AutoPure®

Sphera System Packs

ALT (GPT) IFCC

Introduction

1. AutoPure ALT (GPT) is reagent kit for direct quantitative determination of alanine transaminase (ALT) in human serum and plasma on automated clinical chemistry analyzers.
2. AutoPure ALT (GPT) is a ready-to-use, two liquid reagent system.
3. With AutoPure ALT (GPT), the assay is linear upto 600 IU/l (10.02 µkat/l).

Principle

α- Ketoglutarate reacts with L – alanine in the presence of ALT (GPT) to form pyruvate and L – glutamate.

$$\alpha\text{-Ketoglutarate} + \text{L-alanine} \xrightarrow{\text{ALT (GPT)}} \text{Pyruvate} + \text{L-glutamate}$$

The increase in pyruvate is determined in an indicator reaction catalyzed by lactate dehydrogenase.

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{Lactate dehydrogenase}} \text{L-lactate} + \text{NAD}^+$$

The conversion of NADH to NAD^+ is directly proportional to the ALT (GPT) activity in the specimen and is measured kinetically.

Reagent Storage, Stability & Handling

AutoPure ALT (GPT) is a ready- to- use, two liquid reagent system.

Shelf life
Stable till the expiry date indicated on the label, when stored at $2^0 – 8^0$C.

On – Board Reagent Stability
R1: 30 days at $2^0 – 8^0$ C after opening.
R2 : 30 days at $2^0 – 8^0$ C after opening.
Protect the reagent from light and contamination.

Do not freeze the reagent.

Components & Concentration of Working Solution

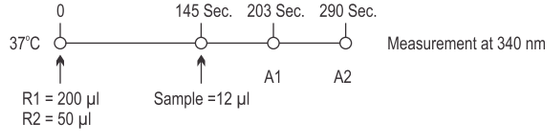
Component	Concentration
R1	
• Tris buffer	100 mmol/l
• L – alanine	⩾ 500 mmol/l
• Lactate dehydrogenase	⩾ 3000 IU/l
• Stabilizers, excipients & surface active agents.	
R2	
• Tris buffer	20 mmol/l
• NADH	⩾ 0.18 mmol/l
• α-Ketoglutarate	⩾ 10 mmol/l
• Stabilizers, excipients & surface active agents.	

Specimen Collection & Preservation

Collect sample using standard sampling tube. Although serum is preferred, heparinized plasma or EDTA plasma can be used. Specimen with any visible haemolysis is not acceptable. ALT (GPT) activity in serum/plasma is stable for 7 days at $2^0 – 8^0$C and 30 days at -10^0C. Centrifuge samples containing precipitate before performing the assay.

Procedure

AutoPure ALT (GPT) can be used on various automated analyzers. The procedure described below is for Sphera auto-analyzer.



Calculations

Fully automated system automatically calculates the ALT (GPT) activity of each sample.
Results in µkat/l = Results in IU/l x 0.0167

Application Sheet

Refer to the application sheet for details. For additional system applications, contact our local Accurex representative.

Calibration

For calibration, it is recommended to use C.f.a.s* or any other suitable calibrator material

Calibration frequency
Re - calibration is recommended

- Whenever the reagent lot is changed.
- As per the requirement of quality control procedures.

*C.f.a.s is brand name of Roche Diagnostics, Germany

Quality Control

Each batch of AutoPure ALT (GPT) is assayed with atleast six quality control sera – Precinorm**, Precipath**, Biorad I***, Biorad II***, Accutrol normal and Accutrol Abnormal prior to release▲.
To ensure the adequate quality control, it is recommended that the laboratory should use the normal and abnormal commercial reference control serum. It should be realized that the use of quality control material checks both the reagent and instrument functions together.
If the control values fall outside the specified limits, each of the below criteria should be cross-checked and corrected:

- Proper instrument function – wavelength setting, light source and temperature control.
- Cleanliness of probes and cuvettes.
- Bacterial contamination of wash water used by the instrument.
- Expiry date of the reagent kit.

**Brand name of Roche Diagnostics, Germany; ***Brand name of Biorad, USA; ▲subject to availability of control serum.

Expected Values

Serum/ Plasma
Men : Upto 41 IU/l (0.68 µkat/l)
Women : Upto 31 IU/l (0.52 µkat/l)

Note:

Expected range varies from population to population. It is therefore recommended that each laboratory should establish its own range. For diagnostic purposes, the ALT (GPT) results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Performance Characteristics

Linearity

With AutoPure ALT (GPT), the assay is linear upto 600 IU/l (10.02 µkat/l). Determine samples with higher concentrations via the rerun function. On instruments without rerun function, manually dilute samples with higher concentrations using 0.9% NaCl or distilled/deionized water (e.g.1 + 3). Multiply the result by the appropriate dilution factor (e.g. 4).

