SGPT/ALT

IFCC Method

Intended Use:
Alanine Amino Transferase (ALT/SGPT) test reagent/kit is a medical device intended for the estimation of Alanine Amino Transferase (ALT/SGPT) in serum or plasma.

Clinical Significance
The group of enzymes called transaminase exist in tissues of many organs. Necrotic activity in these organs causes a release of abnormal quantities of enzyme into the blood where they are measured.
Since heart tissue is rich in AST increased serum levels appear in patients after myocardial infarction, as well as in patients with muscle disease, muscular dystrophy and dermatomyositis.
The liver is specially rich in ALT, being the enzyme measurement used primarily as a test for infectious and toxic hepatitis, although high levels of both ALT and AST may also be found in cases of liver cell damage and acute pancreatitis, suggesting that the obstruction of the biliary tree by the edematous pancreas and the presence of associate hepatic disease may contribute to elevated AST levels in these patients.
Slight or moderate elevations of AST and ALT activities may be observed after intake of alcohol and after administration of various drugs, such as salicylates, opiates and ampicillin.

Principle
Alanine aminotransferase (ALT/GPT) catalyzes the transfer of the amino group from alanine to oxoglutarate with the formation of glutamate and pyruvate. The latter is reduced to lactate by lactate dehydrogenase (LDH) in the presence of reduced nicotinamide adenine dinucleotide (NADH).
The reaction is monitored kinetically at 340 nm by the rate of decrease in absorbance resulting from the oxidation of NADH to NAD^+, proportional to the activity of ALT present in the sample.

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

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

Reagent Composition

R1 SGPT/ALT Reagent	Tris Buffer - 25 mmol/L LDH - 2000 U/L L-Alanine - 200 mmol/L NADH - 0.15mmol/L α-Ketoglutarate - 12 mmol/L
R2 BUFFER	Stabilizers

Working Reagent Preparation
For sample start assays a single reagent is required. Reconstitute one vial of enzyme reagent (R1) with equivalent volume of diluent (R2). This reagent is stable for at least 4 weeks when stored at 2-8^{0}C.

Stability and Storage
Store at 2-8ºC.
All the kit contents are stable until the expiry date stated on the label. Do not use reagents beyond the expiration date. Store the vials tightly closed protected from light and prevent contaminations during the use.

Discard if signs of deterioration appear:
- Presence of particles and turbidity.

Instability or Deterioration of Reagents
When the Spectrophotometer has been set to zero with distilled water, absorbance readings of the working reagent lower than 0.800 OD indicates deterioration.

Materials required
- Photometer or spectrophotometer with a thermostat cell compartment set at 25/30/37ºC, capable of reading at 630 nm.
- Stopwatch, strip-chart recorder or printer.
- Cuvettes with 1-cm path length
- Pipettes to measure reagent and samples.

Sample and Stability
Serum or plasma
Serum, EDTA or heparinized plasma free of hemolysis.
SGPT/ALT is stable in serum or plasma 24 hours at room temperature and for 1 week at 2-8ºC.

Known interference substances:
Lipemia (intra lipid >15 g/L) does not interfere.
Bilirubin (>30 mg/dl) does not interfere.
Hemoglobin (>10 g/dl) does not interfere.
Other drugs and substances may interfere3,4.

Assay Procedure
Pipette into a clean dry test tube labeled as Test (T):

Addition Sequence	Test (T) 37^{0}C
Working Reagent	1000µl
Sample	100µl

Mix well and read the initial absorbance A_0 after 1 min. and repeat the absorbance reading after 1,2 & 3 minutes. Calculate the mean absorbance change per min. (ΔA/min).

Calculation
SGPT/ALT Activity in IU/L 37^{0}C = ΔA/min. x 1746 x tF

TEMPERATURE CONVERSION FACTORS

Assay	Desired Reporting Temperature 37°C
25^{0}C	1.82
30^{0}C	1.38
37^{0}C	1.00

Quality Control
To ensure adequate quality control (QC), each run should include a set of controls (normal and abnormal) with assayed values handled as unknowns.
If the values are found outside of the defined range, check the instrument, reagents and procedure.
Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

Linearity
The procedure is linear up to 450 IU/L at 37^{0}C, if the absorbance change (ΔA/min.) exceeds 0.250, use only the value of the first two minutes to calculate the result, or dilute the sample 1+9 with normal saline (NaCl 0.9%) and repeat the assay (Results x 10).

Reference Values

Serum	(Males)	:	up to 40 IU/L at 37^{0}C
	(Females)	:	up to 31 IU/L at 37^{0}C

It is recommended that each laboratory establishes its own normal range representing its patient population.

General System Parameters

Mode	Kinetic
Reaction	Decreasing
Wavelength	340 nm
Blank with	Distilled Water
Sample Volume	100µl
Reagent Volume	1000µl
Delay Time	60 sec
Measuring Time	180 sec
No. of readings	4
Factor	1746
Linearity limit	Up to 450
Unit	IU/L

Notes

1. This method may be used with different instruments. Any application to an instrument should be validated to demonstrate that results meet the performance characteristics of the method. It is recommended to validate periodically the instrument. Contact to the distributor for any question on the application method.
2. Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

References

1. Winn-Deen E S, David H, Sigler G, and Chavez R. Clin Chem 1988;34:2005.
2. International Federation of Clinical Chemistry (IFCC). Clin Chem Lab Med 1998;36:185.
3. Young DS. Effects of drugs on clinical laboratory tests, 5th ed. AACC Press, 2000.
4. Tietz. Textbook of Clinical Chemistr, 2rnd Edition. Burtis CA, Ashwood ER. W.B. Saunders Co. 1994.
5. IFCC methods for the measurements of catalytic concentrations of enzymes, J.Clin. Chem. Clin. Biochem. (1986) 24:481.

For *in vitro* Diagnostic use only.