Enzymatic Assay of GLUTAMIC-PYRUVIC TRANSAMINASE
(EC 2.6.1.2)

PRINCIPLE:

L-Alanine + a-Ketoglutaric Acid $\xrightarrow{GPT}$ Pyruvate + L-Glutamate

Pyruvate + ß-NADH $\xrightarrow{\text{Lactic Acid Dehydrogenase}}$ Lactate + ß-NAD

Abbreviations used:
GPT = Glutamic-Pyruvic Transaminase
ß-NADH = ß-Nicotinamide Adenine Dinucleotide, Reduced Form
ß-NAD = ß-Nicotinamide Adenine Dinucleotide

CONDITIONS: T = 37°C, pH = 7.4, A_{340nm}, 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 a-Ketoglutaric Acid Solution (a-KGA)
(Prepare 10 ml in Reagent A using a-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 ß-Nicotinamide Adenine Dinucleotide, Reduced Form Solution (ß-NADH)
(Dissolve the contents of one 10 mg vial of ß-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 (a-KGA)	3.0
Reagent C (L-Alanine)	6.0
Reagent D (ß-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:

	Test	Blank
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 ΔA_{340nm}/minute using the maximum linear rate for both the Test and Blank.

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CALCULATIONS:

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

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 µMole 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 ß-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 µmole 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.