Interference

There is no significant interference in samples containing upto 60 mg/dl of bilirubin. Avoid haemolysis as it interferes with the assay.

Precision

Reproducibility was determined using quality control sera as shown below:

n=15

	Within run			Between run		
Quality Control Material	Mean IU/l	SD IU/l	%CV	Mean IU/l	SD IU/l	%CV
Low Control Serum	32.7	0.55	1.7	32.5	0.49	1.5
High Control Serum	115	1.27	1.1	115	1.05	0.9

Co - Relation Studies

A comparison of the ALT (GPT) determination using AutoPure ALT (GPT) and AutoZyme SGPT reagent gave the following co-relation (IU/l):

Linear Regression
y = 0.994x + 0.383
r = 0.9942
Sy.x = 0.86
No. of samples measured : 65
The sample concentrations measured were between 11 and 40 IU/l.

References

1. Tietz N.W., ed. ***Clinical Guide to Laboratory Tests***, 3rd ed. Philadelphia, Pa : W.B. Saunders , 1995 : 20 - 21.

2. Bergmeyer H.U., Horder M, Rej R. Approved recommendation. (1985) on IFCC methods for the measurement of catalytical concentration of enzymes, Part 3. IFCC method for L- alanine aminotransferase. ***J.Clin. Chem. Clin.Biochem*** 1986; 24 : 481 - 489.
3. Fischbach F, Zawta B. Age – dependent Reference Limits of Serveral Enzymes in Plasma at Different Measuring Temperatures. ***Clin Lab.*** 1992; 38 : 555 – 561.
4. Penttila , I.M. et al., Scand, ***J. Clin.Lab. Invest*** 35, 275 (1975).
5. Hafkenshelid, J. C.M. et al. ***J. Clin. Chem. Clin. Biochem.*** 17, 219 (1979).

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Application sheet

Designations

Code: ALT Name: GPT/ALT Type: Kinetic Group: ACCUREX

1 - Pipetting

Reagent 1 ID: ALT R1
Reag 1 bottle: Large
Reagent 2 ID: ALT R2
Reag 2 bottle: Small

	1st	2nd
Sample vol:	µl	12 µl
Reagent 1 vol:	200 µl	
Reagent 2 vol:	50 µl	µl
Diluent vol:	0 µl	

2 - Time

Incubation 1: 145 Sec
Incubation 2: 58 Sec
Reading: 87 Sec

3 - Wavelengths

Wavelength 1: 340 nm
Wavelength 2: 0 nm

4 - Washing

Needle: 1
Cuvette: 1

5 - Incompatibility

1 2 3 4

6 - Links

Blank OD min: 0.7 Abs
Blank OD max: 2 Abs
Reaction slope: Negative
OD Range min: 0 Abs
OD Range max: ** 0.17 Abs
Linearity: 25 %
Subst Depletion: * 0.2 Abs
Blank OD: 0 Abs
Min conc: 0 IU/L
Max conc: 600 IU/L

7 - Autodilution

Rate: * 4
Max OD: ** 0.17 Abs

8 - Dilutions

Serum: ☑ 1:1 ☐ 1:2 ☐ 1:4 ☐ 1:10 ☐ 1:40 ☐ 1:100
Urine: ☑ 1:1 ☐ 1:2 ☐ 1:4 ☐ 1:10 ☐ 1:40 ☐ 1:100

9 - Pathological ranges

Minimum	Sample type	Maximum
	Male	41
	Female	31

Add Remove Edit

10 - Results units

Units 1: IU/L
Units 2:
Conversion : 1
Decimal digits: 1

* User defined ** User defined based on calibration

Designations

Code: ALT Name: GPT/ALT Type: Kinetic Group: ACCUREX

OK

Calibration

General
Number of standards: 3
Validity (hours): 240
Factor:
Interpolation type: Average

Standards — Single

	Calibrator	Dilution rate
1	CFAS	1
2	CFAS	1
3	CFAS	1

Current Calibration

	Lot	Primary concent	Final concent	OD value	Date
1					
2					
3					

Reagent blank ☐ Blank Correction Validity 9999 hours OD OD 0 Date

Print calibration Curve chart

Correlation
Y = 1.000 X + 0.000