SGOT (ASAT) KIT

(Mod. IFCC Method)

(For veterinary invitro diagnostic use only)

INTENDED USE

QUADRAPED™ SGOT (ASAT) kit is used for the determination of SGOT (ASAT) Activity in serum.

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.

L-Aspartate + α Ketoglutarate —*SGOT*→ **Oxaloacetate + L-Glutamate**

Oxaloacetate + NADH + H^+ —*MDH*→ **Malate + NAD^+**

EXPECTED VALUES

Species	SGOT ASAT (U/L)
Dog	13 - 15
Cat	7 - 38
Cow	60 - 125
Horse	160 - 412
Pig	32 - 84
Sheep	60 - 280
Goat	167 - 513
Rabbit	35 - 130
Buffalo	20 - 50

It is recommended that each laboratory establish its own range as reference ranges may vary between laboratories.

PRESENTATION

REF	1126180025
Pack Size	25 ml
L1 Enzyme Reagent	20 ml
L2 Starter Reagent	5 ml

COMPOSITION

Tris Buffer 80mM; pH 7.8; L Aspartate 200mM; LDH 1000U ; MDH 600U; NADH 0.18mM; Ketoglutarate 12mM.; Non Reactive Stabilizers, Detergents and Preservatives.

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.

SAMPLE WASTE AND DISPOSAL

Do not reuse the reagent containers, bottles, caps or plugs due to the risks of contamination and the potential to compromise reagent performance.

Appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.

Handle specimens, solid and liquid waste and test components in accordance with local regulations and NCCLS guidelines M29, or other published biohazard safety guidelines.

MATERIALS REQUIRED BUT NOT PROVIDED

Photometer analyzer with standard thermostatic cuvette holder, micropipette and appropriate laboratory equipment.

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 min. and add		
Starter Reagent (L2)	0.2 ml	0.2 ml

Mix well and read the initial absorbance A_0 after 1 min. & repeat the absorbance reading after every 1, 2, & 3 mins. Calculate the mean absorbance change per min. (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 min. and add		
Sample	0.2 ml	0.1 ml

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

CALCULATIONS

Substrate/ Sample start

SGOT (ASAT) Activity in U/L

25°C / 30°C = ΔA / min. x 952
37°C = ΔA / min. x 1746

QUALITY CONTROL

The following process is recommended for QC during the assay of SGOT (ASAT). *Define and establish acceptable range for your laboratory.

1. Two levels of control (Normal and Abnormal) are to be run on a daily basis.
2. If QC results fall outside acceptance criteria, recalibration may be necessary.
3. Review QC results and run acceptance criteria following a change of reagent lot.

SPECIFIC PERFORMANCE CHARACTERISTICS

LOD - 8.6 U/L
LOQ - 17.1 U/L
Lower Limit - 8.6 U/L
Higher Limit - 500 U/L

If the absorbance change (ΔA / min.) exceeds 0.250, use only the value of the first 2 mins. to calculate the result, or dilute the sample 1+9 with normal saline (NaCl 0.9%) and repeat the assay (Results x 10).

Interferences:

Sample when spiked with interferent such as upto 20 mg/dl Bilirubin, 1000 mg/dl intralipid does not affect the ability of the kit to determine the SGOT (ASAT) concentration.

Precision:

Within run

Within run	n	Mean	SD	% CV
Sample 1	10	35	1.20	3.45
Sample 2	10	147	1.23	0.83
Sample 3	10	35	1.65	4.76

Between run

Between run	n	Mean	SD	% CV
Sample 1	10	35	0.98	2.81
Sample 2	10	147	1.54	1.05
Sample 3	10	35	1.67	4.77

Method comparison:

Comparative studies were done to compare our reagent with another commercial SGOT (ASAT) Assay. No significant differences were observed. Details of the comparative studies are available on request.

TEMPERATURE CONVERSION FACTORS

Assay Temperature	Desired Reporting Temperature		
	25°C	30°C	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

NOTE

In vitro diagnostic reagent for laboratory and professional use only Not for medicinal use. The reagent contain sodium azide 0.1% as preservative. Avoid contact with skin and mucosa. On disposal flush with large quantities of water. Only clean and dry glassware must be used. 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. Do not use turbid, deteriorated or leaking reagents.

REFERENCES

1. IFCC methods for the measurement of catalytic concentrations of enzymes, J. Clin. Chem. Clin Biochem. (1986) 24: 497.
2. Wallnofer H. E. Schmidt and F. W. Schmidt, eds (1974). Synopsis Der Leberkrankheiten. Georg Theme Veriag Stuttgart Thefeld W. et. al. (1974) Dtsh. Med. Wschr. 99:343.
3. Duncan and Prasse's Veterinary Laboratory Medicine: Clinical Pathology, Kenneth S. Latimer, ISBN Jane Wardrop , 6th Edition - 2010.
4. Clinical Biochemistry of Domestic Animals, Sixth Edition, 2008 by Kaneko J.J., Harvey J.W. & Bruss M.L.
5. Data on file: Coral Clinical Systems.

System Parameters

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

SYMBOL KEYS

8°C / 2°C Store at 2-8°C	Manufacturer	IVD *In vitro* Diagnostic Medical Device	L1 Enzyme Reagent	Mod. IFCC Modified IFCC Method	
Use by (Last day of stated month)	Consult Instructions for use	LOT Batch Number			
Date of Manufacture	REF Catalogue Number	This way up	L2 Starter Reagent	H315	

Manufactured by:

Coral Clinical Systems

A Division of Tulip Diagnostics (P) Ltd.

BUILDING E, PLOT NO. M-46/47, PHASE III B, VERNA INDUSTRIAL ESTATE, VERNA, GOA-403 722, INDIA.

REGD. OFFICE : GITANJALI, TULIP BLOCK, DR. ANTONIO DO REGO BAGH, ALTO SANTACRUZ, BAMBOLIM COMPLEX P.O., GOA-403 202, INDIA.

QGOT2/0625/VER-01

QUADRAPED™: A TDPL Veterinary Sciences Trademark