

Alanine Aminotransferase - Pyridoxal Phosphate Activated**Order information**

REF	CONTENT	Analyzer(s) on which cobas c pack(s) can be used
20764957 322	Alanine Aminotransferase (500 tests)	System-ID 07 6495 7 COBAS INTEGRA 400 plus COBAS INTEGRA 800
10759350 190	Calibrator f.a.s. (12 × 3 mL)	System-ID 07 3718 6
10759350 360	Calibrator f.a.s. (12 × 3 mL, for USA)	System-ID 07 3718 6
12149435 122	Precinorm U plus (10 × 3 mL)	System-ID 07 7999 7
12149435 160	Precinorm U plus (10 × 3 mL, for USA)	System-ID 07 7999 7
12149443 122	Precipath U plus (10 × 3 mL)	System-ID 07 8000 6
12149443 160	Precipath U plus (10 × 3 mL, for USA)	System-ID 07 8000 6
10171743 122	Precinorm U (20 × 5 mL)	System-ID 07 7997 0
10171735 122	Precinorm U (4 × 5 mL)	System-ID 07 7997 0
10171778 122	Precipath U (20 × 5 mL)	System-ID 07 7998 9
10171760 122	Precipath U (4 × 5 mL)	System-ID 07 7998 9
05117003 190	PreciControl ClinChem Multi 1 (20 × 5 mL)	System-ID 07 7469 3
05947626 190	PreciControl ClinChem Multi 1 (4 × 5 mL)	System-ID 07 7469 3
05947626 160	PreciControl ClinChem Multi 1 (4 × 5 mL, for USA)	System-ID 07 7469 3
05117216 190	PreciControl ClinChem Multi 2 (20 × 5 mL)	System-ID 07 7470 7
05947774 190	PreciControl ClinChem Multi 2 (4 × 5 mL)	System-ID 07 7470 7
05947774 160	PreciControl ClinChem Multi 2 (4 × 5 mL, for USA)	System-ID 07 7470 7
20764965 322	Pyridoxal Phosphate (10 × 10 mL)	System-ID 07 6496 5

English**System information**

Test ALTPL, test ID 0-595

Intended use

In vitro test for the quantitative determination of the catalytic activity of ALT (EC 2.6.1.2; L-alanine: 2-oxoglutarate aminotransferase) in human serum and plasma on COBAS INTEGRA systems.

This method sheet describes the application for ALT activated by pyridoxal phosphate (test ALTPL, 0-595). The application for ALT without pyridoxal phosphate is described in the method sheet Alanine Aminotransferase.

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.

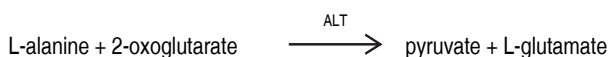
Although both serum aspartate aminotransferase (AST) and ALT become elevated whenever disease processes affect liver cell integrity, ALT is the more liver-specific enzyme. Moreover, elevations of ALT activity persist longer than elevations of AST activity.

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

Method according to the International Federation of Clinical Chemistry (IFCC), with pyridoxal-5'-phosphate.^{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 at 340 nm.

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

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

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

Precautions and warnings

Pay attention to all precautions and warnings listed in Section 1 / Introduction of this Method Manual.

Reagent handling

Ready for use

Storage and stability

Shelf life at 2-8 °C

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

COBAS INTEGRA 400 plus system

On-board in use at 10-15 °C

12 weeks

COBAS INTEGRA 800 system

On-board in use at 8 °C

12 weeks

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 (free from hemolysis)

Plasma (free from hemolysis): Li-heparin or EDTA plasma. Do not use other anticoagulants.

Nonhemolyzed serum is the specimen of choice.

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 -70 °C⁶

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

Pyridoxal Phosphate, Cat. No. 20764965 322, system-ID 07 6496 5 The pyridoxal phosphate solution is ready to use and is placed in its predefined rack position.

One bottle is sufficient for about 500 tests and stable on-board for 8 weeks.

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.

