

Alanine Aminotransferase acc. to IFCC without pyridoxal phosphate activation**Order information**

REF	CONTENT	Analyzer(s) on which cobas c pack(s) can be used
05850797 190	Alanine Aminotransferase acc. to IFCC (1100 tests)	System-ID 05 7501 0 Roche/Hitachi cobas c 701/702
Materials required (but not provided):		
10759350 190	Calibrator f.a.s. (12 x 3 mL)	Code 401
10759350 360	Calibrator f.a.s. (12 x 3 mL, for USA)	Code 401
12149435 122	Precinorm U plus (10 x 3 mL)	Code 300
12149435 160	Precinorm U plus (10 x 3 mL, for USA)	Code 300
12149443 122	Precipath U plus (10 x 3 mL)	Code 301
12149443 160	Precipath U plus (10 x 3 mL, for USA)	Code 301
05117003 190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 391
05947626 190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 391
05947626 160	PreciControl ClinChem Multi 1 (4 x 5 mL, for USA)	Code 391
05117216 190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 392
05947774 190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 392
05947774 160	PreciControl ClinChem Multi 2 (4 x 5 mL, for USA)	Code 392
05172152 190	Diluent NaCl 9 % (119 mL)	System-ID 08 6869 3

English**System information**

ALTL: ACN 8685

Intended use

In vitro test for the quantitative determination of alanine aminotransferase (ALT) in human serum and plasma on Roche/Hitachi **cobas c** systems.

Summary^{1,2}

The enzyme alanine aminotransferase (ALT) has been widely reported as present in a variety of tissues. The major source of ALT is the liver, which has led to the measurement of ALT activity for the diagnosis of hepatic diseases. Elevated serum ALT is found in hepatitis, cirrhosis, obstructive jaundice, carcinoma of the liver, and chronic alcohol abuse. ALT is only slightly elevated in patients who have an uncomplicated myocardial infarction.

Although both serum aspartate aminotransferase (AST) and ALT become elevated whenever disease processes affect liver cell integrity, ALT is the more liver-specific enzyme. Moreover, elevations of ALT activity persist longer than elevations of AST activity.

In patients with vitamin B₆ deficiency, serum aminotransferase activity may be decreased. The apparent reduction in aminotransferase activity may be related to decreased pyridoxal phosphate, the prosthetic group for aminotransferases, resulting in an increase in the ratio of apoenzyme to holoenzyme.

Test principle

This assay follows the recommendations of the IFCC, but was optimized for performance and stability.^{3,4}

ALT catalyzes the reaction between L-alanine and 2-oxoglutarate. The pyruvate formed is reduced by NADH in a reaction catalyzed by lactate dehydrogenase (LDH) to form L-lactate and NAD⁺.



The rate of the NADH oxidation is directly proportional to the catalytic ALT activity. It is determined by measuring the decrease in absorbance.

Reagents - working solutions

R1 TRIS buffer: 224 mmol/L, pH 7.3 (37 °C); L-alanine: 1120 mmol/L; albumin (bovine): 0.25 %; LDH (microorganisms): ≥ 45 μkat/L; stabilizers; preservative

R3 2-Oxoglutarate: 94 mmol/L; NADH: ≥ 1.7 mmol/L; additives; preservative

R1 is in position B and R3 is in position C.

Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:

Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:

Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

Reagent handling

Ready for use

Storage and stability**ALT**

Shelf life at 2-8 °C:

See expiration date on **cobas c** pack label.

On-board in use and refrigerated on the analyzer:

4 weeks

On-board on the Reagent Manager:

24 hours

Diluent NaCl 9 %

Shelf life at 2-8 °C:

See expiration date on **cobas c** pack label.

On-board in use and refrigerated on the analyzer:

4 weeks

On-board on the Reagent Manager:

24 hours

Specimen collection and preparation

For specimen collection and preparation only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.

Serum.

Plasma: Li-heparin and K₂-EDTA plasma.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems

Alanine Aminotransferase acc. to IFCC without pyridoxal phosphate activation

from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Separate the serum or plasma from the clot or cells promptly.

Centrifuge samples containing precipitates before performing the assay.

See the limitations and interferences section for details about possible sample interferences.

Sample stability claims were established by experimental data by the manufacturer or based on reference literature and only for the temperatures/time frames as stated in the method sheet. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory.

