 50 mL

Australia

International

U.S.A.

Phone	1800 333 110	61 3 9790 4100	(800) 558 9115
Facsimile	(03) 9790 4155	61 3 9790 4155	(412) 788 6833