Enzymatic Assay of L-ALANINE DEHYDROGENASE
(EC 1.4.1.1)

PRINCIPLE:

L-Alanine + ß-NAD + H_2O $\xrightarrow{\text{L-Alanine Dehydrogenase}}$ Pyruvate + ß-NADH + NH_3

Abbreviations used:
ß-NAD = ß-Nicotinamide Adenine Dinucleotide, Oxidized Form
ß-NADH = ß-Nicotinamide Adenine Dinucleotide, Reduced Form

CONDITIONS: T = 25°C, pH = 10.0, A_{340nm}, Light Path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 50 mM Sodium Bicarbonate Solution ($NaHCO_3$)
(Prepare 100 ml in deionized water using Sodium Bicarbonate, Prod. No. S-8875.)

B. 50 mM Sodium Carbonate Solution (Na_2CO_3)
(Prepare 150 ml in deionized water using Sodium Carbonate, Anhydrous, Prod. No. S-2127.)

C. 50 mM Sodium Carbonate Buffer, pH 10.0 at 25°C
(Prepare 200 ml by adding 100 ml of Reagent B to 100 ml of Reagent A. Adjust to pH 10.0 at 25°C with Reagent B.)

D. 500 mM L-Alanine Solution (L-ALA)
(Prepare 1.0 ml in deionized water using L-Alanine, Prod. No. A-7627.)

E. 30 mM ß-Nicotinamide Adenine Dinucleotide, Oxidized Form Solution (ß-NAD)
(Dissolve the contents of one 20 mg vial of ß-Nicotinamide Adenine Dinucleotide, Stock No. 260-120, in the appropriate volume of deionized water. **PREPARE FRESH.)**

F. L-Alanine Dehydrogenase Enzyme Solution
(Immediately before use, prepare a solution containing 0.2 - 0.5 unit/ml of L-Alanine Dehydrogenase in cold Reagent C.)

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PROCEDURE:

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

	Test	Blank
Reagent C (Buffer)	2.70	2.70
Reagent D (L-ALA)	0.10	0.10
Reagent E (ß-NAD) 0.10	0.10	

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

Reagent C (Buffer)	------	0.10
Reagent F (Enzyme Solution)	0.10	------

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

CALCULATIONS:

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

3 = Volume (in milliliters) of assay
df = Dilution factor
6.22 = Millimolar extinction coefficient of ß-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 µmole of L-alanine to pyruvate and NH_3 per minute at pH 10.0 at 25°C.

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FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 47 mM sodium bicarbonate, 17 mM L-alanine, 1.0 mM ß-nicotinamide adenine dinucleotide and 0.02 - 0.05 unit of L-alanine dehydrogenase.

REFERENCE:

Bergmeyer, H.U. (1983) *Methods of Enzymatic Analysis*, 2nd edition, Volume I, 427.

NOTES:

1. This assay is based on the cited reference.

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

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.