

ALT (GPT) 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 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.

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

1. L-Alanine + 2-Oxoglutarate \xrightarrow{ALT} Pyruvate + L-Glutamate
2. Pyruvate + NADH \xrightarrow{LDH} L-Lactate + NAD
3. Sample Pyruvate + NADH \xrightarrow{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

<u>Ingredientes activos</u>	<u>Concentración</u>
Reagent 1:	
NADH	0.42 mmol/L
LDH (microbial)	>2000 U/L
2-Oxoglutarate	18.8 mmol/L
Tris Buffer	33 mmol/L
Reagent 2:	
L-Alanine	1300 mmol/L
Tris Buffer	443.5 mmol/L

pH 7.7 ± 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 ALT(GPT) 2 part Liquid reagent Material Safety Data Sheet.

REAGENT PREPARATION

Reagents are supplied ready to use.

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)

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.

Indications of Reagent Deterioration:

- Turbidity;
- Reagent 1 absorbance < 2.3 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: Serum samples may be stored for at least 3 days at room temperature (18-25°C) and for at least 1 week at 4°C⁵

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 1.8 AU
(340nm, 1cm lightpath)	High 2.4 AU
Linearity	Up to 1500 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

$$\text{Factor} = \frac{TV \times 1000}{6.3 \times SV \times P}$$

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:

$\Delta\text{Abs}/\text{min} = 0.08$
 Factor = 3333 (See note 5)
 ALT = $0.08 \times 3333 = 267 \text{ U/L}$

NOTES

- The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
- If the change in absorbance is greater than 0.45/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.
- Calculated factor above is applicable when run monochromatically.
- Unit Conversion: $\text{U/L} \times 16.67 \times 10^{-3} = \mu\text{kat/L}$

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 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 300 mg/dL.
Free bilirubin: No interference from free bilirubin up to 684 $\mu\text{mol/L}$ (40 mg/dL).
Conjugated Bilirubin: No interference from conjugated bilirubin up to 684 $\mu\text{mol/L}$ (40 mg/dL).
Pyruvate: No interference from pyruvate up to 2.3 mmol/L (20 mg/dL).
- Haemolyzed serum specimens should not be used. ALT activity levels in erythrocytes are some 7 times higher, than those in sera.⁶
- Avoid the use of lipaemic samples.
- Young DS⁷ has published a comprehensive list of drugs and substances which may interfere with this assay.

EXPECTED VALUES⁸

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 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.⁹

PERFORMANCE DATA

The following data was obtained using the ALT(GPT) 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.¹⁰

Within Run:	LEVEL I	LEVEL II
Number of data points	80	80
Mean (U/L)	34	99
Mean ($\mu\text{kat/L}$)	0.568	1.65
SD (U/L)	1.16	1.28
SD ($\mu\text{kat/L}$)	0.019	0.021
CV (%)	3.4	1.3

Total:	LEVEL I	LEVEL II
Number of data points	80	80
Mean (U/L)	34	99
Mean ($\mu\text{kat/L}$)	0.568	1.65
SD (U/L)	1.47	3.48
SD ($\mu\text{kat/L}$)	0.025	0.058
CV (%)	4.3	3.5

METHOD COMPARISON

Comparison studies were carried out using a similar commercially available ALT(GPT) 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	65
Range of sample results	6 - 186 U/L (0.100 - 3.11 $\mu\text{kat/L}$)
Mean of reference method results	47 U/L (0.785 $\mu\text{kat/L}$)
Mean of ALT(GPT) results	38 U/L (0.635 $\mu\text{kat/L}$)
Slope	0.80
Intercept	-0.03 U/L (-0.001 $\mu\text{kat/L}$)
Correlation coefficient	0.998

LINEARITY

When run as recommended the assay is linear up to 1500 U/L (25.1 $\mu\text{kat/L}$).

ANALYTICAL SENSITIVITY

When run as recommended the sensitivity of the assay is 0.30 $\Delta\text{mA}/\text{min}$ per U/L (18.0 $\Delta\text{mA}/\text{min}$ per $\mu\text{kat/L}$).

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**REF****Reorder Information**

Catalogue No.	REAG 1	REAG 2
TR18920	1 x 125 mL	1 x 35 mL
TL18901 (iLab 600)	5 x 80 mL	5 x 20 mL
TH18901 (Hitachi)	4 x 80 mL	4 x 20 mL
TY18901 (Hitachi)	4 x 53 mL	4 x 15 mL
7500-104A	4 x 500 mL	
7500-204A		2 x 250 mL