Enzymatic Assay of Glutamic Oxalacetic Transaminase (GOT, EC 2.6.1.1)

Description
This procedure may be used for Glutamic Oxalacetic Transaminase (GOT) products.

The continuous spectrophotometric rate determination (A_{340}, Light path = 1 cm) is based on the following reactions:

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

$$\text{Oxalacetate} + \beta\text{-NADH} \xleftrightarrow{\text{MDH}} \text{Malate} + \beta\text{-NAD}^+$$

where:
GOT – Glutamic Oxalacetic Transaminase
β-NADH – β-Nicotinamide Adenine Dinucleotide, Reduced Form
MDH – Malic Dehydrogenase

Unit Definition: One unit will convert 1.0 μmole of α-ketoglutarate to L-glutamate per minute at pH 7.5 at 37°C, in the presence of L-aspartic acid.

Precautions
Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Reagents and Equipment Required
Potassium phosphate, monobasic (Catalog Number P5379)
α-Ketoglutaric acid (Catalog Number K1750)
L-Aspartic acid, potassium salt (Catalog Number A6558)
β-Nicotinamide adenine dinucleotide, reduced form, disodium salt, hydrate (Catalog Number N8129)
Malic Dehydrogenase (Catalog Number M2634)

Preparation Instructions
Use ultrapure water (≥18 MΩ×cm resistivity at 25°C) for the preparation of reagents.

Buffer (100mM Potassium Phosphate, pH 7.5 at 37°C) – Prepare a 13.6 mg/ml solution in ultrapure water using Potassium phosphate, monobasic (Catalog Number P5379). Adjust to pH 7.5 at 37°C with 1M KOH.

α-Ketoglutarate solution (100mM) – Prepare a 14.6 mg/ml solution in ultrapure water using α-Ketoglutaric acid (Catalog Number K1750). Adjust to pH 7.5 at 37°C with 1M KOH.

L-Asp Diluent (350mM Potassium phosphate, pH 7.5 at 37°C) – Prepare a 47.6 mg/ml solution in ultrapure water using Potassium phosphate, monobasic (Catalog Number P5379). Adjust to pH 7.5 at 37°C with 1M KOH.

L-Asp solution (400mM L-Aspartic ccid) – Prepare a 68.5 mg/ml solution in L-Asp Diluent using L-Aspartic acid, potassium salt (Catalog Number A6558).

β-NADH solution (1.90mM β-Nicotinamide Adenine Dinucleotide, Reduced form) – Prepare a 1.90mM solution in Buffer using β-Nicotinamide adenine dinucleotide, reduced form, disodium salt, hydrate (Catalog Number N8129). **Prepare Fresh** and correct for water and solvent content.

MDH Solution (Malic Dehydrogenase) – Prepare a 50-100 units/mL solution in cold ultrapure water using Malic Dehydrogenase (Catalog Number M2634). **Prepare Fresh**.

GOT solution (Glutamic Oxalacetic Transaminase Enzyme Solution) – Immediately before use, prepare a solution containing 0.3–0.6 unit/ml of Glutamic Oxalacetic Transaminase in cold ultrapure water.

Procedure
In a 3.00 mL reaction mix, the final concentrations are 124mM Potassium Phosphate, 6.6mM α-Ketoglutaric acid, 60mM L-Aspartic acid, 0.16mM β-NADH, 5–10 units Malic Dehydrogenase, and 0.015–0.06 unit Glutamic Oxalacetic Transaminase.

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

Reagent	Test 1	Test 2	Test 3	Blank
Buffer	1.40	1.40	1.40	1.40
α-Ketoglutarate solution	0.20	0.20	0.20	0.20
L-Asp solution	0.50	0.50	0.50	0.50
β-NADH solution	0.26	0.26	0.26	0.26
MDH Solution	0.10	0.10	0.10	0.10
Ultrapure Water	0.49	0.47	0.44	0.54

2. Mix by inversion and equilibrate to 37°C. Monitor the A_{340} until constant, using a suitably thermostatted spectrophotometer. Then add (in milliliters):

Reagent	Test 1	Test 2	Test 3	Blank
GOT solution	0.05	0.07	0.10	–

3. Immediately mix by inversion and record the decrease in A_{340} for ~5 minutes. Obtain the ΔA_{340}/minute using the maximum linear rate for both the Tests and Blank.

Results
Calculations

1. Units/ml enzyme = $\dfrac{(\Delta A_{340}/\text{min Test} - \Delta A_{340}/\text{min Blank})\ (3)\ (df)}{(V_e)\ (6.22)}$

where:
3 = Total volume (in milliliters) of assay
df = Dilution factor
6.22 = Millimolar extinction coefficient of β-NADH at 340nm
V_e= Volume (in milliliter) of enzyme used

2.

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