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GPT / ALT End-Point Colorimetric

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

BS.1/PT02.050.0100	200 test	BS.1/PT02.100.0200	400 test
BS.1/PT02.125.0250	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

Alanine aminotranferase (ALT) or Glutamate pyruvate transaminase (GPT) catalyzes the reversible transfer of an amino group from L-alanine to 2-oxoglutarate forming glutamate and pyruvate.

L-Alanine + 2-Oxoglutarate $\xrightleftharpoons{\text{ALT}}$ **L-Glutamate + Pyruvate**

The ALT activity is measured by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenylhydrazine.

REAGENT COMPOSITION

R1:Buffer enzyme	
Phosphate buffer	100 mmol/L
DL- Alanine	200 mmol/L
2-Oxoglutarate	6 mmol/L
Sodium Azide	12 mmol/L
R2: Substrate	
2,4-dinitrophenyl-hydrazine	2 mmol/L

PRECAUTIONS AND WARNINGS

Reagent to be handled by entitled and professionally educated person. Do not ingest or inhale as reagent 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 contain sodium azide which may react with copper or lead plumbing.
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.

Deterioration
The ***Bioscien*** ALT reagent normally clear. Do not use the ALT regents if precipitate forms.

SPECIMEN COLLECTION AND PRESERVATION [1]

Serum or Plasma
Use only non haemolyzed serum. The only acceptable anticoagulants are heparin and EDTA. The biological half-life of ALT in serum is 17 hours.
Stability: 3 day at 15-25 ºC , 7 days at 4-8 ºC , 12 weeks at -20 ºC.

SYSTEM PARAMETERS

Wavelength	546 nm (530 – 550 nm)
Optical path	1Cm
Assay type	End Point
Direction	Increase
Sample Reagent Ratio	1:60
Temperature	37ºc to 20-25ºc
Zero adjustment	Reagent or Sample blank
Sensitivity	4 U/L
Linearity	94 U/L

ASSAY PROCEDURE

1-Measurement against Reagent

	Reagent Blank	*Sample*
R1	250 µl	250µl
Distilled water	50µl	
Specimen		50µl
Mix and incubate for exactly 30 minutes at 37 ºc		
R2	250 µl	250µl
Mix and incubate for exactly 20 minutes at 20-25 ºc		
Sodium hydroxide	2.5 ml	2.5 ml

Mix, and incubate for 5 minutes at 20-25 ºc then measure absorbance of specimen against reagent blank at 546 nm.

2-Measurement against Sample Blank

	Sample Blank	*Sample*
R1	250 µl	250µl
Specimen		50µl
Mix and incubate for exactly 30 minutes at 37 ºc		
R2	250 µl	250µl
Specimen	50µl	
Mix and incubate for exactly 20 minutes at 20-25 ºc		
Sodium hydroxide	2.5 ml	2.5 ml

Mix, and incubate for 5 minutes at 20-25 ºc then measure absorbance of specimen against sample blank at 546 nm.

CALCULATION

Absorbance	U/L	Absorbance	U/L
0.025	4	0.275	48
0.050	8	0.300	52
0.075	12	0.325	57
0.100	17	0.350	62
0.125	21	0.375	67
0.150	25	0.400	72
0.175	29	0.425	77
0.200	34	0.450	83
0.225	39	0.475	88
0.250	43	0.500	94

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.

Sensitivity
When run as recommended, the minimum detection limit of this assay is 7.0 U/L.

Linearity
The reaction is linear up to ALT concentration of 94 U/L; specimens showing higher concentration should be diluted 1+9 with physiological saline and repeat the assay (result×10).

INTERFERING SUBSTANCES [3,5]

Hemolysis
Positive interference because ALT released from erythrocytes
Icterus
No significant interference.
Lipemia
Lipemic specimens may cause high absorbance flagging. Diluted sample is recommended.

Note
High concentration of aldehydes, ketones, or Oxo-acids in some sera may cause false high transaminases levels. Measurement against a serum blank instead of a reagent blank avoids the risk of finding such artifacts.

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]

Up to 12 U/L

DYNAMIC RANGE

4 – 94 U/L.

REFERENCES

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3. IFCC Method for L- Alanine aminotranferase. Jcli, chem.,clin.Biochem.(1986),24,p.481-495.
4. Sherwin JE. Liver function. In:kaplan LA, Pesce AJ, eds. Clinical chemistry, theory, analysis, and correlation. St louis: Mosby; 1984:420-438.
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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