



ALT(SGPT) TEST

ALT

BCG Dye-Binding Method

INTENDED USE

For the quantitative determination of Alanine aminotransferase activity in serum.

INSTRUCTIONS AND CLINICAL SIGNIFICANCE

The enzyme Alanine aminotransferase (ALT or SGPT) – transfers the amino group from Alanine to α -ketoglutarate,

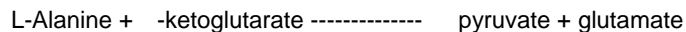
is widely distributed in animal tissue, with the highest concentration present in liver and kidney. Acute destruction of tissue results in the release of ALT into the blood. Serum ALT levels up to 1000 times normal have been observed in cases of acute hepatitis. Less elevated values have been associated with infectious mononucleosis, extra-hepatic obstructive jaundice and cirrhosis.

ALT activity in serum is usually determined by UV kinetic rate method based on the rate of NADH oxidation by a coupled Lactic dehydrogenase (LDH) reaction which converts the product pyruvate to Lactic acid and is proportion to the decrease in absorbance at 340 nm.

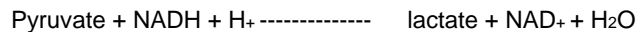
PRINCIPLE

ALT catalyzes the transfer of the amino group from L-Alanine to α -ketoglutarate. The pyruvate formed in this reaction is reduced to lactate in the presence of LDH with the concomitant oxidation of NADH to NAD⁺. The rate of decrease in absorbance at 340 nm of NADH is taken as a measure of ALT activity. The reaction formula is as follows:

ALT



LDH



SPECIMEN COLLECTION AND PREPARATION

Serum or plasma collected with heparin, oxalate, or citrate are suitable for ALT assay. The blood specimen should be drawn and handled carefully to avoid hemolysis. Hemolysis falsely elevates the ALT activity. Serum should be removed from the clot or cells without delay.

Serum ALT activity is not too stable and should be kept at 4 °C for short period frozen at -20 °C or lower for longer periods.

REAGENT

1. Package: R1: 2 x 40 ml R2: 2 x 10 ml Common

R1: 4 x 60 ml R2: 4 x 15 ml Hitachi 7170 or 917

R1: 4 x 80 ml R2: 4 x 20 ml Hitachi 7060 or 912

R1: 8 x 40 ml R2: 2 x 40 ml Hitachi 7020

R1: 4 x 50 ml R2: 2 x 25 ml Toshiba

Components: All components concentration refer to final reaction mixture are as follows:

L-Alanine: 500 mM; α -ketoglutarate: 12 mM;

NADH: 0.23mM; LDH: 1000 μ /l;

Tris buffer: pH 7.5; Stabilizers and preservatives.

Store at 2~8 °C

PRECAUTIONS

1. For in vitro diagnostic use only.



2. Since all specimens are potentially infectious, they should be handled with appropriate precautions and practices in accordance with Biosafety level 2 as recommended by USA NIH manual Biosafety in Microbiological and Biomedical Laboratories, and in accordance with National or local regulations related to the safety precautions of such materials.

3. Each laboratory has to perform the quality control test to assure the results being reliable before running the specimen tests.

PROCEDURE

Wavelength: 340 nm; Cuvette: 1 cm light path; Incubation: 37 .

Measure against water.

The reagents are ready-to-use .

1. Transfer 0.8ml of R1 and 0.2 ml of R2 into a clean cuvette and bring it to 37 .

2. Add 0.1 ml of serum and read absorbance after 1 minute incubation-Absorbance .

3. After another 1 minute, read the absorbance again-Absorbance .

4. Absorbance II - Absorbance I = A/min

5. The Hitachi reagents are used directly on Hitachi analyzers in accordant with Hitachi parameters.

RESULT CALCULATION

$$A/min \times 1000 \times 1.1$$

$$ALT \text{ activity (U/L)} = \text{-----} = A/min \times (-1768)$$

$$6.22 \times 0.1 \times 1.0$$

A/min: Change in absorbance per minute.

1000: convert ml to L.

1.1: reagent volume (1 ml) plus serum volume (0.1 ml).

0.1: serum volume.

6.22: milimolar absorptivity of NADH at 340 nm

EXPECTED VALUES

Up to 46 U/L (37)

Quality control: Frozen aliquots of pooled sera or commercially available quality control material of known activity should be assayed concurrently with patient specimens to ensure proper performance of the procedure.

Both a low and an elevated control serum must be assayed with each run to assure performance within acceptable limits.

PERFORMANCE

Specificity: A change in absorbance of 0.001 A per minute at 340 nm corresponds to 1.7 μ /l of ALT activity.

Linearity: 600U/L. Those specimens with ALT activities higher than this limit should be diluted with normal saline and re-assayed.

Precision:

	Within run		Between run	
	Level	Level	Level	Level
Samples				
Number n	20	20	20	20
Mean U/L	35	119	36	133
SD U/L	1.103	2.558	1.627	4.256
CV %	3.15	2.15	4.52	3.20

REFERENCES

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