Stability:	3 days at 15-25 °C ⁵
	7 days at 2-8 °C ⁵
	> 7 days at (-60)-(-80) °C

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- See "Order information" section
- General laboratory equipment

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum and plasma**cobas c 701/702 test definition**

Assay type	Rate A		
Reaction time / Assay points	10 / 24-38		
Wavelength (sub/main)	700/340 nm		
Reaction direction	Decrease		
Units	U/L (µkat/L)		
Reagent pipetting	Diluent (H ₂ O)		
R1	65 µL	36 µL	
R3	19 µL	22 µL	
<i>Sample volumes</i>	<i>Sample</i>	<i>Sample dilution</i>	
		<i>Sample</i>	<i>Diluent (NaCl)</i>
Normal	10 µL	–	–
Decreased	10 µL	15 µL	135 µL
Increased	20 µL	–	–

Calibration

Calibrators	S1: H ₂ O S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	2-point calibration
	- after reagent lot change
	- as required following quality control procedures

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Traceability: This method has been standardized against the original IFCC formulation using calibrated pipettes together with a manual photometer providing absolute values and the substrate-specific absorptivity, ϵ .⁶

Quality control

For quality control, use control materials as listed in the "Order information" section.

In addition, other suitable control material can be used.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

Roche/Hitachi **cobas c** systems automatically calculate the analyte activity of each sample.

Conversion factor: U/L x 0.0167 = µkat/L

Limitations - interference

Criterion: Recovery within $\pm 10\%$ of initial value at an ALT activity of 30 U/L (0.5 µkat/L).

Icterus:⁷ No significant interference up to an I index of 60 (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).

Hemolysis:⁷ No significant interference up to an H index of 90 (approximate hemoglobin concentration: 56 µmol/L or 90 mg/dL).

Contamination with erythrocytes will elevate results, because the analyte level in erythrocytes is higher than in normal sera. The level of interference may be variable depending on the content of analyte in the lysed erythrocytes.

Lipemia (Intralipid):⁷ No significant interference up to an L index of 150. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Lipemic samples may cause > Abs flagging. Choose diluted sample treatment for automatic rerun.

Drugs: No interference was found at therapeutic concentrations using common drug panels.^{8,9}

Exception: Calcium dobesilate can cause artificially low ALT results at therapeutic concentrations.

Cyanokit (Hydroxocobalamin) may cause interference with results.

Physiological plasma concentrations of Sulfasalazine or Sulfapyridine may lead to false results.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.¹⁰

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi **cobas c** systems. All special wash programming necessary for avoiding carry-over is available via the **cobas** link, manual input is required in certain cases. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/SmpCln1+2/SCCS Method Sheet and for further instructions refer to the operator's manual.

Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.

Limits and ranges**Measuring range**

5-700 U/L (0.08-11.7 µkat/L)

Determine samples having higher activities via the rerun function. Dilution of samples via the rerun function is a 1:10 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 10.

Lower limits of measurement

Lower detection limit of the test

5 U/L (0.08 µkat/L)

Alanine Aminotransferase acc. to IFCC without pyridoxal phosphate activation

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying 3 standard deviations above that of the lowest standard (standard 1 + 3 SD, repeatability, n = 21).

Values below the lower detection limit (< 5 U/L) will not be flagged by the instrument.

Expected values¹¹

Acc. to the optimized standard method (comparable to the IFCC method without pyridoxal phosphate activation¹²):

Males	up to 41 U/L	(up to 0.68 μ kat/L)
Females	up to 33 U/L	(up to 0.55 μ kat/L)

Calculated values: A factor of 1.85 is used for the conversion from 25 °C to 37 °C.¹³

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using human samples and controls in an internal protocol. Repeatability (n = 21), intermediate precision (3 aliquots per run, 1 run per day, 20 days). The following results were obtained:

<i>Repeatability</i>	<i>Mean</i>	<i>SD</i>	<i>CV</i>
	<i>U/L (μkat/L)</i>	<i>U/L (μkat/L)</i>	<i>%</i>
Precinorm U	46.3 (0.773)	0.8 (0.013)	1.6
Precipath U	123 (2.05)	1 (0.02)	0.5
Human serum A	21.5 (0.359)	0.7 (0.012)	3.1
Human serum B	80.3 (1.34)	0.4 (0.01)	0.5
Human serum C	546 (9.12)	3 (0.05)	0.6
<i>Intermediate precision</i>	<i>Mean</i>	<i>SD</i>	<i>CV</i>
	<i>U/L (μkat/L)</i>	<i>U/L (μkat/L)</i>	<i>%</i>
Precinorm U	39.3 (0.66)	0.6 (0.01)	1.4
Precipath U	120 (2.00)	1 (0.02)	1.0
Human serum 3	24.0 (0.40)	0.6 (0.01)	2.6
Human serum 4	98.1 (1.64)	3.2 (0.05)	3.3

Results for intermediate precision were obtained on the master system **cobas c 501** analyzer.

