ALANINE AMINOTRANSFERASE (ALT) REAGENTS C164-0B

Contents	Product No.	Package
ALT KIT - B	**C164-0B**	
ALT Substrate R1	C164-05	6 x 45.0 mL
ALT Activator R2	C164-06	6 x 5.0 mL

REAGENT PREPARATION

The Catachem ALT reagents are packaged ready to use.
No preparation of either reagent is required.

REAGENT STORAGE AND STABILITY

Store the unopened reagent kit at 2-8°C.
When stored as directed, both unopened reagents are stable until the expiration date stated on the label.

NOT FOR USE IN UNPROFESSIONAL SETTINGS

FOR TECHNICAL ASSISTANCE:
Email: catachem@catacheminc.com
Contact Form: www.catacheminc.com
Call: 203-262-0330

ALANINE AMINOTRANSFERASE (ALT) C164-0B MANUAL/AUTOMATED APPLICATION

Intended Use
For **IN VITRO** quantitative determination of ALT (SGPT) 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.

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

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

Reagent Content
When reconstituted according to the directions, the concentrations of the active ingredients in the reagent(s) will be approximately as follows:

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	

Preparation of Working Reagents
If running as a 2-component system, reagents are packaged ready to use. If desired, a single working reagent can be prepared by completing the following steps: Prepare Catachem ALT Working Reagent by adding 1 part ALT activator (R2) to 9 parts ALT Sample Diluent (Substrate). Mix well by inverting the bottle several times.

Reagent Storage and Stability
Store the unopened ALT reagents at 2-8˚C. When stored as directed, these reagents are stable until the expiration date stated on the label. Once opened these reagents are stable for 30 days at 2- 8 ˚C if capped when not in active use. If used as a single reagent the ALT single Working Reagent is stable for 30 days at 2-8˚C when prepared and stored as directed. The Catachem ALT Reagents have been tested to reflect shipping conditions and are stable for the lifespan of the product if frozen up to 5 times or upon reaching temperatures up to 40°C for up to one week.

Specimen Collection and Preparation
Test sera should be fresh, clear, and non-hemolyzed. When blood is drawn, it should be processed as soon as possible and the serum should be isolated from the clot without delay. In separated non-hemolyzed 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 ensure optimal performance of these reagents and this procedure, we recommend systematic calibration using Catachem's Catacal (C1200-10). Assay performance should be monitored by running normal/abnormal controls concomitantly with samples. Catachem has optimized this assay using Catatrol Level I (C1200-11) and Catatrol Level II (C1200-12) and recommends their use for daily QC.

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 analytical measuring range of this assay, as performed below, is 5 U/L to 40 U/L at 37˚C. These values serve as suggested reference points only. For veterinary samples, ranges will be dependent on the species under test. It is recommended that each laboratory establishes the normal ranges for the species under

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study and for the geographic area in which the laboratory is located.

Procedure

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

Materials Required (Not Provided)

Spectrophotometer with a 340nm wavelength
Matching cuvettes 1 cm light path
Timer to time incubation period
Pipette 1.0 mL for reagent
Pipette 0.05 mL for sample

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	5 minutes
Reagent Volume	1.0 mL
Sample Volume	0.05 mL
Total Volume	1.05 mL
Sample-to-reagent ratio	1:21

Assay Procedure

1. Prepare the required volume of ALT Working Reagent.
2. Set spectrophotometer wavelength at 340nm and zero the instrument with the cuvette containing water.
3. Pipette 1.0 ml of Working Reagent into two 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. Determine the Delta absorbance per minute for 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	= Pathlength (cuvette width in cm)
1000	= converts U/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 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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