



ALT (GPT)

Alanine aminotransferase

E.C.2.6.1.2.

UV Method (According to IFCC)

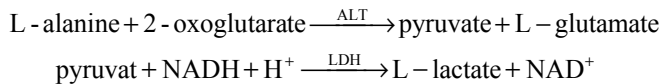
Cat.No. 101-0326 Size 20 x 2.5 ml

Cat.No. 101-0255 Size 10 x 10 ml

Cat.No. 101-0228 Size 8 x 100 ml

PRINCIPLE:

ALT catalyzes the transfer of amino group from L-alanine to 2-oxoglutarate resulting in the formation of pyruvate and L-glutamate. The pyruvate thus formed undergoes reduction with simultaneous oxidation of NADH to NAD in the lactate dehydrogenase catalyzed indicator reaction. Oxidation of NADH causes a decrease in absorbance at 340 nm (334 or 365 nm) and the rate of absorbance change is directly proportional to ALT activity.



SAMPLE:

Serum, heparinized or EDTA plasma
Stability 7 days at +2 °C to +8 °C.

REAGENTS:

- Reagent 1 (Buffer/Substrate)
 - Tris buffer pH 7.3 100 mmol/L
 - L-alanine 500 mmol/L
- Reagent 2 (Enzyme/Coenzyme/ α -oxoglutarate)
 - α -oxoglutarate 15 mmol/L
 - LDH ≥ 1200 U/L
 - NADH 0.18 mmol/L

Store at +2 °C to +8 °C.

REAGENT PREPARATION :

Dissolve the contents of Reagent 2 with the corresponding volume of Reagent 1 (buffer/substrate).

Working reagent is stable for 30 days at +2 °C to +8 °C or 4 days at +15 °C to +25 °C.

PROCEDURE:

Method:	Kinetic (decreasing)
Wavelength:	340, 334, 365 nm
Preincubation:	1 min
Temperature:	25 °C, 30 °C, 37 °C
Read:	every 60 sec. during 3 min.
Cuvette:	1 cm light path
Zero:	air or H ₂ O

Pipette into cuvette:	
Sample	0.1 ml
Working reagent	1.0 ml
Mix well, read initial absorbance after 1 min. Read again after every 60 s during 3 min. Calculate $\Delta A/\text{min}$.	

CALCULATION:

$$\frac{\Delta A/\text{min} \times 10^6 \times TV}{6.3 \times 10^3 \times l \times V} = \Delta A/\text{min} \times F = \text{U/L}$$

Where is :

ΔA	= change in absorbance
min	= minute
6.3×10^3	= molar absorptivity of NADH at 340 nm
10^6	= conversion of mol to μmol
l	= light path in cm
TV	= total reaction volume in ml
V	= sample volume in ml

$\Delta A_{340 \text{ nm}}/\text{min} \times 1746 = \text{U/L ALT}$

$\Delta A_{334 \text{ nm}}/\text{min} \times 1780 = \text{U/L ALT}$

$\Delta A_{365 \text{ nm}}/\text{min} \times 3235 = \text{U/L ALT}$

$\text{U/L} \times 16.67 = \text{nkat/l}$

EXPECTED VALUES:

	25 °C	30 °C	37 °C	UNIT
Men	up to 22 up to 367	up to 29 up to 483	up to 40 up to 667	U/L nkat/L
Women	up to 16 up to 267	up to 22 up to 367	up to 31 up to 517	U/L nkat/L

LINEARITY:

up to 270 U/L (4500 nkat/L)

NOTE:

- If the absorbance change per minute ≥ 0.150 at 340 nm and 334 nm or ≥ 0.08 at 365 nm dilute the sample 1:10 with physiological solution and reassay (result x 10).
- Avoid hemolysis as it interferes with assay.
- Solution 1 contains sodium azide. Avoid ingestion or contact with skin or mucous membranes.

QUALITY CONTROL:

CONTRO-N	20 x 5 ml	Cat. No. 101-0083
CONTRO-P	20 x 5 ml	Cat. No. 101-0084

REFERENCES:

- Bergmeyer, H.U. et al., Clin.Chem 24, 58-73, (1978).
- Bergmeyer H.U. and Horder M., Clin.Chem Acta 105, 147 F (1980).