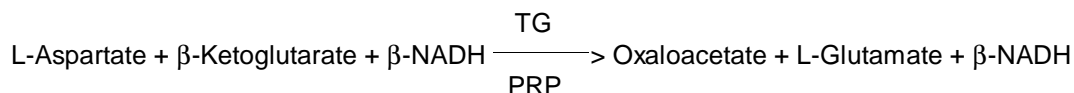


SIGMA QUALITY CONTROL TEST PROCEDURE

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.

Sigma warrants that the above procedure information is currently utilized at Sigma and that all Sigma-Aldrich, Inc. products conform to the information in this and other Sigma-Aldrich, Inc. publications. Purchaser must determine the suitability of the information and product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packaging slip.