

ALT (GPT) Reagent

Alanine Aminotransferase

PRODUCT SUMMARY

Stability	:	50 days 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

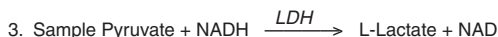
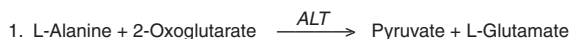
This reagent is intended for the in vitro quantitative determination of ALT (L-Alanine:2-Oxoglutarate Aminotransferase EC2.6.1.2) in human serum.

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.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 ALT 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

	Concentration
L-Alanine	440 mmol/L
NADH	> 0.18 mmol/L
LDH (microbial)	> 1820 U/L
2-Oxoglutarate	16.5 mmol/L
Tris Buffer	88 mmol/L

Also contains non-reactive fillers and stabilizers.

pH 7.5 ± 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) Reagent Material Safety Data Sheet. **The Packaging of This Product Contains Dry Natural Rubber.** Exercise precaution when handling crimps and broken glass vials, as sharp edges can injure the user.

R22 Harmful if swallowed

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

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		Manufactured by
	Consult instructions for use		Xn - Harmful

REAGENT PREPARATION

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

STABILITY AND STORAGE

Prior to Use:

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 50 days.

Indications of Reagent Deterioration:

- Turbidity,
- Absorbance <1.1 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.

Plasma: Not recommended.⁵

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

- 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	30/37°C
Wavelength	340 nm
Assay Type	Rate/Kinetic
Direction	Decrease
Sample : Reagent Ratio	1:10
eg: Sample Vol	30 µL
Reagent Vol	300 µL
Delay/Lag Time	60 seconds
Read Time	60 seconds
Reagent Blank	Low 1.1 AU
(1cm lightpath, 340nm)	High 2.0 AU
Linearity	0 - 450 U/L
(refer to Linearity section)	
Sensitivity	0.57 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:

Δ Abs/min = 0.10
Factor = 1746
ALT = $0.10 \times 1746 = 175$ 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 $\times 16.67 \times 10^{-3} = \mu$ 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 haemoglobin, bilirubin, pyruvate and lipaemia were carried out. The following results were obtained:
Haemoglobin: No interference from haemoglobin up to 1000 mg/dL.
Bilirubin: No interference from bilirubin up to 1000 μ mol/L (60 mg/dL).
Pyruvate: No interference from pyruvate up to 1.25 mmol/L.
Lipaemia: No interference from lipaemia, measured as triglycerides, up to 10.7 mmol/L (950 mg/dL).
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 DS⁸ 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
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.¹⁰

* These values have not been validated.

PERFORMANCE DATA

The following data was obtained using the ALT(GPT) reagent on a well maintained automated clinical chemistry analyser. Users should establish product performance on their specific analyser used.

IMPRECISION

Within Run:

	LEVEL I	LEVEL II
Number of Samples	20	20
Mean (U/L)	24.3	105
SD (U/L)	0.37	0.67
CV%	1.52	0.64

Between Day:

	LEVEL I	LEVEL II
Number of Samples	20	20
Mean (U/L)	24.0	103
SD (U/L)	1.16	3.00
CV%	4.83	2.91

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	60
Range of sample results	4 - 617 U/L
Mean of reference method results	88 U/L
Mean of ALT (GPT) reagent results	84 U/L
Slope	0.991
Intercept	-3.1 U/L
Correlation coefficient	0.999

LINEARITY

When run as recommended, the assay is linear up to 450 U/L (7.5 μ kat/L).

SENSITIVITY

When run as recommended the sensitivity of this assay is 0.57 Δ mA/min per U/L.

REFERENCES

1. Zilva JF, Pannall PR. "Plasma Enzymes in Diagnosis" in Clinical Chemistry in Diagnosis and Treatment. Lloyd-Luke London. 1979: Chap 17:338.
2. Wroblewski F, LaDue JS. Proc Sec Exp Biol and Med 1956; 34:381.
3. Henry RJ, et al. Am Jnl Clin Path 1960; 34:381.
4. Bergmeyer HU, et al. Clin Chem 1978; 24:58-73.
5. IFCC Expert Panel on enzymes Part 3. J. Clin. Chem. Clin. Biochem. 1986; 24:481-95.
6. Murray RL. "Alanine aminotransferase" in Clinical Chemistry. Theory, analysis and correlation. Kaplan LA, Pesce AJ (Eds), CV Mosby St Louis 1984:1090.
7. Burtis CA, Ashwood ER, "Tietz textbook of Clinical Chemistry" Second Edition, 1994; 795.
8. Young DS, Effects of Drugs on Clinical Laboratory Tests. Third Edition. 1990: 3:6-12.
9. Tietz Textbook of Clinical Chemistry. Burtis CA and Ashwood ER (Eds). Second Edition, WB Saunders Company, 1994; 2177.
10. Wachtel M et al, Creation and Verification of Reference Intervals. Laboratory Medicine 1995; 26:593-7.



Fisher Diagnostics
a division of Fisher Scientific Company, LLC
a part of Thermo Fisher Scientific Inc.
Middletown, VA 22645-1905 USA
Phone: 800-528-0494
540-869-3200
Fax: 540-869-8132



MDCI Ltd.
Arundel House
1 Liverpool Gardens
Worthing, West Sussex BN11 1SL UK



© 2009 Thermo Fisher Scientific Inc. All rights reserved.

REF

Reorder Information

Catalogue No.	Configuration
1160-200	20 x 10 mL
TR18515	20 x 20 mL
TR18503/1160-500	10 x 50 mL
TR18504	10 x 200 mL