LiquiMAX SGOT (AST)

MODIFIED REITMAN & FRANKEL'S COLORIMETRIC - DNPH METHOD

ORDER INFORMATION

Ref. No.	Pack Size
AVGOT1 - 30	30 Tests

INTENDED USE :

LiquiMAX SGOT (AST) is an in-vitro diagnostic kit is use for the quantitative determination of Serum Glutamate Oxaloacetate Transaminase activity in serum.

PRODUCT FEATURES

1. Three Liquid Reagents.
2. Avoids cumbersome graph preparation. No need to plot any standard curve.
3. No inhibitory intermediate products produced during the assay.
4. Results correlate with UV-Kinetic Method.
5. Calibrator is calibrated against WHO AST (E.C.2.6.1.1) enzyme calibrator.
6. For colorimeters only.
7. Tailor made for tropical Conditions.
8. Can be used on any colorimeter and photometer.

CLINICAL SIGNIFICANCE

Serum Glutamate Oxaloacetate Transaminase (SGOT), also called as Aspartate aminotransferase (AST), belongs to the transferase class of enzymes. This enzyme shows high levels of activity in the heart, liver, skeletal muscles and kidneys. Since its level seems to be increasing enormously following Myocardial Infarction (MI), it can be used as supporting evidence in the diagnosis of MI (Especially 20-36 hrs after MI). Elevated levels are also seen in Viral / Toxic Hepatitis, Hepatic and Cardiac Necrosis, Muscular Dystrophy and Pulmonary Embolism.

PRINCIPLE:

$$\text{L-Aspartate} + \text{2-Oxogluatarate} \xrightarrow{\text{SGOT}} \text{Oxaloacetate} + \text{L-Glutamate.}$$

$$\text{Oxaloacetate} + \text{2,4 DNPH} \longrightarrow \text{Brownish red coloured complex.}$$

SGOT (AST) catalyses the transfer of amino group from Aspartic acid to 2-Oxoglutarate to form Oxaloacetate and L-Glutamate. The Oxaloacetate thus formed reacts with 2,4 Dinitrophenyl Hydrazine (2,4 DNPH) to form a corresponding Hydrazone, a brownish red colored complex in an alkaline medium. The color intensity is directly proportional to the SGOT concentration in the serum and is measured photometerically at 505 nm (490-546).

STORAGE AND STABILITY

Dilute Reagent-4 (Alkaline Reagent) 1:10 with deionised water (1 part Alkaline Reagent and 9 parts Deionized water). All the other reagents are ready to use. All the reagents are stable at 2-8ºC up to the expiry date mentioned on the label when properly stored.

KIT COMPONENTS

1. Substrate Reagent R1
2. Color Reagent R2
3. Calibrator Reagent R3
4. Alkaline Reagent R4

COMPOSITION:

	Contents	Active Ingredients	Composition
Reagent-1	Substrate Reagent	L-AspartiC 2-Oxoglutarate	Acid 120 mm/l 12 mmol/l
Reagent-2	Color Reagent	2,4-DNPH	6 mmol/l
Reagent-3	Calibrator	Sodium Pyruvate	Conc : 160 U/L
Reagent-4	Alkaline Reagent	Sodium Hydroxide	4N

REAGENT RECONSTITUTION & STABILITY

Reagent are liquid stable no need for reconstitution.

When the reagent is stored properly at 2-8°C & the contamination avoided, it is stable up to the expiry date mention on the label & kit box.

MATERIAL REQUIRED BUT NOT PROVIDED

Laboratory Instrumentation, Spectrophotometer UV/VIS with thermostatic cuvette holder or clinical chemistry analyzer: semi auto, calibrated micropipettes, glass or high quality polystyrene cuvettes, test tube/rack, heating bath controls, saline.

REAGENT DETERIORATION

Discard any turbid reagent if blank reagent absorbance exceeds 1.2 at 505 nm against distilled water.

WARNING & PRECAUTIONS

- Reagent may contain some non reactive and preservative components. It is recommended to handle carefully, avoiding contact with skin and ingestion.
- Specimen should be considered infectious and handled appropriately.
- Contamination by soap or glycerol will affect this assay.
- Perform the test according to the general " Good Laboratory Practice" GLP

SPECIMEN:

Clear, unhemolysed serum is preferred.

