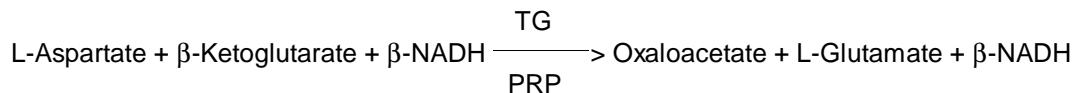


SIGMA QUALITY CONTROL TEST PROCEDURE

Product Information

**Enzymatic Assay of
TRANSAMINASE, Broad-Range****PRINCIPLE:****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.)

**Enzymatic Assay of
TRANSAMINASE, Broad-Range**

PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
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 $A_{340\text{nm}}$ 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 $A_{340\text{nm}}$ for approximately 5 minutes. Obtain the $\Delta A_{340}/\text{min}$ using the maximum linear rate for both the Test and the Blank.

CALCULATIONS:

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

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

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

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

**Enzymatic Assay of
TRANSAMINASE, Broad-Range**

UNIT DEFINITION:

One unit will produce 1.0 μ mole 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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