SGOT (ASAT)KIT

(Mod. IFCC method)

For the determination of SGOT (ASAT) activity in serum.
(For Invitro Diagnostic Use Only)

Summary

SGOT is an enzyme found mainly in heart muscle, liver cells, skeletal muscle and kidneys. Injury to these tissues results in the release of the enzyme in blood. Elevated levels are found in myocardial infarction, Cardiac operations, Hepatitis, Cirrhosis, acute pancreatitis, acute renal diseases, primary muscle diseases. Decreased levels may be found in Pregnancy, Beri Beri and Diabetic ketoacidosis.

Principle

SGOT (ASAT) catalyzes the transfer of amino group between L-Aspartate and α Ketoglutarate to form Oxaloacetate and Glutamate. The Oxaloacetate formed reacts with NADH in the presence of Malate Dehydrogenase to form NAD.The rate of oxidation of NADH to NAD is measured as a decrease in absorbance which is proportional to the SGOT (ASAT) activity in the sample.

$$\text{L-Aspartate} + \alpha\text{ Ketoglutarate} \xrightarrow{SGOT} \text{Oxaloacetate} + \text{L-Glutamate}$$

$$\text{Oxaloacetate} + \text{NADH} + H^{+} \xrightarrow{MDH} \text{Malate} + NAD^{+}$$

Normal reference values

Serum (males) : upto 37 U/L at 37°C
(females) : upto 31 U/L at 37°C

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

Contents	25 ml	75 ml	2 x 150 ml
L1 : Enzyme Reagent	20 ml	60 ml	2 x 120 ml
L2 : Starter Reagent	5 ml	15 ml	2 x 30 ml

Storage / stability

Contents are stable at 2-8°C till the expiry mentioned on the labels.

Reagent Preparation

Reagents are ready to use.

Working reagent : For sample start assays a single reagent is required. Pour the contents of 1 bottle of L2 (Starter Reagent) into 1 bottle of L1 (Enzyme Reagent). This working reagent is stable for at least 3 weeks when stored at 2-8°C. Alternatively for flexibility as much of working reagent may be made as and when desired by mixing together 4 parts of L1 (Enzyme Reagent) and 1 part of L2 (Starter Reagent). Alternatively 0.8 ml of L1 and 0.2 ml of L2 may also be used instead of 1 ml of the working reagent directly during the assay.

Sample material

Serum. Free from hemolysis. SGOT (ASAT) is reported to be stable in serum for 3 days at 2-8°C.

Procedure

Wavelength / filter : 340 nm
Temperature : 37°C / 30°C / 25°C
Light path : 1 cm

Substrate Start Assay :

Pipette into a clean dry test tube labelled as Test (T) :

Addition Sequence	(T) 25°C / 30°C	(T) 37°C
Enzyme Reagent (L1)	0.8 ml	0.8 ml
Sample	0.2 ml	0.1 ml
Incubate at the assay temperature for 1 minute and add		
Starter Reagent (L2)	0.2 ml	0.2 ml

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

Sample Start Assay :

Pipette into a clean dry test tube labelled as Test (T) :

Addition Sequence	(T) 25°C / 30°C	(T) 37°C
Working Reagent	1.0 ml	1.0 ml
Incubate at the assay temperature for 1 minute and add		
Sample	0.2 ml	0.1 ml

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

Calculations

Substrate / Sample start

SGOT (ASAT) Activity in U/L 25°C / 30°C = ΔA / min. x 952
SGOT (ASAT) Activity in U/L 37°C = ΔA / min. x 1746

TEMPERATURE CONVERSION FACTORS

Assay Temperature	Desired 25°C	Reporting 30°C	Temperature 37°C
25°C	1.00	1.37	2.08
30°C	0.73	1.00	1.54
37°C	0.48	0.65	1.00

Linearity

The procedure is linear upto 500 U/L at 37°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).

Note

Samples having a very high activity show a very low initial absorbance as most of the NADH is consumed prior to the start of measurement. If this is suspected then dilute the sample and repeat the assay.

The working reagent or the combined reagent should have an absorbance above 1.000 against distilled water at 340 nm. Discard the reagent if the absorbance is below 1.000.

References

IFCC methods for the measurment of catalytic concentrations of enzymes, J. Clin. Chem. Clin Biochem. (1986) 24 : 497

System Parameters

Reaction	:	U.V. Kinetic
Wavelength	:	340 nm
Zero Setting	:	Distilled Water
Incub. Temp.	:	37°C
Incub. Time	:	---
Delay Time	:	60 sec.
Read Time	:	180 sec.
No. of read.	:	4
Interval	:	60 sec.
Sample Vol.	:	0.10 ml
Reagent Vol.	:	1.00 ml
Standard	:	---
Factor	:	1746
React. Slope	:	Decreasing
Linearity	:	500 U/L
Units	:	U/L

GOT(IFCC):02(P)

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