Alanine aminotransferase Assay Kit (GPT/ALT)

Method: IFCC

Cat .No	Size	Instrument
GB000G	R1: 4×100 ml R2: 2×50 ml	For Hitachi 717 &ShimadzuCL7200/8000
GS001G	R1: 6×60 ml R2: 2×45 ml	For Hitachi917 &OlympusAU640/400/600
GH001G	R1: 2×50 ml R2: 1×25 ml	For Hitachi902
GX001G	R1: 2×80 ml R2: 2×40 ml	For SYNCHRON CX4-5-7-9/LX20/DXC600-800
GT001G	R1: 5×48 ml R2: 2×30 ml	For TOSHIBA

INTENDED USE

For the quantitative *in vitro* of alanine aminotransferase in human serum.

CLINICAL SIGNIFICANCE

Alanine Aminotransferase (ALT), also referred to as glutamate pyruvate transaminase (GPT), is an enzyme involved in amino acid metabolism. It is found in many tissues, and the highest levels are found in liver and kidney tissues. Tissues destruction leads to the release of the intracellular enzyme into the circulating blood. Markedly elevated serum ALT levels may be found in a variety of diseases which involve the liver, such as hepatitis, mononucleosis, and cirrhosis. These very high levels of ALT are not usually observed in other disease processes, e.g., myocardial infarction; thus, ALT is regarded as a reasonably specific indicator of liver disease.

ASSAY PRINCIPLES [1, 2, 3]

ALT present in the sample catalyzes the transfer of the amino group from L-alanine to α-ketoglutarate, forming pyruvate and L-glutamate. Pyruvate in the presence of NADH and lactate dehydrogenase is reduced to L-lactate. In this reaction NADH is oxidized to NAD. The reaction is monitored by measuring the rate of decrease in absorbance at 340 nm due to the oxidation of NADH to NAD.

$$\text{L-alanine} + \alpha\text{-ketoglutarate} \xrightarrow{\text{ALT}} \text{pyruvate} + \text{L-glutamate}$$

$$\text{pyruvate} + \text{NADH} + H^+ \xrightarrow{\text{LDH}} \text{L-lactate} + NAD^+ + H_2O$$

REAGENT COMPOSITION

Contents	Concentration of Solutions
Reagent 1 (R1)	
TrisBuffer (pH=7.50)	100 mmol/L
L-alanine	500 mmol/L
LDH	≥1200 U/L
Reagent 2 (R2)	
NADH	0.18 mmol/L
α-ketoglutarate	15 mmol/L

SAMPLE COLLECTION AND PREPARATION

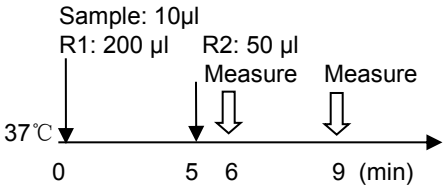
Serum samples.
Use fresh patient serum.
Serum samples are stable for a week at 2-8 ℃.

STABILITY AND PREPARATION OF REAGENTS

All reagents are ready to use.
Stable up to the expiry date when stored at 2-8℃.
The reagents are stable for 1 month on-board the analyzer after opening and kept at 2-8℃.

ASSAY PROCEDURE

Test Procedure for Analyzers (HITACHI 917)
Assay Mode: Rate A 20-34
Wave Length (main/sub): 340 nm/405 nm



1. Mix 10 μl sample with 200 μl R1 and incubate at 37℃ for 5 minutes.
2. Add 50 μl R2 into cuvette, mix and incubate at 37℃ for 1 minute.
3. Read initial absorbance and start timer simultaneously, read again after 1, 2 and 3 minutes.
4. Calculate absorbance change per minute (ΔA/min).

CALIBRATION

Recommend that this assay should be calibrated using Gcell calibration serum, calibration trace to JSCC-TS01. Randox calibration also can be used, Randox calibration choosing method: (IFCC Tris buffer no P5P/IFCC 37℃)

CALCULATION OF RESULTS

Calculation using calibration

$$\text{Concentration} = \frac{\Delta A_{\text{sample}}/\text{min}}{\Delta A_{\text{calibrator}}/\text{min}} \times \text{calibrator value}$$

Calculation using factor (ε=6.22)

$$\text{ALT (U/L)} = \frac{\Delta A/\text{min} \times V_t}{\varepsilon \times V_s \times L} \times 1000 = \Delta A/\text{min} \times K$$

K= 4180

QUALITY CONTROL

Randox Assayed Multisera are recommended for daily quality control. Values obtained should fall within a specified range. If these values fall outside the range, the following steps should be taken:

1. Check instrument settings and light source.
2. Check reaction temperature.
3. Check expiration date of kit and contents.

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NORMAL VALUE

	30℃	37℃
Men:	0-30 U/L	0-42 U/L
Women:	0-23 U/L	0-32 U/L

It is recommended that each laboratory establish its own reference range to reflect the age, sex, diet and geographical location of the population.

SPECIFIC PERFORMANCE CHARACTERISTICS

LINEARITY

The method is linear up to 1000 U/L. If the Sample above this concentration should be diluted it with 0.9% NaCl and repeat assay. Multiply the result by dilution factor.

PRECISION

The CV of the test should be less than 10%

Intra assay precision		
N=20	level 1	level 2
Mean(U/L)	39.3	141.5
SD	0.5	0.8
CV(%)	1.2	0.5

Inter assay precision			
N=5	Batch 1	Batch 2	Batch 3
Mean(U/L)	37.7	37.3	38.0
$\bar{x}$	37.7		
(Xmax-Xmin)/$\bar{x}$	(38.0-37.3)/37.7*100=1.77%		

INTERFERENCE

The following analytes were tested up to the levels indicated and found not to interfere:

Hemoglobin	up to 300 mg/dl
Intralipid	up to 300 mg/dl
Bilirubin	up to 40 mg/dl
Ascorbic Acid:	up to 50 mg/dl

SAFETY PRECAUTIONS AND WARNINGS

1. For in vitro diagnostic use only. Do not pipette by mouth. Exercise the normal precautions required for handling laboratory reagents.
2. Reagent contains Sodium Azide. Avoid ingestion or contact with skin or mucous membranes. In case of skin contact, flush affected area with copious amounts of water. In case of contact with eyes or if ingested, seek immediate medical attention.
3. Sodium Azide reacts with lead and copper plumbing, to form potentially explosive azides. When disposing of such reagents flush with large volumes of water to prevent azide from building up. Exposed metal surfaces should be cleaned with 10% sodium hydroxide.
4. Specimens should be treated as potentially infectious (HIV, Hepatitis B virus, Hepatitis C virus, etc.) and handled with appropriate caution.
5. Reagents with different lot numbers should not be interchanged or mixed.

REFERENCES

1. Wroblewski F, La Due J.S: Ann Intern Med. 1956; 45:801.
2. Wroblewski F, La Due J.S: Proc Soc Exp Biol Med 1956; 91:569.
3. Bergmeyer HU, Bowers GN Jr, et al: Clin Chem 1977; 23: 887.

INDEX OF SYMBOLS

	Manufacture
REF	Catalogue Number
LOT	Lot number
	Date of manufacture
	Use by(Expiration date)
IVD	For In-Vitro Diagnostic use only
2℃ 8℃	Stored at 2-8℃
	Attention:See instruction for use
EC REP	Authorized Representative in the European Company

Manufacture：Beijing Strong Biotechnology, Inc.
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