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ALANINE AMINOTRANSFERASE (ALT/GPT)

ALT reagent is for the in- vitro quantitative determination of ALT activity in human serum or plasma on both automated and manual systems.

REF:BS.1/PT05.010.0050	50 test	REF:BS.1/PT04.025.0100	100 test
REF:BS.1/PT02.025.0050	50 test	REF:BS.1/PT04.050.0200	200 test
REF:BS.1/PT05.020.0100	100 test	REF:BS.1/PT05.100.0500	500 test

CLINICAL SIGNIFICANCE [1]

The ALT is a cellular enzyme, found in highest concentration in liver and kidney. High levels are observed in hepatic disease like hepatitis, diseases of muscles and traumatisms; it's better application is in the diagnosis of the diseases of the liver.
When they are used in conjunction with AST aid in the diagnosis of infarcts in the myocardium, since the value of the ALT stays within the normal limits in the presence of elevated levels of AST.
Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

METHOD PRINCIPLE [3,4]

Alanine aminotranferase (ALT)or Glutamate pyruvate transaminase (GPT) catalyses the reversible transfer of an amino group from alanine to α-ketoglutarate forming glutamate and pyruvate.
The pyruvate produced is reduced to lactate by lactate dehydrogenase (LDH) and NADH:

$$\text{Alanine} + \alpha\text{-Ketoglutarate} \xrightleftharpoons{\text{ALT}} \text{Glutamate} + \text{Pyruvate}$$

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \xrightleftharpoons{\text{LDH}} \text{LDH Lactate} + \text{NAD}^+$$

The rate of decrease in concentration of NADH, measured photometrically, is proportional to the catalytic concentration of ALT present in the sample.

REAGENT COMPOSITION

R1: Buffer Enzyme	
Tris buffer (pH 7.4)	100 mmol/L
L- Alanine	800 mmol/L
LDH	≥2000 U/L
Sodium Azide	8 mmol/L
R2: Coenzyme	
NADH	≥ 0.18 mmol/L
2 – Oxoglutarate	18 mmol/L
Sodium Azide	8 mmol/L

PRECAUTIONS AND WARNINGS

Reagent to be handled by entitled and professionally educated person. Do not ingest or inhale as reagent (R) contains sodium azide which is classified as dangerous substance for environment.

Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses,).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Respect country requirement for waste disposal.

S56: dispose of this material and its container at hazardous or special waste collection point.
S57: use appropriate container to avoid environmental contamination.
S61: avoid release in environment.

For further information, refer to the GPT reagent material safety data sheet.

REAGENT PREPARATION, STORAGE AND STABILITY

Bioscien. reagents (R1) and (R2) contains sodium azide which may react with copper or lead plumbing.
Preparing the working solution according to the number of tests required by mixing 4 volumes of reagent 1 (R1) and 1 volume of reagent 2 (R2), e.g. 400 µl R1 +100 µl R2.
All reagents are supplied ready-to-use and stable up to the expiry date labeled on the bottles when properly stored refrigerated at 2–8ºC.Working solution is stable for 4 weeks at specified temperature.

Deterioration
The ***Bioscien*** ALT reagent normally clear. Do not use ALT reagent if it is turbid or if the absorbance is less than 0.1 at 340nm.

SPECIMEN COLLECTION AND PRESERVATION [1]

Serum or Plasma
Anticoagulants currently in use like EDTA, oxalate and fluoride do not affect the results. Hemolysis interferes with the assay.

SYSTEM PARAMETERS

Wavelength Optical path	340 nm (334 – 365 nm) 1Cm
Assay type	Kinetic
Direction	Decrease
Sample Reagent Ratio e.g.: Reagent volume Sample volume	1:10 1 ml 100µl
Temperature	37º c to 30ºc
Equilibration time	60 Sec
Read time	1-3 mins
Zero adjustment	Against air
Reagent Blank Limits	Low 1.00 AU High 2.5 AU
Sensitivity	5 U/L
Linearity	400 U/L

ASSAY PROCEDURE

Working Reagent	500µl
Specimen	50µl

Mix, read initial absorbance after 60 seconds and start timer simultaneously. Read again after 1, 2 and 3 minutes.
Determine the mean absorbance change per minute (ΔA/min).

