ALANINE AMINOTRANSFERASE (ALT)
MANUAL APPLICATION
C164-0A, C164-0B, V164-0A

Intended Use
For **IN VITRO** quantitative determination of ALT (GPT) in serum or plasma using manual or automated applications.

Clinical Significance
Measurements of ALT activity are used for diagnosing liver and heart diseases, as well as for monitoring the causes and treatments. [1]

Method History
Kinetic procedures for the assay of Alanine Aminotransferase (EC 2.6.1.2) were reported by Wroblewski and Ladue[2]. In 1977, the International Federation for Clinical Chemistry (IFCC) published recommendations on optimum assay conditions for Alanine Aminotransferase (ALT). [3,4] The Catachem Alanine Aminotransferase method for manual or automated applications is based upon the work reported by IFCC.

Method Principle
The Alanine Aminotransferase enzyme catalyzes the conversion of alpha-ketoglutarate to L-glutamate and pyruvate. The pyruvate produced is then quantitatively determined by the LDH-NADH reaction. The decrease in absorbance due to the oxidation of NADH to NAD^+ is monitored at 340nm. The rate of decrease in absorbance of the reaction mixture is directly proportional to the ALT enzyme activity in the serum sample. The reaction scheme below illustrates the reactions that occur in this method.

$$\text{L-alanine} + \text{alpha-KG} \xrightarrow{\text{ALT}} \text{L-glutamate} + \text{pyruvate}$$

$$\text{Pyruvate} + \text{NADH} \xrightarrow{\text{LDH}} \text{Lactate} + NAD^+$$

Reagent Content
The reagents contain approximately:

ALT Substrate Reagent

Each liter contains:	
Buffer	
L-alanine	575 mmol
D-LDH	≥2500 U/L
Alpha-ketoglutarate	13.2 mmol
Non-reactive ingredients and stabilizers	

ALT Activator Reagent

Each liter contains:	
Buffer	
NADH	2.5 mmol/L
Non-reactive ingredients and stabilizers	

Reagent Preparation
The Catachem ALT Substrate Reagent (R1) and the ALT Activator Reagent (R2) are liquid, ready for use and require no preparation.

Reagent Storage and Stability
Store the ALT Reagents at 2-8˚C. When unopened and stored as directed, the reagents are stable until the expiration date stated on the label. Once opened, the Catachem ALT Reagents are stable for at least 30 days when stored as directed, refrigerated at 2-8˚C and capped when not in use.

Specimen Collection and Preparation
Test sera should be fresh, clear, and unhemolyzed. After blood is drawn, it should be processed as soon as possible and the serum should be isolated from the clot without delay. In separated unhemolyzed serum, the ALT concentration is stable for seven days at 2-8˚C and for longer periods of time if stored frozen.[1] Plasma can also be used if collected using ammonium, lithium or sodium heparin. Anticoagulants that should not be used include potassium oxalate/sodium fluoride and sodium citrate.

Precautions
Avoid contact of specimen with skin and eyes. Should contact occur, wash affected area with plenty of water. **DO NOT PIPETTE SPECIMENS BY MOUTH.**

Quality Control
To monitor the performance of the Catachem ALT Reagents and the procedure used, the regular use of a normal and abnormal control serum is recommended.

Interfering Substances
A comprehensive discussion has been reported on the effects of interfering substances on the ALT assay.[5] A summary of the influence of drugs on clinical laboratory procedures may be found by consulting D.S. Young, et al.[5]

Expected Values
The range of expected human values determined for this method is 5-40 U/L at 37˚C. These values are suggested guidelines. It is recommended that each laboratory establish the normal range for the area in which it is located.

Procedure
Important: Read the entire procedure instructions before proceeding with assay.

