ANA BIO ISP SGOT (AST)

(IFCC without P5P activation Method)

For Miura Instruments

Intended Use
GOT (AST) is a reagent kit used for the determination of GOT (AST) activity in serum or plasma based on enzymatic UV-Kinetic method.

Principle
α - Ketoglutarate reacts with L-aspartate in presence of GOT (AST) to form oxaloacetate and L-glutamate. The increase in oxaloacetate is determined in an indicator reaction catalyzed by malate dehydrogenase. The conversion of NADH to NAD^+ is proportional to the activity of GOT (AST) in serum/plasma and is determined kinetically as rate of decrease in absorbance.

$$\text{L - Aspartate} + \alpha\text{ - Kg} \xleftrightarrow{\text{GOT}} \text{Oxaloacetate} + \text{L-Glutamate}$$

$$\text{Oxaloacetate} + \text{NADH} + H^+ \xleftrightarrow{\text{MDH}} \text{L-Malate} + NAD^+$$

Components & Concentration of Reagents

Reagent	Component	Concentration
Reagent 1	TRIS buffer pH 7.8	100 mmol/L
	LDH	≥1000 U/L
	2-Oxoglutarate	15 mmol/L
	MDH	≥400 U/L
	Aspartate	≥200 mmol/L
	Stabilizers, excipients & surface active agents	
Reagent 2	TRIS buffer pH 9.8	20 mmol/L
	NADH	2.60 mmol/L
	Stabilizers, excipients & surface active agents	

Reagent storage and stability
The reagent kit should be stored at 2° - 8°C and is stable till the expiry date indicated on the label.
A slight variation in the composition of the components may occur between batches, but this has no effect on the test results.
After opening, the vial R1 and R2 are stable 30 days if recapped immediately and protect from contamination, evaporation, direct light and stored at correct temperature.

Specimen collection and preservation
Blood should be collected in a clean dry container. Although serum is preferred, plasma with heparin or EDTA can also be used. Samples with any visible haemolysis are not acceptable. GOT (AST) activity in serum/plasma is stable for 1 week at 2° - 8°C and 30 days when stored at -20°C. The samples should be brought to room temperature prior to use.

Automation
This kit, though developed and manufactured to be used as manual assay and with I.S.E. Miura Analyzer, can be used also with other analyzers able to meet the specifications indicated in section "Reaction conditions – Test procedure" Application sheets are available for automatic instruments.
All applications not explicitly approved by KDPL. Cannot be guaranteed in terms of performance, and must there be established by the operator.

Calibration
For Calibration use the "Multicalibrator"

Calibration Stability
For the instrumentation series Miura, the calibration is recommended to be done every 10 days.

Materials required but not supplied in the kit
Calibrators and controls

Assay guidelines for Analyzer I.S.E. Miura

Analyte Name	AST(GOT)	
Method Code	GOT	
Type	Kinetic	
Unit	IU/L	
Filter F1	340 nm	
Blank in	Not Use	
Step	Reaction Volume	U.M.
Volume reagent R1	200	µl
Volume reagent R2	50	µl
Sample Volume	25	µl
First Incubation	60	Sec
Final Incubation	192	Sec.

Normal Range
Guidance value for Men : Up to 37 IU/L
Guidance value for Women : Up to 31 IU/L
Note: Expected range varies from population to population and each laboratory should establish its own normal range.

Limitations
This method is linear up to 500 IU / L. If the activity exceeds 500 IU/L, dilute the sample suitably with normal saline and repeat the assay. Apply proper dilution factor to calculate the final results.

Quality Control
To ensure adequate quality control measures, it is recommended that each batch should include a normal and an abnormal commercial reference control serum. It should be realized that the use of quality control material checks both instrument and reagent functions together. Factors which might affect the performance of this test include proper instrument function, temperature control, cleanliness of glassware, Wavelength setting, Expiration date of reagents and accuracy of prob aspiration.

Accuracy-Recovery
AST/GOT added to a serum matrix containing known amounts of AST gave an average recovery of 95%.

Interference
Triglycerides below 2000 mg/dl does not interfere in the reaction. Bilirubin below 5.8 mg/dl does not interfere in the reaction. Haemoglobin interferes at concentrations above 10.0 g/L. Ascorbic Acid influences the reaction at concentrations over 30 mg/dl.

Precision of the Method

Within-run					
Range	U.M	Mean	S.D.	C.V.(%)	No. run
Low	IU/L	41.23	1.21	2.93	20
High	IU/L	185.25	1.12	0.6	20
Between run					
Range	U.M	Mean	S.D.	C.V.(%)	No. run
Low	IU/L	41.23	0.99	2.40	60
High	IU/L	185.25	1.22	0.66	60

Sensitivity
At 340 nm, the activity of AST/GOT of 5.95 IU/L can estimate.

References

1. Tietz, N.W, ed. Clinical Guide to Laboratory tests, 3rd ed. Philadelphia, pa : W.B. Saunders, 1995: 76 - 77.
2. Bergmmeyer, HU,Horder M,Rej R. Approved recommandation(1985) on IFCC methods for the measurent of catalytical concentration of enzymes, Part 3. IFCC method for alanine aminotransferase. J.Clin. Chem. Clin. Biochem. 1986 ; 24 :497 - 510.
3. Fischbach F, Zawta B. Age – dependant reference limits of several enzymes in plasma at different measuring temperatures. Clin. Lab. 1992 ; 38 :555 - 561.
4. Penttila, I.M., et al. Scand. J. Clin. Lab. Invest. 35, 275 (1975).
5. Hafkensheild. J.C.M., *et. al. J. Clin. Chem. Clin. Biochem*. 17, 219 (1979).

Symbols

IVD	In Vitro Diagnostics		Caution
LOT	Batch No.		Product Expiry Date
CONT	Content		Manufactured By
	Read Instructions		Date of Manufacture
	Storage Temperature		Keep Dry
REF	Catalogue No.		Fragile
			Keep away from sun light

KEE DIAGNOSTICS PVT LTD
(Formerly known as Kee GAD Biogen Pvt. Ltd.) .
CIN: U24231DL2004PTC128343 .
A subsidiary of KEE PHARMA LTD
A-8 . 3RD FLOOR. NARAINA INDUSTRIAL AREA, PHASE 2 . NEW DELHI 110028.
W. www.keediagnostics.in.
E. info@keediagnostics.in, T. 011 43136000.

Kee Diagnostics
because life matters