

ALT (ALAT/GPT)

Alanine Aminotransferase Kit (IFCC Method), without pyridoxal phosphate activation

Order Information

Cat. No.	Package size	Model
ALT-400	R1 8×62 mL+R2 8×18 mL	BS-400
ALT-300	R1 8×45 mL+R2 8×14 mL	BS-300
ALT-200	R1 8×38 mL+R2 8×12 mL	BS-200/BS-120

Intended use

ALT reagent is intended for quantitative determination of Alanine Aminotransferase activity in serum or plasma on photometric systems.

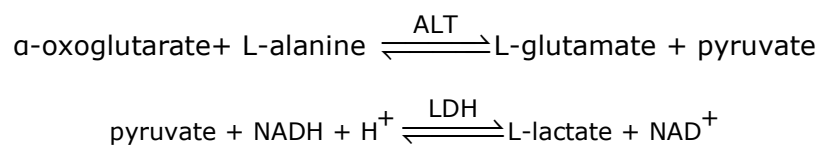
Summary^{1, 2}

Alanine aminotransferase (EC 2.6.1.2, ALT), formerly called Glutamic Pyruvic Transaminase (GPT), is one of liver-specific enzymes. It can catalyze the interconversion of amino acids and α -ketoacids by transfer of amino groups. Elevated ALT levels can indicate myocardial infarction, muscular dystrophy, especially in hepatobiliary diseases. Measurement of ALT is often used in diagnosis and monitoring treatment of liver diseases and heart diseases. The AST/ALT ratio is often used for differential diagnosis in liver diseases: if the AST/ALT ratio < 1, it indicates mild liver damage; otherwise it is associated with severe, often chronic liver diseases.

Method³

UV-assay according to IFCC (International Federation of Clinical Chemistry and Laboratory Medicine) without pyridoxal phosphate activation.

Reaction Principle



Alanine aminotransferase catalyzes the reversible transamination of L-alanine and α -oxoglutarate to pyruvate and L-glutamate. The pyruvate is then reduced to lactate in the presence of lactate dehydrogenase (LDH) with the concurrent oxidation of reduced β -nicotinamide adenine dinucleotide (NADH) to β -nicotinamide adenine dinucleotide (NAD). This change in absorbance is directly proportional to the activity of ALT in the sample.

Reagent

Components and Concentrations

R 1	TRIS buffer	150 mmol/L
	L-Alanine	750 mmol/L
	LDH	≥ 1200 U/L
R 2	α -oxoglutarate	90 mmol/L

	NADH	0.9 mmol/L
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Warnings and Precautions

1. For in vitro diagnostic use only.
2. Take the necessary precautions for the use of laboratory reagents.
3. Sodium azide contained. Do not swallow. Avoid contact with skin and mucous membranes.
4. Disposal of all waste material should be in accordance with local guidelines.
5. Material safety data sheet available on request for professional users.

Reagent Preparation

R1 and R2 are ready to use.

Storage and Stability

Stable up to expiry date indicated on the label, when stored unopened at 2°C–8°C and protected from light.

Once opened, the reagent is stable for 28 days when refrigerated on the analyzer or refrigerator.

Contamination of the reagents must be avoided.

Do not freeze the reagents.

Reagent Blank Absorbance

The absorbance of reagent blank at 340 nm should be ≥ 1.0 A.

Reagent Blank Rate

To determine the reagent blank rate, the sample is replaced by physiological saline (9 g/L NaCl). If the reagent blank rate ($\Delta A/s$) exceeds 0.0005, the measurements must be repeated and if necessary the reagent solution should be discarded.

Materials required but not provided

1. Calibrator and controls as indicated below.
2. NaCl solution 9 g/L.
3. General laboratory equipment.

Specimen collection and preparation

1. Serum, heparin plasma or EDTA plasma is suitable for sample. Whole blood, hemolysis and urine are not recommended for use as a sample. Freshly drawn serum or plasma is preferred.
2. Use the suitable tubes or collection containers and follow the manufacturer's instruction, avoid the effect of the materials of the tubes or collection containers.
3. Centrifuge samples containing precipitate before performing the assay.
4. Stability:
 - 4 hours at 15°C–25°C
 - 5-12 hours at 2°C–8°C
 - 2 days at (-15)°C–(-20)°C.

Assay procedure

	Blank	Sample
Reagent 1	1000 µL	1000 µL
Dist water	100 µL	–
Sample	–	100 µL
Mix, incubate for 5 min, then add:		
Reagent 2	250 µL	250 µL
Mix thoroughly, read the absorbance after 1 min and monitor time. Read the absorbance again for additional 3 min.		
$\Delta A/\text{min} = [\Delta A/\text{min sample}] - [\Delta A/\text{min blank}]$		

Application sheets for BS series analyzers are available in this document. Please refer to the appropriate operation manual for the analyzer-specific assay instructions.

