ANA BIO CLR SGOT (AST)

(UV - Kinetic Method)

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^+ at 340 nm is proportional to the activity of GOT (AST) in serum/plasma and is determined kinetically as rate of decrease in absorbance.

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

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

Reagents provided

1. R1 – Substrate Reagent
2. R2 – Enzyme Reagent

Working Reagent Preparation

Prepare working reagent by mixing **Reagent R_1** and **Reagent R_2** in the ratio 4:1 as per the number of tests required.

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.
The working reagent (4 R_1 + 1 R_2) is stable for 30 days at 2° - 8°C.

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 since erythrocytes contain approximately 10 times the normal activity of GOT (AST) found in serum. GOT (AST) activity in serum/plasma is stable for 1 week at 2° - 8°C and one month when stored at -20°C. The samples should be brought to room temperature prior to use.

Assay guidelines for Analyzers

Reaction type – Slope	UV Kinetic – Decreasing
Wavelength	340 nm
Flow Cell Temperature	37°C
Zero setting with	Distilled water
Delay time	60 seconds
Interval time	60 Seconds
No. of Intervals	3
Sample volume	100 µl (0.1 ml)
Reagent volume	1000 µl (1.0 ml)
Factor	1746
Blank absorbance limit	≥ 0.900 Abs. against water blank.
Normal value	Up to 37 IU/L
Linearity	Up to 500 IU/L

Assay guidelines for Manual Procedure

Prewarm the required amount of working reagent at 37°C before use. Perform the assay as given below:

Reagents	**Test**
Working Reagent	1000 µl (1.0 ml)
Sample	100 µl (0.1 ml)

1. Mix thoroughly and transfer the assay mixture immediately to the thermo stated cuvette and start the stop watch simultaneously.
2. Record the first reading at 60^{th} second and subsequently 3 more readings with 60 seconds interval at 340 nm.

Calculation

∆ OD is the average difference in absorption between the second OD and the first OD and vise versa.
Serum GOT (AST) (IU/L) = (∆ OD / Min) x 1746

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.
- The working solution is considered unsatisfactory and should not be used if the absorbance is less than 0.900 at 340 nm against distilled water as blank.

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 and accuracy of pipetting.

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

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