



## Glutamic Pyruvic Transaminase (ALT)

(L-alanine:2 oxoglutarate aminotransferase; EC 2.6.1.2) Glutamic pyruvic transaminase (GPT) is also known as alanine aminotransferase. It catalyzes the following reaction:  $GPT \text{ L-Alanine} + \alpha\text{-Ketoglutarate} \rightarrow \text{Pyruvate} + \text{L-Glutamate}$ . Transamination reactions play a significant role in intermediary metabolism. Transaminases require pyridoxal phosphate as a coenzyme for their catalytic activity. GPT is found in many animal and plant tissues and its activity is especially high in mammalian heart and liver. The enzyme exists in two distinct isoenzyme forms in mammalian tissues, the mitochondrial form and the cytoplasmic form. GPT from porcine heart has been extensively studied. It has a molecular weight of 100,000. Human GPT levels in serum are used in clinical diagnosis of liver and heart disease (Methods of Enzymatic Analysis, Bergmeyer, H.U. ed. Vol I, 14, 1974. Academic Press, New York).

### ASSAY

The decrease in the absorbance at 340 nm, caused by the oxidation of NADH, is proportional to the catalytic activity of GPT.

### REAGENTS

- 0.1 M Potassium phosphate buffer, pH 7.5.
- 1.15 M L-Alanine (103 mg/ml) in buffer.
- 0.31 M  $\alpha$ -Ketoglutarate (45 mg/ml) in buffer.
- 0.008 M NADH disodium salt (5 mg/ml) in distilled water. Prepare fresh.
- Lactate dehydrogenase (LDH), 250 U/ml in buffer. Prepare fresh immediately prior to assay.
- Enzyme (GPT) solution. Prepare in buffer to yield a final concentration of 0.1-0.2 U/ml. Must be prepared fresh immediately prior to assay.

### PROCEDURE

- Set spectrophotometer (equipped with strip chart recorder and temperature control) at 340 nm and 25°C.
  - Into a cuvette pipette the following reagents in the amounts indicated:  
L-alanine 2.60 ml  
 $\alpha$ -ketoglutarate 0.10 ml  
NADH 0.10 ml  
LDH 0.10 ml  
Mix and incubate in spectrophotometer for 5 minutes to attain temperature equilibration, then record blank rate at 340 nm.
  - Initiate the reaction by adding 0.1 ml of enzyme (GPT) solution to the cuvette.
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- Record the rate of decrease in absorbance at 340 nm for 5-8 minutes.
- Calculate  $\Delta E_{340\text{nm}/\text{min}}$

CALCULATION

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