Materials Required (But Not Provided)
Spectrophotometer with a 340 nm wavelength

Matching cuvettes	1 cm light path
Timer	to time incubation period
Pipettes	0.9 mL & 0.1mL for reagents
Pipette	0.05 mL for sample

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Materials Provided
Catachem ALT Substrate Reagent (R1)
Catachem ALT Activator Reagent (R2)

Analytical Parameters

Wavelength	340 nm
Temperature	37ºC
Path Length	1 cm
Reaction Mode	Rate
Reaction Time	3 minutes
Substrate Reagent (R1) Volume	0.90 mL
Activator Reagent (R2) Volume	0.10 mL
Sample Volume	0.05 mL
Total Volume	1.05 mL
Sample-to-reagent ratio	1:21

Assay Procedure
1. Set spectrophotometer wavelength to 340nm and zero the instrument with the cuvette containing water.
2. Pipette 0.90 mL of ALT Substrate Reagent (R1) into two cuvettes marked “Sample” and “Control”.
3. Pipette 0.10 mL of ALT Activator Reagent (R2) into the cuvettes marked “Sample” and “Control”.
4. Incubate cuvettes for 3 minutes at 37ºC.
5. Pipette 0.05ml of “Control” or “Sample” into their respective cuvettes, mix well.
6. Replace cuvettes in spectrophotometer and continuously monitor change in absorbance for at least 3 minutes.
7. Read the absorbance of both the “Control” and “Sample”.
8. Calculate the ALT concentration (U/L) in the sample(s) as shown in the results calculations below.

Calculations and Results

$$\text{Activity U/L} = \frac{\Delta \text{ OD}}{\text{Min}} \times \frac{\text{TV} \times 1000}{6.22 \times \text{L} \times \text{SV}}$$

Where:
Δ OD/min = change in absorbance per minute
TV ml = total volume in cuvette
SV ml = volume of sample being assayed
6.22 = mmol absorptivity of NADH at 340 nm
L cm = Path Length (cuvette width in cm)
1000 = converts µmol/ml to U/L

Example:

Δ OD/min = 0.01

$$\text{ALT U/L} = \frac{0.01 \times 1.05\text{mL} \times 1000}{6.22 \times 1\text{cm} \times 0.05\text{ mL}}$$

$$= 34 \text{ U/L}$$

Method Performance Characteristics

Sensitivity: The sensitivity of this method is 0.0002 absorbance units per U/L.
Linear Range: In this method there is no significant nonlinearity over the range of 0-1000 U/L.
Precision: Within-run and day-to-day precision is summarized below:

Precision Study

ALT	Within-Run Precision		Total Precision	
Mean	SD	CV	SD	CV
U/L	U/L	%	U/L	%
32	1.50	4.70	2.00	6.00
384	6.60	1.70	6.30	1.60
728	7.10	1.00	7.40	1.00

Correlation
A comparison of this method using an automated analyzer and a reference procedure resulted in the following regression statistics:

Range	=	10-397 U/L
N	=	101
Y	=	0.969x + 3.3
r	=	0.997
Sy.x	=	4.5

References

1. Fundamentals of Clinical Chemistry. Edited by Norbert W. Tietz. WB Saunders, Philadelphia.
2. Wroblewski F, Ladue JS. Serum glutamic pyruvic transaminase in cardiac and hepatic disease. Proc Soc Exp Biol Med 91, 569 (1956).
3. Expert Panel on Enzymes, Committee on Standards (IFCC): Provisional recommendation (1974) on IFCC methods for the measurement of catalytic concentration of enzymes Clin Chim Acta 61, following p 238, F11 to F24 (1975) Clin Chem 22, 384 (1976).
4. Expert Panel on Enzymes, Committee on Standards (IFCC): Provisional recommendation on IFCC methods for the measurement of catalytic concentrations of enzymes: Part 2. IFCC method for aspartate aminotransferase. Clin Chim Acta 70, following p 336, F19 to F24 (1976) Clin Chem 23, 887 (1977).
5. Young DA, Pestaner LC, Gibberman V. Effect of drugs on clinical lab tests.Clin Chem 21 (5):1D-432D (1975).

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