CALCULATION

To calculate the ALT/GPT activity uses the following formula:

U/l = 1780 x ΔA 334 nm /min

U/l = 1746 x ΔA 340 nm /min

U/l = 3235 x ΔA 365 nm /min

µKat/L=IU/60

QUALITY CONTROL

To ensure adequate quality control, it is recommended that normal and abnormal commercial control serum of known concentrations included in each run. If control values are found outside the defined range, check the instrument calibration, and reagent for problems. If control still out of range please contact ***Bioscien*** technical support.

PERFORMANCE CHARACTERISTICS

Precision	Within run (Repeatability)		Run to run (Reproducibility)	
	Normal level	High level	Normal level	High level
n	20	20	20	20
Mean mg/dl	24.6	105.9	25.2	106
SD.	0.93	0.94	1.1	1.05
CV. %	3.78	0.89	3.9	0.95

The results of the performance characteristics depend on the analyzer used.

Accuracy (Methods Comparison)
Result obtained from ***Bioscien*** ALT (4+1) reagent and a commercial reagent of the same methodology was performed on 20 human sera. A correlation of 0.983 was obtained

Sensitivity
When run as recommended, the minimum detection limit of this assay is 5.0 U/L.

Linearity
The reaction is linear up to ALT concentration of 400 U/L; specimens showing higher concentration should be diluted 1+5 with physiological saline and repeat the assay (result×6).

INTERFERING SUBSTANCES [3,5]

Hemolysis
Positive interference because ALT released from erythrocytes
Hemoglobin
No interference up to 300 µmol/L
Turbidity
No interference up to 7.00mmol/L triglycerides
Bilirubin
No interference up to 20mg/dl (342µmol/L)
Icterus
No significant interference.
Lipemia
Lipemic specimens may cause high absorbance flagging. Diluted sample is recommended.
Anticoagulants
Citrate and fluoride inhibit the enzyme activity
Drugs
Calcium desolate and doxycycline HCL cause artificially low ALT values at the tested drug level.

Units: One international unit (IU) is the amount of enzyme that transforms 1 µmol of substrate per minute, in standard conditions.

The concentration is expressed in units per liter of sample (U/L).

EXPECTED VALUES [2,5]

	30ºC	37ºC
Newborns, infants	9-32 U/L	13-45 U/L
Woman	7-28 U/L	10-40 U/L
Man	5-25 U/L	7-35 U/L

Temperature conversion factor

To correct results to other temperatures multiply by:

Assay temperature	**Conversion Factor to**		
	20ºC	30ºC	37ºC
20ºC	1.00	1.32	1.82
30ºC	0.76	1.00	1.39
37ºC	0.55	0.72	1.00

These values are for orientation purpose; each laboratory should establish its own reference range

DYNAMIC RANGE

5 – 400 U/L.

REFERENCES

1. Burtis A. et al. Tietz Textbook of Clinical Chemistry, 3rd edition. AACC 1999.
2. Clinical guide to laboratory test4th ed.N.W.TIETZ(2006)p-64-67
3. IFCC Method for L- Alanine aminotranferase.Jcli,chem.,clin.Biochem.(1986),24,p.481-495
4. Murray R. Alanine aminotransferase. Kaplan A et al. Clin Chem The C.V. Mosby Co. St Louis. Toronto. Princeton 1984; 1088-1090.
5. Young DS. Effects of disease on Clinical Lab. Tests, 4th ed AACC 2001

SYMBOLS IN PRODUCT LABELLING

IVD	For in-vitro diagnostic use	Σ	Number of test in the pack
LOT	Batch Code/Lot number		Caution
REF	Catalogue Number		Do not use if package is damaged
	Temperature Limitation		
	Expiration Date		
	Manufactured by		

EC REP

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