Application for serum and plasma**COBAS INTEGRA 400 plus test definition**

Measuring mode	Absorbance
Abs. calculation mode	Kinsearch
Reaction mode	R1-SD•S-SR
Reaction direction	Decrease
Wavelength A/B	340/378 nm
Calc. first/last	39/64
Unit	U/L

Pipetting parameters

		Diluent (H ₂ O)
R1	59 µL	10 µL
Sample	11 µL	8 µL
Special diluent (SD)	18 µL	
SR	17 µL	9 µL
Total volume	132 µL	

COBAS INTEGRA 800 test definition

Measuring mode	Absorbance
Abs. calculation mode	Kinsearch
Reaction mode	R1-SD/S-SR
Reaction direction	Decrease
Wavelength A/B	340/378 nm
Calc. first/last	54/97
Unit	U/L

Pipetting parameters

		Diluent (H ₂ O)
R1	59 µL	10 µL
Sample	11 µL	
Special diluent (SD)	18 µL	8 µL
SR	17 µL	9 µL
Total volume	132 µL	

Calibration

Calibrator	Calibrator f.a.s. Use deionized water as zero calibrator.
Calibration mode	Linear regression
Calibration replicate	Duplicate recommended
Calibration interval	Each lot and as required following quality control procedures

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

Reference range	Precinorm U, Precinorm U plus or PreciControl ClinChem Multi 1
Pathological range	Precipath U, Precipath U plus or PreciControl ClinChem Multi 2
Control interval	24 hours recommended
Control sequence	User defined
Control after calibration	Recommended

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 INTEGRA analyzers automatically calculate the analyte activity of each sample. For more details, please refer to Data Analysis in the Online Help (COBAS INTEGRA 400 plus/800 analyzers).

Conversion factor: $U/L \times 0.0167 = \mu\text{kat/L}$

Limitations - interference

Criterion: Recovery within $\pm 10\%$ of initial value.

Serum, plasma

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:⁸ No significant interference up to an H index of 130 (approximate hemoglobin concentration: 81 µmol/L or 130 mg/dL).

Lipemia (Intralipid):⁸ 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 specimens may cause > Abs flagging. Choose diluted sample treatment for automatic rerun.

Anticoagulants: Citrate and fluoride inhibit the enzyme activity.

Drugs: No interference was found at therapeutic concentrations using common drug panels.^{9,10} Exceptions: Calcium dobesilate and doxycycline HCl cause artificially low ALT values at the tested drug level. Isoniazid can cause artificially low ALT results at therapeutic concentrations. Furosemid can cause artificially high ALT results at therapeutic concentrations. Hydroxocobalamin (Cyanokit) may cause false-low 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 COBAS INTEGRA

analyzers. Refer to the Method Manual, Introduction, Extra Wash Cycles for further instructions.

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

Limits and ranges

Measuring range

2-700 U/L (0.03-11.7 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:
2 U/L (0.03 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 a zero sample (zero sample + 3 SD, repeatability, n = 30).

Expected values

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

Males	10-50 U/L	(0.167-0.835 µkat/L)
Females	10-35 U/L	(0.167-0.585 µkat/L)

Consensus values with pyridoxal phosphate activation:¹³

Males	up to 50 U/L	(up to 0.835 µkat/L)
Females	up to 35 U/L	(up to 0.585 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 COBAS INTEGRA 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 and intermediate precision (2 aliquots per run, 2 runs per day, 20 days). The following results were obtained:

	Level 1	Level 2
Mean	40.6 U/L (0.678 µkat/L)	132 U/L (2.20 µkat/L)
CV repeatability	1.5 %	0.7 %
CV intermediate precision	2.0 %	1.3 %

Method comparison

ALT values for human serum and plasma samples obtained on a COBAS INTEGRA 700 analyzer using the COBAS INTEGRA Alanine Aminotransferase reagent ALT and pyridoxal phosphate as special diluent (y) were compared with those determined using commercially available reagents for ALT (with pyridoxal phosphate) on a COBAS INTEGRA analyzer (x) and on an alternative manufacturer's clinical chemistry system (x). Samples were measured in duplicate. Sample size (n) represents all replicates.

COBAS INTEGRA analyzer

Sample size (n)	228
Correlation coefficient	(r) 1.00 (r _s) 0.998
Linear regression	y = 1.061x - 2.32 U/L
Passing/Bablok ¹⁴	y = 1.048x - 1.82 U/L

Alternative system

Sample size (n)	222
Correlation coefficient	(r) 0.999 (r _s) 0.997
Linear regression	y = 1.032x + 0.318 U/L
Passing/Bablok ¹⁴	y = 1.028x + 0.547 U/L

The sample activities were between 4.1 and 446 U/L (0.068 and 7.45 µkat/L).

References

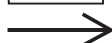
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A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard.

CONTENT



Contents of kit

Volume after reconstitution or mixing

ALTL**Alanine Aminotransferase - Pyridoxal Phosphate Activated****cobas[®]**
Enzymes**FOR US CUSTOMERS ONLY: LIMITED WARRANTY**

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