ALANINE AMINOTRANSFERASE (ALT/GPT)

COD 12533 5 x 40 mL + 5 x 10 mL

Only for *in vitro* use in the clinical laboratory

BioSystems

ALANINE AMINOTRANSFERASE (ALT/GPT)
IFCC

INTENDED USE

Reagent for the measurement of alanine aminostransferase (ALT or GPT) concentration in human serum or plasma. The obtained values are useful as an aid in the diagnosis and monitoring of disorders of liver diseases, especially acute ones.

This reagent is for use in the BioSystems A25 and A15 analyzers or in other analyzer with similar performance characteristics.

CLINICAL SIGNIFICANCE

The aminoatransferases catalyze the formation of glutamic acid from 2-oxoglutarate by transfer of amino groups. ALT is normally present in various tissues but its higher concentrations are found in liver and kidney.

The serum concentration of ALT is elevated in hepatitis and other forms of hepatic disease associated with necrosis: infectious mononucleosis, cholestasis, cirrhosis, metastasic carcinoma of the liver, delirium tremens, and after administration of various drugs, such as opiates, salycilates or ampicillin[1,2].

Serum ALT concentration can also be elevated in skeletal or cardiac muscle disease[1,2].

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

PRINCIPLE OF THE METHOD

Alanine aminotransferase (ALT or GPT) catalyzes the transfer of the amino group from alanine to 2-oxoglutarate, forming pyruvate and glutamate. The catalytic concentration is determined from the rate of decrease of NADH, measured at 340 nm, by means of the lactate dehydrogenase (LDH) coupled reaction[3-6].

$$\text{Alanine + 2 - Oxoglutarate} \xrightarrow{\text{ALT}} \text{Pyruvate + Glutamate}$$

$$\text{Pyruvate + NADH + H}^+ \xrightarrow{\text{LDH}} \text{Lactate + NAD}^+$$

CONTENTS AND COMPOSITION

A. Reagent: 5 x 40 mL. Tris 150 mmol/L, L-alanine 750 mmol/L, lactate dehydrogenase > 1350 U/L, pH 7.3.

B. Reagent: 5 x 10 mL. NADH 1.9 mmol/L, 2-oxoglutarate 75 mmol/L, sodium hydroxide 148 mmol/L, sodium azide 9.5 g/L.

WARNING: H302: Harmful if swallowed. EUH031: Contact with acids liberates toxic gas. P301+P312: IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell. P330: Rinse mouth.

For further warnings and precautions, see the product safety data sheet (SDS).

STORAGE AND STABILITY

Store at 2-8 ºC.

Components are stable once opened until the expiry date marked in the label if they are stored well closed and care is taken to prevent contamination during their use.

On board stability: Reagents open and kept in the refrigerated compartment of the analyzer are stable 15 days.

Indications of deterioration: Absorbance of the blank below the limit indicated in "Test Parameters".

ADDITIONAL MATERIALS REQUIRED (NOT PROVIDED)

- C. Reagent (cod 11667): Pyridoxal phosphate ALT 10 mmol/L. 5 mL.
- Biochemistry Calibrator (BioSystems cod. 18011) or Biochemistry Calibrator Human (BioSystems cod. 18044).

REAGENT PREPARATION

Reagents are provided ready to use.

Working Reagent: Pour the contents of the Reagent B into the Reagent A bottle. Mix gently. Other volumes can be prepared in the proportion: 4 mL Reagent A + 1 mL Reagent B. Stable for 1 month at 2-8ºC.

Working Reagent with Pyridoxal Phosphate (Note 1): Mix as follows: 10 mL of Working Reagent + 0.1 mL of Reagent C (cod 11666). Stable for 6 days at 2-8ºC. Stable for 6 days at 2-8ºC and for 6 days in the refrigerated compartment of the analyzer.

SAMPLES

Serum and plasma collected by standard procedures.

Alanine aminotransferase in serum and plasma is stable for 7 days at 2-8ºC. Use heparin or EDTA as anticoagulant[8].

CALIBRATION

A reagent blank should be done every day and a calibration at least every 15 days (6 days for Working Reagent with Pyridoxal Phosphate), after reagent lot change or as required by quality control procedures.