Method comparison

ALT values for human serum and plasma samples obtained on a Roche/Hitachi **cobas c 701** analyzer (y) were compared with those determined using the corresponding reagent on a Roche/Hitachi **cobas c 501** analyzer (x).

Sample size (n) = 69

Passing/Bablok ¹⁴	Linear regression
y = 0.976x + 1.61 U/L	y = 0.971x + 2.82 U/L
τ = 0.985	r = 0.999

The sample activities were between 5.60 and 637 U/L (0.094 and 10.6 μ kat/L).

References

- Sherwin JE. Liver function. In: Kaplan LA, Pesce AJ, eds. Clinical Chemistry, theory, analysis, and correlation. St. Louis: Mosby 1984;420-438.
- Moss DW, Henderson AR, Kachmar JF. Enzymes. In: Tietz NW, ed. Fundamentals of Clinical Chemistry, 3rd ed. Philadelphia, PA: WB Saunders 1987;346-421.




- Bergmeyer HU, Hørder M, Rej R. Approved recommendation (1985) on IFCC methods for the measurement of catalytic concentration of enzymes. Part 3. IFCC method for alanine aminotransferase. J Clin Chem Clin Biochem 1986;24:481-495.
- ECCLS. Determination of the catalytic activity concentration in serum of L-alanine aminotransferase (EC 2.6.1.2, ALAT). Klin Chem Mitt 1989;20:204-211.
- Heins M, Heil W, Withold W. Storage of Serum or Whole Blood Samples? Effects of Time and Temperature on 22 Serum Analytes. Eur J Clin Chem Clin Biochem 1995;33:231-238.
- Schumann G, Bonora R, Ceriotti F, et al. IFCC Primary Reference Procedures for the Measurement of Catalytic Activity Concentrations of Enzymes at 37 °C – Part 4. Reference Procedure for the Measurement of Catalytic Activity Concentration of Alanine Aminotransferase. Clin Chem Lab Med 2002;40(7):718-724.
- Glick MR, Ryder KW, Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. Clin Chem 1986;32:470-475.
- Breuer J. Report on the Symposium "Drug Effects in Clinical Chemistry Methods". Eur J Clin Chem Clin Biochem 1996;34:385-386.
- Sonntag O, Scholer A. Drug interference in clinical chemistry: recommendation of drugs and their concentrations to be used in drug interference studies. Ann Clin Biochem 2001;38:376-385.
- Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. Clin Chem Lab Med 2007;45(9):1240-1243.
- Thefeld W, Hoffmeister H, Busch EW, et al. Referenzwerte für die Bestimmungen der Transaminasen GOT und GPT sowie der alkalischen Phosphatase im Serum mit optimierten Standardmethoden. Dtsch Med Wschr 1974;99(8):343-351.
- Klein G, Lehmann P, Michel E, et al. Vergleich der IFCC-Methoden für ALAT, ASAT und GGT bei 37 °C mit den eingeführten Standardmethoden bei 25 °C und 37 °C. Lab Med 1994;18:403-404.
- Zawta B, Klein G, Bablok W. Temperaturumrechnung in der klinischen Enzymologie? Klin Lab 1994;40:23-32.
- Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols used):

	Contents of kit
	Volume after reconstitution or mixing
	Global Trade Item Number

FOR US CUSTOMERS ONLY: LIMITED WARRANTY

Roche Diagnostics warrants that this product will meet the specifications stated in the labeling when used in accordance with such labeling and will be free from defects in material and workmanship until the expiration date printed on the label. THIS LIMITED WARRANTY IS IN LIEU OF ANY OTHER WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR PARTICULAR PURPOSE. IN NO EVENT SHALL ROCHE DIAGNOSTICS BE LIABLE FOR INCIDENTAL, INDIRECT, SPECIAL OR CONSEQUENTIAL DAMAGES.

COBAS, COBAS C, PRECICONTROL, PRECINORM and PRECIPATH are trademarks of Roche.

All other product names and trademarks are the property of their respective owners.

0005850797190c701V8.0

ALT

Alanine Aminotransferase acc. to IFCC without pyridoxal phosphate activation

Additions, deletions or changes are indicated by a change bar in the margin.

© 2020, Roche Diagnostics

 0123



Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com

+800 5505 6606



Distribution in USA by:
Roche Diagnostics, Indianapolis, IN
US Customer Technical Support 1-800-428-2336

cobas[®]