台塑生醫科技股份有限公司
FORMOSA BIOMEDICAL TECHNOLOGY CORP.

# 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 pyuvate to Lactic acid and is proportion to the decrease in absorptance 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

L-Alanine + α-ketoglutarate ----------→ pyruvate + glutamate

LDH

Pyruvate + NADH + $H^+$ ----------→ lactate + $NAD^+$ + $H_2O$

**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℃ for short period frozen at -20℃ or lower for longer periods.

**REAGENT**

1. Package: R1: 2×40 ml R2: 2×10 ml Common
R1: 4×60 ml R2: 4×15 ml Hitachi 7170 or 917
R1: 4×80 ml R2: 4×20 ml Hitachi 7060 or 912
R1: 8×40 ml R2: 2×40 ml Hitachi 7020
R1: 4×50 ml R2: 2×25 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; Stabalizers and preservatives.
Store at 2~8 ℃

**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 Ⅰ.

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

4. Absorbance II - Absorbance I = △A/min

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

**RESULT CALCULATION**

$$\text{ALT activity (U/L)} = \frac{\triangle A/\text{min} \times 1000 \times 1.1}{6.22 \times 0.1 \times 1.0} = \triangle A/\text{min} \times (-1768)$$

△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 | |
|---|---|---|---|---|
| Samples | Level Ⅰ | Level Ⅱ | Level Ⅰ | Level Ⅱ |
| 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**

1. The Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology. 1974.

Recommended methods for the determination of four enzymes in blood. Scand J. Clinical Lab. Invest. 32:291.

2. Demetriou JA, Drewes pA, Gin JB, 1974. Enzymes. In Clinical Chem., Principles and Technics. 2 nd ed. RJ Henry. DC Cannon, JW Winkelman , Editors, Harper & Row, Hagerstown, Maryland.p. 879-888.

3. Fhenly KS and H.M. P0olland, 1955. J. Clinical. Med. 46:785

4. Worblewsk Fand J.S. LaDue, 1956. Proc. Soc. Exp Biol. Med 91: 569.

5. Young D.S., et.al. 1975. Clinical Chem. 21:1D.