QUALITY CONTROL

It is recommended to use the Biochemistry Control Serum level I (cod. 18005, 18009 and 18042) and II (cod. 18007. 18010 and 18043) to verify the accuracy of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if control results are not within the acceptable limits.

REFERENCE VALUES

Reaction temperature	37ºC	30ºC
Without pyr-P, up to[3,6]	41 U/L = 0.68 µkat/L	29 U/L = 0.48 µkat/L
With pyr-P, up to[3,4]	65 U/L = 1.08 µkat/L	35 U/L = 0.58 µkat/L

These ranges are given for orientation only; each laboratory should establish its own reference ranges.

METROLOGICAL CHARACTERISTICS

The metrological characteristics described below have been obtained using an A25 analyzer. Results are similar with A15.

- Detection limit: 3.1 U/L = 0.05 µkat/L.
- Linearity limit: 500 U/L = 8.33 µkat/L.
- Precision:

Mean concentration	Repeatability (CV)	Within-laboratory (CV)
48 U/L = 0.80 µkat/L	1.4 %	2.5 %
208 U/L = 3.47 µkat/L	0.7 %	2.2 %

- Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents. Details of the comparison experiments are available on request.

LIMITATIONS OF THE PROCEDURE

- Interferences: bilirubin (up to 20 mg/dL), hemolysis (hemoglobin up to 1000 mg/dL) and lipemia (triglycerides up to 200 mg/dL) do not interfere. Other drugs and substances may interfere[7].

NOTES

1. The IFCC recommended method specifies the addition of pyridoxal phosphate.

BIBLIOGRAPHY

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2. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 4th ed. Burtis CA, Ashwood ER, Bruns DE. WB Saunders Co, 2005.
3. Sociedad Española de Química Clínica, Comité Científico, Comisión de Enzimas. Metódo recomendado para la determinación en rutina de la concentración catalítica de la alanina aminotransferasa en suero sanguíneo humano. *Quim Clin* 1987; 6: 241-244.
4. 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 concentration of alanine aminotransferase. *Clin Chem Lab Med* 2002; 40: 718-724.
5. IFCC reference procedures for measurement of catalytic concentrations of enzymes: corrigendum, notes and useful advice. *Clin Chem Lab Med* 2010; 48: 615-621.
6. Gella FJ, Olivella T, Cruz Pastor M, Arenas J, Moreno R, Durban R and Gómez JA. A simple procedure for routine determination of aspartate aminotransferase and alanine aminotransferase with pyridoxal phosphate. *Clin Chim Acta* 1985; 153: 241-247.
7. Young DS. Effects of drugs on clinical laboratory tests, 5th ed. AACC Press, 2000.
8. Word Health Organization (WHO). Use of anticoagulants in diagnostic laboratory investigations. Document WHO/DIL/LAB/99.1, Rev.2; 2002.

TEST PARAMETERS

These reagents may be used in several automatic analyzers. Specific instructions for application in many of them are available on request.

R1: use Reagent A, or Reagent A with Pyridoxal Phosphate, R2: use Reagent B.

	A25	A15
GENERAL		
Name	**ALT**	**ALT**
Sample type	SER	SER
Analysis mode	kinetic mon.	kinetic mon.
Units	U/L	U/L
Turbidimetry test	no	no
Decimals	0	0
Type of reaction	decreasing	decreasing
PROCEDURE		
Reading mode	monoch.	monoch.
Main filter	340	340
Reference filter	-	-
Sample	25	25
Vol. R1	300	300
Vol. R2	-	-
Washing	1.2	1.2
Reading 1 (cycle)	7 (12)*	5 (8)*
Reading 2 (cycle)	18	12
Reagent 2 (cycle)	-	-
Predilution factor	-	-
CALIBRATION AND BLANK		
Calibration type	multiple	multiple
Number of calibrators	-	-
Calibration curve	-	-
OPTIONS		
Blank absorbance limit	1.400	1.400
Kinetic blank limit	-	-
Linearity limit	500	500
Substrate depletion	0.5	0.5

*Application for Working Reagent with Pyridoxal Phosphate.

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