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Glutamic Oxaloacetic Transaminase (AST)

(L-Aspartate: a-Ketoglutarate amino transferase, EC 2.6.1.1) Glutamic oxaloacetic transaminase (GOT), also known as aspartate aminotransferase, catalyzes the following reaction: GOT L-Asp + a-Ketoglutarate ------> Oxaloacetate + L-Glu The enzyme is widely distributed in plants and animals, but it occurs in concentrated form in mammalian heart and liver. GOT requires pyridoxal phosphate as a coenzyme for its activity. It exists as two isozyme forms, the mitochondrial form (M-GOT) and the cytosol form (S-GOT). GOT from porcine heart has a molecular weight in the range of 91,000-94,000. Serum GOT levels in healthy subjects are low, but the levels are significantly elevated in a number of clinical conditions such as acute and chronic hepatitis, obstructive jaundice, carcinoma of liver, myocardial infraction and muscular dystrophy (Methods of Enzymatic Analysis, Bergmeyer, H.U. ed; Vol I, 6, 1974, Academic Press, New York). Therefore, determination of serum GOT level has great clinical and diagnostic significance. In addition, GOT has applications in coupled enzyme reactions for measurement of metabolite levels in biological fluids.

ASSAY

The assay is based on a coupled reaction where the oxaloacetate formed is reduced to malate in the presence of NADH and malate dehydrogenase. The decrease in the absorbance at 340 nm caused by the oxidation of NADH is proportional to the catalytic activity of GOT (Amador, E. and W.E.C. Wacker, Clin Chem, 8, 343, 1962).

REAGENTS

1. 0.1 M Potassium phosphate buffer, pH 7.5.
2. 0.008 M NADH, disodium salt (5 mg/ml) in buffer. Prepare fresh.
3. 0.3 M L-Aspartate-monopotassium salt (51.4 mg/ml) in buffer.
4. 0.60 M a-Ketoglutarate (87 mg/ml) in buffer.
5. Malate dehydrogenase (MDH). Using phosphate buffer, prepare a solution to yield a concentration of 200-250 U/ml. Prepare fresh prior to assay.
6. Glutamate-oxaloacetate transaminase (GOT) enzyme solution. Using phosphate buffer, prepare a GOT solution with a concentration in the range of 0.2-0.4 U/ml. Must be prepared fresh prior to the assay.

PROCEDURE

1. Set spectrophotometer (equipped with strip chart recorder and temperature control) at 340 nm and 25°C.
2. Into a cuvette pipette the following reagents in the amounts indicated:

   Phosphate buffer 1.00 ml

NADH 0.10 ml

L-Aspartate 1.50 ml

a-ketoglutarate 0.20 ml

MDH 0.10 ml

Mix and incubate in spectrophotometer at 25°C for 5 minutes.

3. Record absorbance at 340 nm (blank), if any.
4. Initiate the reaction by adding 0.1 ml of enzyme (GOT) solution. Record absorbance at 340 nm for 5 minutes.
5. Calculate ΔE340 nm/min

CALCULATION

$$\text{Activity (U/mg)} = \frac{(\Delta E_{340nm/min})(\text{Total Vol.})(\text{Enz. Diln.})}{(6.22)(\text{Enz. Vol.})(\text{mg Enz./ml})}$$