OTTO Scientific™

ALT(SPGT) Assay Kit

IFCC without pyridoxal phosphate

Cat.No: OttoBC128

Summary & Explanation

Alanine aminotransferase (glutamate-pyruvate-transaminase) belongs to the group of transaminases which catalyze the conversion of amino acids to the corresponding a-keto acids via the transfer of amino groups; they also catalyze the reverse process. Although higher activities exist in the liver, minor activity can also be detected in the kidneys, heart, skeletal muscle, pancreas, spleen, and lungs. Elevated levels of transaminases are indicative of myocardial infarction, hepatopathies, muscular dystrophy, and damage to internal organs. Increased ALT activity in the serum, however, is a rather specific indicator of damage to the liver parenchyma, while AST is not necessarily a liver-specific parameter. The International Federation of Clinical Chemistry (IFCC) recommended standardized methods for the determination of ALT with optimized substrate concentrations, use of TRIS buffer, simultaneous preincubation of serum with buffer (to avoid competing reactions with NADH), substrate start, and pyridoxal phos-phate activation. The method described here is derived from the IFCC reference method.

Test Principle

UV test according to the IFCC method.

L-Alanin + 2-Oxoglutarate <—> L-Glutamate + Pyruvat

Pyruvat + NADH + H + <—> D-Lactate+ NAD+

The enzyme alanine aminotransferase (EC 2.6.1.2; L-Alanine:2-Oxoglutarate Aminotransferase, ALT or A1aAT; Glutamate Pyruvate Transaminase, GPT) catalyzes the tran- saminase reaction between L-Alanine and 2-Oxoglutarate. The pyruvate formed, is reduced to lactate in the presence of LDH. As the reactions proceed, NADH is oxidized to NAD+ . The disappearance of NADH per unit time is followed by measuring the decrease in absorbance at 340 nm.

Kit Companents

Content	Explanation	Shelf life
Reagent-1	1x30ml	6 months
Reagent-2	1x10ml	6 months
Calibrator	1x0.5ml	6 months
Q.Control	1x0.5ml	6 months

Storage & Stability

Reagent: Stable up to expiry date when stored capped and at +4°C even after start using

Calibration and Quality Control: Reconstitute the contents of with 0.5 ml of redistilled water . Stable for 2 days when stored at +4°C

Reactivity

Universal

Specimen

Collect serum using standard sampling tubes.
Heparin or EDTA plasma.
Stability: 7 days at +2°C - +8°C, 30 days at - 70°C

Assay Range

3 - 400 U/l

Refference Range

Each laboratory is recommended to establish their own reference values.

Analytical Performance

Inter Assay Coefficent of Variation (CV) % 2.7
Intra Assay Coefficent of Variation (CV) % 2.8

Procedure

Wavelength	340nm (±10nm)
Sample or Standard	25µl
Reagent-1	200µl
Mix, incubate 1-5 min. Then Add;	
Reagent-2	50 µl
Mix, incubate for 1 min. and start stopwatch simultaneously. Read again after exactly 1, 2 and 3 minutes and calculate A/min.	
Zero Adjustment	Sample blank

Calculation

Hg 365 nm 3235 x A/min
Hg 340 nm 1746 x A/min
Hg 334 nm 1780 x A/min

Warning

For in vitro use only
Do not pipette by mouth
Do not use reagents beyond the expiry date.

References

1.J. Clin.Chem.Clin.Biochem 8 (1970) 658; 10 (1972) 182

2.Tietz Textbook of Clinical Chemistry, Second Edition, Burtis-Ashwood (1994).

3. HU Bergmeyer - Methods of enzymatic analysis, (1987). CCLM 2002; 40(7):725-733, Schumann et al.

4. IFCC reference procedure for aspartate aminotransferase.