

**Enzymatic Assay of GLUTAMIC-PYRUVIC TRANSAMINASE  
(EC 2.6.1.2)**

**PRINCIPLE:**

L-Alanine +  $\alpha$ -Ketoglutaric Acid  $\xrightarrow{\text{GPT}}$  Pyruvate + L-Glutamate  
Pyruvate +  $\beta$ -NADH  $\xrightarrow{\text{Lactic Acid Dehydrogenase}}$  Lactate +  $\beta$ -NAD

Abbreviations used:

GPT = Glutamic-Pyruvic Transaminase

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

$\beta$ -NAD =  $\beta$ -Nicotinamide Adenine Dinucleotide

**CONDITIONS:** T = 37°C, pH = 7.4, A<sub>340nm</sub>, Light path = 1 cm

**METHOD:** Continuous Spectrophotometric Rate Determination

**REAGENTS:**

- A. 100 mM Tris Buffer, pH 7.4 at 37°C.  
(Prepare 100 ml in deionized water using Trizma Base, Prod. No. T-1503. Adjust to pH 7.4 at 37°C with 1 M HCl.)
- B. 100 mM  $\alpha$ -Ketoglutaric Acid Solution ( $\alpha$ -KGA)  
(Prepare 10 ml in Reagent A using  $\alpha$ -Ketoglutaric Acid, Monosodium Salt, Prod. No. K-1875.)
- C. 1 mM L-Alanine Solution  
(Prepare 10 ml in Reagent A using L-Alanine, Prod. No. A-7627.)
- D. 6.4 mM  $\beta$ -Nicotinamide Adenine Dinucleotide, Reduced Form Solution ( $\beta$ -NADH)  
(Dissolve the contents of one 10 mg vial of  $\beta$ -Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Preweighed Vial, Stock No. 340-110, in the appropriate volume of Reagent A.)

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**REAGENTS:** (continued)

- E. Lactic Dehydrogenase Enzyme Solution (LDH)  
(Immediately before use, prepare a solution containing 400 - 600 units/ml in cold deionized water using Lactic Dehydrogenase, Prod. No. L-2500.)
- F. Glutamic-Pyruvic Transaminase Enzyme Solution  
(Immediately before use, prepare a solution containing 0.3 - 0.6 units/ml of Glutamic-Pyruvic Transaminase in cold deionized water.)

**PROCEDURE:**

Prepare a reaction cocktail by pipetting (in milliliters) the following reagents into a suitable container:

Reagent A (Buffer)	18.5
Reagent B ( $\alpha$ -KGA)	3.0
Reagent C (L-Alanine)	6.0
Reagent D ( $\beta$ -NADH)	0.5
Reagent E (LDH)	1.0

Mix and adjust to pH 7.4 at 37°C with 1 M NaOH or 1 M HCl, if necessary.

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

	<u>Test</u>	<u>Blank</u>
Reaction Cocktail	2.90	2.90

Equilibrate to 37°C. Monitor the  $A_{340nm}$  until constant, using a suitably thermostatted spectrophotometer. Then add:

Deionized Water	-----	0.10
Reagent F (Enzyme Solution)	0.10	-----

Immediately mix by inversion and record the decrease in  $A_{340nm}$  for approximately 5 minutes. Obtain the  $r A_{340nm}/\text{minute}$  using the maximum linear rate for both the Test and Blank.

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**CALCULATIONS:**

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

3 = Total volume (in milliliters) of assay

df = Dilution factor

6.22 = Millimolar extinction coefficient of NADH at 340nm

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

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

**UNIT DEFINITION:**

One unit will convert 1.0  $\mu$ Mole of a-ketoglutarate to L-glutamate per minute at pH 7.4 at 37°C, in the presence of L-alanine.

**FINAL ASSAY CONCENTRATION:**

In a 3.00 ml reaction mix, the final concentrations are 93 mM Tris, 10 mM a-ketoglutarate, 200 mM L-alanine, 0.11 mM  $\beta$ -NADH, 13 - 20 units lactic dehydrogenase and 0.03 - 0.06 units glutamic pyruvic transaminase.

**NOTES:**

1. Lactic Dehydrogenase Unit Definition: One unit will reduce 1.0  $\mu$ mole of pyruvate to L-lactate per minute at pH 7.5 at 37°C.
2. 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.**