


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

REF		CONTENT		Analyzer(s) on which cobas c pack(s) can be used
05850797190	05850797500	Alanine Aminotransferase acc. to IFCC (1100 tests)	System-ID 05 7501 0	cobas c 701/702

Materials required (but not provided):

10759350190	Calibrator f.a.s. (12 x 3 mL)	Code 401	
12149435122	Precinorm U plus (10 x 3 mL)	Code 300	
12149443122	Precipath U plus (10 x 3 mL)	Code 301	
05117003190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 391	
05947626190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 391	
05117216190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 392	
05947774190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 392	
05172152190	Diluent NaCl 9 % (119 mL)	System-ID 08 6869 3	

English**System information****ALT**: ACN 8685**Intended use**In vitro test for the quantitative determination of alanine aminotransferase (ALT) in human serum and plasma on **cobas c** systems.**Summary**

Alanine aminotransferase (ALT) measurements, performed with this device, in human serum and plasma are used as an aid in diagnosis of hepatocellular injury and in monitoring chronic liver injury.

The enzyme alanine aminotransferase (ALT) is present in highest concentrations in the liver, in the cytosol of the hepatocytes, although it is also found in the kidney, and, in much smaller quantities, in heart and skeletal muscle cells.¹ ALT catalyzes the transfer of amino groups from L-alanine to α -ketoglutarate, resulting in the converted products L-glutamate and pyruvate. This is a critical process of the tricarboxylic acid cycle, in which the coenzyme pyridoxal phosphate (also known as pyridoxal-5-phosphate or active vitamin B6) is required. When liver injury occurs, ALT is released from injured liver cells and causes a significant serum elevation.¹ Measurement of ALT activity is therefore used for the diagnosis of hepatic diseases such as acute and chronic viral hepatitis, nonalcoholic fatty liver disease (NAFLD), alcohol-related liver disease, ischemic hepatopathy, autoimmune hepatitis, biliary injury, suspected malignant infiltration, cholestasis.¹ Serum elevations of ALT activity are rarely observed in conditions other than parenchymal liver disease.² In addition, ALT testing is recommended for monitoring chronic hepatitis status and progression.³

Although both serum aspartate aminotransferase (AST) and ALT become elevated whenever disease processes affect liver cell integrity, evidence suggests that ALT is a more specific marker of hepatic injury than AST. Moreover, elevations of ALT activity persist longer than elevations of AST activity.^{1,4}

In patients with vitamin B6 deficiency (insufficient endogenous pyridoxal phosphate), serum aminotransferase activity may be decreased. The addition of pyridoxal phosphate to this assay causes an increase in aminotransferase activity (activation higher for AST than for ALT). Pyridoxal phosphate activation prevents falsely low aminotransferase activity in patient samples with insufficient endogenous pyridoxal phosphate (vitamin B6 deficiency).⁵

Test principle

This assay follows the recommendations of the IFCC, but was optimized for performance and stability.^{6,7}

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): $\geq 45 \mu\text{kat/L}$; stabilizers; preservative

R3 2-Oxoglutarate: 94 mmol/L; NADH: $\geq 1.7 \text{ mmol/L}$; 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.

Reagent handling

Ready for use

Storage and stability

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 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.

Alanine Aminotransferase acc. to IFCC without pyridoxal phosphate activation

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

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

Freeze only once.

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 .⁵

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

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 ± 10 % of initial value at an ALT activity of 30 U/L (0.5 µkat/L).

Icterus:⁹ No significant interference up to an I index of 60 (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).

Hemolysis:⁹ 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):⁹ 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.

Drugs: No interference was found at therapeutic concentrations using common drug panels.^{10,11}

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.¹²

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 **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)

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 values¹³

Acc. to the optimized standard method (comparable to the IFCC method without pyridoxal phosphate activation¹⁴):

Males up to 41 U/L (up to 0.68 µkat/L)

Alanine Aminotransferase acc. to IFCC without pyridoxal phosphate activation

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.¹⁵

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:

Repeatability	Mean	SD	CV
	U/L (μ kat/L)	U/L (μ kat/L)	%
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
Intermediate precision	Mean	SD	CV
	U/L (μ kat/L)	U/L (μ kat/L)	%
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 **cobas c 501** analyzer.

Method comparison

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

Sample size (n) = 69

Passing/Bablok ¹⁶	Linear regression
$y = 0.976x + 1.61$ U/L	$y = 0.971x + 2.82$ U/L
$\tau = 0.985$	$r = 0.999$

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

References

- Kim WR, Flamm SL, Di Bisceglie AM, et al. Public Policy Committee of the American Association for the Study of Liver Disease. Serum activity of alanine aminotransferase (ALT) as an indicator of health and disease. *Hepatology* 2008 Apr;47(4):1363-1370. doi: 10.1002/hep.22109.
- Panteghini M, Bais R. Amino Transferases. In: Burtis CA, Ashwood ER, Bruns DE. *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics*. 5th ed. 2012;573-576.
- Guidelines for the Prevention, Care and Treatment of Persons with Chronic Hepatitis B Infection. Geneva: World Health Organization; 2015 Mar.
- Kwo PY, Cohen SM, Lim JK. ACG Clinical Guideline: Evaluation of Abnormal Liver Chemistries. *Am J Gastroenterol* 2017 Jan;112(1):18-35. doi: 10.1038/ajg.2016.517.


- Schumann G, Bonora R, Ceriotti F, et al. IFCC primary reference procedures for the measurement of catalytic activity concentrations of enzymes at 37 degrees C. International Federation of Clinical Chemistry and Laboratory Medicine. Part 4. Reference procedure for the measurement of catalytic concentration of alanine aminotransferase. *Clin Chem Lab Med* 2022 Jul;40(7):718-724. doi: 10.1515/CCLM.2002.124.
- Bergmeyer HU, Hørdler 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.
- 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):

CONTENT	Contents of kit
	Volume for reconstitution
GTIN	Global Trade Item Number

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

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

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

© 2023, Roche Diagnostics

05850797500V10.0

ALT

Alanine Aminotransferase acc. to IFCC without pyridoxal phosphate activation

CE 0123



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

+800 5505 6606



cobas[®]