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.

$$\text{L-alanine} + \text{2-oxoglutarate} \xrightarrow{\text{ALT}} \text{pyruvate} + \text{L-glutamate}$$

$$\text{pyruvat} + \text{NADH} + \text{H}^+ \xrightarrow{\text{LDH}} \text{L-lactate} + \text{NAD}^+$$

SAMPLE:

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

REAGENTS:

1. Reagent 1 (Buffer/Substrate)

Tris buffer pH 7.3	100 mmol/L
L-alanine	500 mmol/L

2. 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_2O

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 ΔA/min.	

CALCULATION:

$$\frac{\Delta A/\text{min} \times 10^6 \times TV}{6.3x10^3 \times l \times V} = \Delta A/\text{min} \times F = U/L$$

Where is :

ΔA	=	change in absorbance
min	=	minute
6.3 x 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

$\Delta A_{340\ nm}$/min x 1746 = U/L ALT
$\Delta A_{334\ nm}$/min x 1780 = U/L ALT
$\Delta A_{365\ nm}$/min x 3235 = U/L ALT

U/L x 16.67 = 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:

1. 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).
2. Avoid hemolysis as it interferes with assay.
3. 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:

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