(Serum should be removed from the clot as soon as possible after collection because of the approximately 10 fold greater concentrations of SGOT in erythrocytes than in serum). For similar reason hemolysis of the specimen must be avoided.

TEST PROCEDURE:

Pipette into test tubes labeled as Blank, Calibrator, Control, Test and proceed as per given below.

Reagent	Blank	Calibrator	Control	Test
Substrate Reagent	0.25 ml	0.25 ml	0.25 ml	0.25 ml
Deionised Water	50 µl	-	-	-
Serum Sample	-	-	-	50 µl
Calibrator (Conc. 160 U/L)	-	50 µl	-	-

Mix and incubate at 37ºC for 60 minutes.

Color Reagent	0.25 ml	0.25 ml	0.25 ml	0.25 ml
Serum Sample (Same Serum Sample Which is used above)	-	-	50 µl	-

Mix and incubate at 37ºC for 20 minutes.

Alkaline Reagent (Prediluted)	1.5 ml	1.5 ml	1.5 ml	1.5 ml

Read absorbances of all the tubes against distilled water at 505 nm (490-546).

For samples above the linearity limit, dilute suitably with saline and re assay. Multiply by the dilution factor to calculate the end result.

CALCULATION:

$$\text{SGOT (AST) activity in U/L (160 U/L)} = \frac{\text{Abs of Test} - \text{Abs of Control}}{\text{Abs of Calibrator} - \text{Abs of Blank}} \times \text{Conc of Calibrator}$$

EXPECTED VALUE

Male : Up to 40 U/L at 37ºC
Female : Up to 37 U/L at 37ºC

(The expected value should be used as a reference only. It is recommended that each laboratory should establish its own normal range.)

QUALITY CONTROL & CALIBRATION:

To ensure adequate quality control, the use of commercial reference control serum is recommended with each assay batch. Use of quality control material checks both the instrument as well as reagent functions.

PERFORMANCE CHARACTERISTICS

1. Linearity

Linearity : 300 U/L

2. Sensitivity/ Limit of Detection (LOD)

The lower limit of detection is 0.2 mg/dl

3. Interferences

No interference was observed by ascorbic acid up to 32 mg/dl, bilirubin up to 43 mg/dl, hemoglobin up to 500 mg/dl and lipemia up to 2200 mg/dl.

4. Precision:

Intra-Assay (N=20)

Sample	Mean (IU/L)	SD (IU/L)	CV (%)
sample 1	43.5	2.05	4.71
sample 2	83.4	1.95	2.34
sample 3	127.0	2.92	2.30

Inter-Assay (N=20) Sample	Mean (IU/L)	SD (IU/L)	CV (%)
sample 1	39.8	1.13	2.83
sample 2	86.0	1.04	1.21
sample 3	135.0	1.51	1.12

5. Method Comparison:

A comparison of the LiquiMAX SGOT (AST) with a commercial obtainable assay (x) gave the following result with 58 samples:
y = 0.946 x + 1.385; r= 0.998

LIMITATIONS

The test has been developed to determine AST activities, which correspond to a maximal △A/min of 0.379 (Equivalent to 750 IU/ml) at 340 nm. If such value is exceeded the sample should be diluted 1+9 with NaCl solution (9 g/l) and results be multiplied by 10.

WASTE DISPOSAL

Reagents must be disposed off in accordance with local regulations.

REFERENCES:

1. Reitman, S. Frankel, S. Am.J. Clin. Path. 25:56 (1957).
2. Henry, J.B., Clinical Diagnosis and Managementby Laboratory Method P. 361 (1974).

Symbols Used on Pack

REF	Catalogue Number		Warning/Caution
LOT	Batch No.	IVD	In vitro diagnostic device
	Manufacturing Date		Storage Limit
	Expiry Date		Consult instruction for use
	Manufacturer		Keep away from sunlight
	Keep Dry		Do not use if package is damaged
	Contains sufficient no. of test		

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