

Alanine Aminotransferase acc. to IFCC with pyridoxal phosphate activation**Order information**

REF	CONTENT	Analyzer(s) on which cobas c pack(s) can be used
04467388 190	Alanine Aminotransferase acc. to IFCC 275 tests	System-ID 07 6858 8 Roche/Hitachi cobas c 311 , cobas c 501/502
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
10171743 122	Precinorm U (20 x 5 mL)	Code 300
10171735 122	Precinorm U (4 x 5 mL)	Code 300
10171778 122	Precipath U (20 x 5 mL)	Code 301
10171760 122	Precipath U (4 x 5 mL)	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
04489357 190	Diluent NaCl 9 % (50 mL)	System-ID 07 6869 3

English**System information**

For **cobas c 311/501** analyzers:

ALTLP: ACN 684

For **cobas c 502** analyzer:

ALTPM: ACN 8681

Intended use

In vitro test for the quantitative determination of alanine aminotransferase (ALT) with pyridoxal phosphate activation 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.

The addition of pyridoxal phosphate to the assay causes an increase in aminotransferase activity. The activation is 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).

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⁺. Pyridoxal phosphate serves as a coenzyme in the amino transfer reaction. It ensures full enzyme activation.



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

R2 Pyridoxal phosphate: 730 µmol/L; additives; preservative

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

R1 is in position A, R2 is in position B, and R3 is in position C.

Precautions and warnings

For in vitro diagnostic use.

Exercise the normal precautions required for handling all laboratory reagents.

Disposal of all waste material should be in accordance with local guidelines. 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**ALTLP**

Shelf life at 2-8 °C:

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

On-board in use and refrigerated on the analyzer:

12 weeks

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:

12 weeks

Alanine Aminotransferase acc. to IFCC with pyridoxal phosphate activation**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.

Centrifuge samples containing precipitates before performing the assay.

Stability: 3 days at 15-25 °C^{5,6}
 7 days at 2-8 °C^{5,6}
 > 7 days at (-60)-(-80) °C⁶

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 311 test definition**

Assay type	Rate A		
Reaction time / Assay points	10 / 29-57		
Wavelength (sub/main)	700/340 nm		
Reaction direction	Decrease		
Units	U/L (µkat/L)		
Reagent pipetting	Diluent (H ₂ O)		
R1	59 µL	32 µL	
R2	20 µL	–	
R3	20 µL	20 µL	
<i>Sample volumes</i>	<i>Sample</i>	<i>Sample dilution</i>	
		<i>Sample</i>	<i>Diluent (NaCl)</i>
Normal	9 µL	–	–
Decreased	9 µL	15 µL	135 µL
Increased	9 µL	–	–

cobas c 501 test definition

Assay type	Rate A		
Reaction time / Assay points	10 / 44-70		
Wavelength (sub/main)	700/340 nm		
Reaction direction	Decrease		
Units	U/L (µkat/L)		
Reagent pipetting	Diluent (H ₂ O)		
R1	59 µL	32 µL	
R2	20 µL	–	
R3	20 µL	20 µL	

<i>Sample volumes</i>	<i>Sample</i>	<i>Sample dilution</i>	
		<i>Sample</i>	<i>Diluent (NaCl)</i>
Normal	9 µL	–	–
Decreased	9 µL	15 µL	135 µL
Increased	9 µL	–	–

cobas c 502 test definition

Assay type	Rate A	
Reaction time / Assay points	10 / 44-70	
Wavelength (sub/main)	700/340 nm	
Reaction direction	Decrease	
Units	U/L (µkat/L)	
Reagent pipetting	Diluent (H ₂ O)	
R1	59 µL	32 µL
R2	20 µL	–
R3	20 µL	20 µL

<i>Sample volumes</i>	<i>Sample</i>	<i>Sample dilution</i>	
		<i>Sample</i>	<i>Diluent (NaCl)</i>
Normal	9 µL	–	–
Decreased	9 µL	15 µL	135 µL
Increased	18 µL	–	–

Calibration

Calibrators	S1: H ₂ O S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	2-point calibration <ul style="list-style-type: none"> • 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 .⁷

Quality control

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

In addition, other suitable control material can be used.

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

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.

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 35 U/L (0.58 µkat/L).

Icterus:⁸ No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).

Hemolysis:⁹ No significant interference up to an H index of 170 (approximate hemoglobin concentration: 106 µmol/L or 170 mg/dL). Contamination with erythrocytes will elevate results, because the analyte level in erythrocytes is higher than in normal sera. The level of interference

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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. Choose diluted sample treatment for automatic rerun.

Drugs: No interference was found at therapeutic concentrations using common drug panels.^{9,10}

Exception: Calcium dobesilate and Isoniazid can cause artificially low and Furosemide artificially high 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.¹¹

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. The latest version of the carry-over evasion list can be found with the NaOHD-SMS-SmpCln1+2-SCCS Method Sheets. For further instructions refer to the operator's manual. **cobas c** 502 analyzer: All special wash programming necessary for avoiding carry-over is available via the **cobas** link, manual input is not required.

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).

Expected values

Acc. to IFCC/Standard Method 94 with pyridoxal phosphate activation measured at 37 °C:¹²

Males	10-50 U/L	(0.17-0.83 µkat/L)
Females	10-35 U/L	(0.17-0.58 µkat/L)

Consensus values with pyridoxal phosphate activation:¹³

Males	up to 50 U/L	(up to 0.83 µkat/L)
Females	up to 35 U/L	(up to 0.58 µkat/L)

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 with repeatability (n = 21) and 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>		
Precinorm U	42.1 (0.70)	0.6 (0.01)		1.3
Precipath U	124 (2.07)	1 (0.01)		0.5
Human serum 1	122 (2.03)	1 (0.01)		0.6
Human serum 2	7.33 (0.12)	0.94 (0.02)		12.9
<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>		
Precinorm U	41.9 (0.70)	0.7 (0.01)		1.6
Precipath U	124 (2.07)	1 (0.02)		1.0
Human serum 3	26.3 (0.44)	0.8 (0.01)		2.9
Human serum 4	122 (2.03)	4 (0.07)		3.6

Method comparison

ALT values for human serum and plasma samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined using the corresponding reagent on a Roche/Hitachi 917 analyzer (x).

Sample size (n) = 198

Passing/Bablok ¹⁴	Linear regression
y = 1.000x - 0.438 U/L	y = 0.994x - 1.72 U/L
τ = 0.931	r = 0.995

The sample activities were between 5.10 and 469 U/L (0.085 and 7.83 µkat/L).

References

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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.

Symbols

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

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

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