Calibration

1. It is recommended to use the Human multi-calibrator from Randox for calibration. Traceability of the multi-calibrator can refer to the calibrator instructions for use of Randox Company.
2. Calibration frequency:
After reagent lot changes.
As required following quality control procedures.

Quality control

At least two levels of control material should be analyzed with each batch of samples. These controls should be run with each new calibration, each new reagent cartridge and after specific maintenance or troubleshooting procedures as detailed in the appropriate system manual.

It is recommended to use the Human Assayed Control level II and III from Randox to verify the performance of the measurement procedure.

Each laboratory should establish its own internal quality control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

Calculation

The analyzer calculates the activity of each sample automatically with a specified valid calibration factor from calibration process.

Conversion factor of traditional units (U/L) into SI-units (µkat/L):

$$1 \text{ U/L} = 16.67 \times 10^{-3} \text{ µkat/L,}$$

$$1 \text{ µkat/L} = 60 \text{ U/L}$$

Reference Intervals

Each laboratory should establish its own reference intervals based upon its patient population. The reference intervals measured at 37 °C listed below were taken from literature ³.

Sample Type	Conventional Units	S.I.Units
Male	≤45 U/L	≤0.75 µkat/L
Female	≤34 U/L	≤0.57 µkat/L

Performance Characteristics

Representative performance data obtained from Mindray system is given below. Results may vary if a different instrument, individual laboratory or a manual procedure is used.

Interferences/Specificity

The following substances were tested for interference with this methodology. Criterion: Recovery within $\pm 10\%$ of initial value.

Substance	Level Tested	Observed Effect
Ascorbic acid	30 mg/dL	NSI*
Bilirubin	40 mg/dL	NSI
Hemoglobin	500 mg/dL	NSI
Lipemia	500 mg/dL	NSI

* NSI: No Significant Interference (within $\pm 10\%$)

Reportable Range

The Mindray System (Mindray BS series analyzers / Mindray ALT Reagent) provides the following analytical ranges:

Sample Type	Conventional Units	S.I.Units
Serum / Plasma	4–500 U/L	0.07–8.33 μ kat/L

If the value of sample exceeds 500 U/L, the sample should be diluted with 9 g/L NaCl solution (e.g. 1+ 9) and repeat the assay using this dilution, the result should be multiplied by 10.

Sensitivity/Detection Limit

The lowest measurable ALT activity that can be distinguished from zero is 4 U/L (0.07 μ kat/L) with 99.7% confidence.

Precision

Precision performance using the NCCLS Approved Guideline EP5-A to assay serum control appears in the table below⁴.

Type of Precision	Control II			Control III		
	Mean (U/L)	SD (U/L)	CV%	Mean (U/L)	SD (U/L)	CV%
Within-run	32.5	0.62	1.91	124.6	0.75	0.60
Between-run		0.36	1.10		0.59	0.48
Between-day		1.25	3.85		3.08	2.47
Total		1.44	4.44		3.22	2.59

Method Comparison

A comparison between Mindray system (Mindray BS series analyzers /Mindray ALT reagent) (y) and Hitachi/Roche system (Hitachi /Roche ALT, IFCC) (x) using 40 samples gave following correlation (U/L): $y = 1.0508x + 1.5685$, $r = 0.999$. Details of the comparison experiments are available on request.

References

1. Thomas L. Alanine aminotransferase (ALT), Aspartate aminotransferase (AST). In:Thomas L, editor. Clinical

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- Laboratory Diagnostics. 1st ed. Frankfurt: TH-Books Verlagsgesellschaft, 1998:55-65.
2. Greiling H, Gressner AM, eds. Lehrbuch der Klinischen Chemie und Pathobiochemie, 3rd ed. Stuttgart/New York: Schattauer Verlag, 1995.
 3. Schumann G, Bonora R, Ceriotti F, et al. IFCC primary reference procedure for the measurement of catalytic activity concentrations of enzymes at 37°C. Part 4. Reference Procedure for the Measurement of Catalytic Concentration of Alanine Aminotransferase. Clin Chem Lab Med 2002;40:718-724.
 4. National Committee for Clinical Laboratory Standards, Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline, NCCLS publication EP5-A 1999, Vol.19, No.2.

Parameters for BS Series Analyzers

Parameters Screen		BS-120	BS-200	BS-300	BS-400
Reac. Type		Kinetics			
Direction		Decrease			
Unit		U/L			
Precision		0.1			
Pri. Wave		340			
Sec. Wave		405			412
Sample Volume (μL)		20			
R1 (μL)		200			
R2 (μL)		50			
Reac. Time	From	3	4	5	50
	to	13	15	20	69
Incuba. Time		16	8	10	/
Blank. Time		/			
Linearity Range	From	4			
	to	500			
Substrate Limit		5000			
Factor					
Calibration Screen					
Rule		Two point linear			
Replicates		3-5			



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