



SIGMA QUALITY CONTROL TEST PROCEDURE

Product Information

Enzymatic Assay of GLUTAMIC-OXALACETIC TRANSAMINASE (EC 2.6.1.1)

PRINCIPLE:

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

Oxalacetate + β -NADH $\xrightarrow{\text{MDH}}$ Malate + β -NAD⁺

Abbreviations:

GOT = Glutamic-Oxalacetic Transaminase

MDH = Malic Dehydrogenase

β -NAD⁺ = β -Nicotinamide Adenine Dinucleotide

β -NADH = β -Nicotinamide Adenine Dinucleotide, Reduced Form

CONDITIONS: T = 37°C, pH = 7.8, A_{340nm}, 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_{340nm} 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_{340nm} for approximately 15 minutes. Obtain the maximum linear rate.

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CALCULATIONS:

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

3 = Total volume (in milliliters) of assay

df = Dilution factor

6.22 = Millimolar extinction coefficient of β -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 μ mole of α -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 α -Ketoglutarate, 0.6 units/ml malic dehydrogenase, 0.2 mM β -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.

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