Spectrum For Diagnostic Industries

Alanine aminotransferase (ALT/GPT) - Colorimetric

IVD

REF.	Pack size
182 02 125	(2 x 125 ml) 250 tests

Intended Use

ALT reagent is intended for the in-vitro quantitative and diagnostic determination of ALT in human serum.

Introduction

Transaminases are widely distributed in human tissues. Both AST and ALT are normally present in human plasma, bile, cerebrospinal fluid and saliva but none is found in urine unless a kidney lesion is present. In viral hepatitis and other forms of liver disease associated with hepatic necrosis, levels of serum AST and ALT are elevated even before the clinical signs and symptoms of disease, such as jaundice.

Method

ALT – (colorimetric method).

Principle

L-Alanine + 2-Oxoglutarate $\xrightarrow{ALT}$ Pyruvate + L-Glutamate

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

Reagents

Reagent 1 (Buffer)

Phosphate buffer	100 mmol/L
DL- Alanine	200 mmol/L
2 – Oxoglutarate	6 mmol/L
Sodium Azide	12 mmol/L

Reagent 2

2,4-dinitrophenylhydrazine	2.0 mmo/L

Additional Reagent

Sodium hydroxide	0.4 mol/L.

Reagents preparation, storage and stability

The reagents are supplied ready-to-use and stable till the expiration date labeled on the bottles when stored at 2 – 8 ^{o}C.Once opened, the reagent is stable for 6 months at the specified temperature.

Deterioration

Failure to recover control values within assigned range may indicate reagent deterioration.

Precautions and Warnings

Do not ingest or inhalate. 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.

Specimen collection and preservation

Use only non-haemolyzed serum. The biological half-life of ALT in serum is 47 hours. The only acceptable anticoagulants are heparin and EDTA.

Stability: 3 days at 15 - 25 ^{o}C or
7 days at either 4 - 8 ^{o}C or at -20 ^{o}C

Procedure

Wavelength	546 nm (530-550 nm)
Optical path	1 cm
Assay type	End-point
Direction	Increase
Sample: Reagent Ratio	1 : 60
Temperature	37 ^{o}C and 20 – 25 ^{o}C
Zero adjustment	Reagent or Sample blank

1. Measurement against Reagent Blank

Pipette into test tubes

	Reagent blank	Specimen
Reagent1 (buffer)	0.5 ml	0.5 ml
Specimen	_____	100 µl
Distilled water	100 µl	_____

Mix and incubate for exactly 30 minutes at 37 ^{o}C

Reagent2	0.5 ml	0.5 ml

Mix and incubate for exactly 20 minutes at 20 – 25 ^{o}C

Sodium hydroxide	5.0 ml	5.0 ml

Mix, measure absorbance of specimen against reagent blank at 546 nm after 5 minutes.

2. Measurement against Sample Blank

	Sample blank	Specimen
Reagent1 (buffer)	0.5 ml	0.5 ml
Specimen	_____	100 µl

Mix and incubate for exactly 30 minutes at 37 ^{o}C

Reagent 2	0.5 ml	0.5 ml
Specimen	100 µl	_____

Mix and incubate for exactly 20 minutes at 20 – 25 ^{o}C

Sodium hydroxide	5.0 ml	5.0 ml

Mix, measure absorbance of specimen against sample blank at 546 nm after 5 minutes.

Calculation

The ALT activity in the serum can be determined from the following table:

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

Normal and abnormal control serum of known concentration should be analyzed with each run.

Sensitivity

4 U/L.

Linearity

The assay is linear up to 94 U/L. If the absorbance exceeds 0.5 at 546 nm (ALT 94 U/L) samples should be diluted 1 + 9 using sodium chloride and repeat the assay (result × 10).

Interference

Serum, plasma

Haemolysis
Erythrocyte contamination may elevate results, since ALT activities in erythrocytes are 3 to 5 times than those in normal sera.

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 transaminase levels. Measurement aganist a serum blank instead of a reagent blank avoids the risk of finding such artifacts.

Performance characteristics

Precision
Within run (Repeatiblity)

	Level 1	Level 2
n	20	20
Mean (U/L)	4	48
CV%	1.5	0.73

Run to run (Reproducibility)

	Level 1	Level 2
n	20	20
Mean (U/L)	5	50
CV%	2	1.3

Expected Values

Serum : up to 12 U/L.

Analytical Range

4-94 U/L..

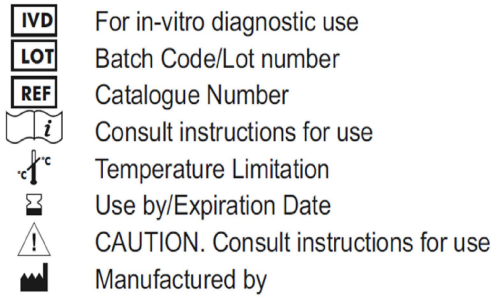
Waste Disposal

This product is made to be used in professional laboratories.
Please consult local regulations for a correct 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. Refer to special instructions/safety data sheets.

References

1. ECCLS. Determination of the catalytic activity concentration in serum on L- aspartate aminotransferase (EC 2.6.1.1,AST) Clin Chem. 1989;20:204-211.
2. Henry RJ, et al. Am j Clin Path 1960 :34:381
3. Young DS. Effects of drugs on clinical laboratory tests.Third edition. 1990 :3:6-12.

SYMBOLS IN PRODUCT LABELLING



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