SIGMA QUALITY CONTROL TEST PROCEDURE

ProductInformation

Enzymatic Assay of
TRANSAMINASE, Broad-Range

PRINCIPLE:

$$\text{L-Aspartate} + \beta\text{-Ketoglutarate} + \beta\text{-NADH} \xrightarrow[\text{PRP}]{\text{TG}} \text{Oxaloacetate} + \text{L-Glutamate} + \beta\text{-NADH}$$

CONDITIONS: T=25°C, pH=7.0, A_{340nm}, Light path=1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 50 mM Potassium Phosphate Buffer, pH 7.0 at 25°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 7.0 at 25°C with 1 M KOH.)

B. 200 mM L-Aspartate Solution, pH 7.0 at 25°C (L-Asp)
(Prepare 10 ml in Reagent A using L-Aspartic Acid, Monosodium Salt, Hydrate, Sigma Prod. No. A-6683.)

C. 200 mM α-Ketoglutaric Acid Solution (α-KG)
(Prepare 10 ml in Reagent A using α-Ketoglutaric Acid, Monopotassium Salt, Sigma Prod. No. K-2000.)

D. 10 mM Pyridoxal 5-Phosphate Solution (PRP)
(Prepare 10 ml in deionized water using Pyridoxal 5-Phosphate, Sigma Prod. No. P-9255).

E. 6.4 mM β-Nicotinamide Adenine Dinucleotide, Reduced Form, Solution (β-NADH)
(Prepare 5 ml in deionized water using β-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Prod. No. N-8129.)

F. Malic Dehydrogenase Enzyme Solution (MDH)
(Use Malic Dehydrogenase, Sigma Prod. No. M-9004).

G. Transaminase Broad-Range Enzyme Solution (TBR)
(Immediately before use, prepared solution containing 0.50 – 2.0 units/ml in cold Reagent A.)

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PROCEDURE:

Pipette (in milliliters) the following reagents into a suitable cuvette:

	Test	Blank
Reagent A (Buffer)	2.30	2.30
Reagent C (α-KG)	0.30	0.30
Reagent B (L-ASP)	0.30	0.30
Reagent D (PRP)	0.06	0.06
Reagent E (β-NADH)	0.045	0.045
Reagent F (MDH)	0.01	0.01

Mix by inversion and equilibrate to 25°C. Monitor the A_{340nm} until constant using a suitably thermostatted spectrophotometer. Then add:

Reagent G (TBR)	0.025	------
Reagent A (Buffer)	------	0.025

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

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{340nm}/\text{min Test} - \Delta A_{340nm}/\text{min Blank})(3.04)(df)}{(6.22)(0.025)}$$

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

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

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

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UNIT DEFINITION:

One unit will produce 1.0 µmole of oxaloacetate from L-aspartate during the transamination of 2-ketoglutarate per minute at pH 7.0 at 25°C in the presence of pyridoxal phosphate.

FINAL CONCENTRATION:

In a 3.04 ml reaction mix, the final concentrations are 38 mM potassium phosphate, 19.7 mM L-aspartate, 19.7 mM α-ketoglutaric acid, 0.2 mM pyridoxal 5-phosphate, 0.1 mM β-nicotinamide adenine dinucleotide, reduced form, 20 units malic dehydrogenase, 0.0125– 0.05 units transaminase.

REFERENCE:

1. This assay is based on the cited reference.

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

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