

**Enzymatic Assay of GLUTAMIC-OXALACETIC TRANSAMINASE  
(EC 2.6.1.1)**

**PRINCIPLE:**

L-Aspartate +  $\alpha$ -Ketoglutarate  $\xrightarrow{\text{GOT}}$  L-Glutamate + Oxalacetate

Oxalacetate +  $\beta$ -NADH  $\xrightarrow{\text{MDH}}$  Malate +  $\beta$ -NAD<sup>+</sup>

Abbreviations:

GOT = Glutamic-Oxalacetic Transaminase

MDH = Malic Dehydrogenase

$\beta$ -NAD<sup>+</sup> =  $\beta$ -Nicotinamide Adenine Dinucleotide

$\beta$ -NADH =  $\beta$ -Nicotinamide Adenine Dinucleotide, Reduced Form

**CONDITIONS:** T = 37°C, pH = 7.8, A<sub>340nm</sub>, Light path = 1 cm

**METHOD:** Continuous Spectrophotometric Rate Determination

**REAGENTS:**

- A. Reaction Cocktail  
(Reconstitute AST 10 Reagent, Stock No. 58-10, in 10 ml of deionized water.)
- B. Glutamic-Oxalacetic Transaminase Solution  
(Immediately before use prepare a solution containing 0.3 - 0.6 units per ml in cold deionized water.)

**PROCEDURE:**

Pipette (in milliliters) the following reagent into suitable cuvettes:

Reagent A (Reaction Cocktail)	2.9
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Equilibrate to 37°C. Monitor the A<sub>340nm</sub> until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent B (Enzyme Solution)	0.1
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Immediately mix by inversion and record the decrease in A<sub>340nm</sub> for approximately 15 minutes. Obtain the maximum linear rate.

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**CALCULATIONS:**

$$\text{Units/ml enzyme} = \frac{(\text{r } A_{340\text{nm}}/\text{min})(3)(\text{df})}{(6.22)(0.1)}$$

3 = Total volume (in milliliters) of assay

df = Dilution factor

6.22 = Millimolar extinction coefficient of  $\beta$ -NADH at 340 nm

0.1 = Volume (in milliliter) of enzyme used

$$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

**UNIT DEFINITION:**

One unit will convert 1.0  $\mu$ mole of  $\alpha$ -ketoglutarate to L-glutamate per minute at pH 7.5 at 37°C, in the presence of L-aspartic acid.

**FINAL ASSAY CONCENTRATION:**

In a 3.0 ml reaction mix, the final concentrations are 193 mM L-Aspartate, 12 mM  $\alpha$ -Ketoglutarate, 0.6 units/ml malic dehydrogenase, 0.2 mM  $\beta$ -nicotinamide adenine nucleotide, 0.03 - 0.06 unit Glutamic-Oxalacetic Transaminase.

**NOTES:**

1. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

**This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.**