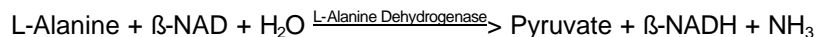


Enzymatic Assay of L-ALANINE DEHYDROGENASE (EC 1.4.1.1)

PRINCIPLE:



Abbreviations used:

β -NAD = β -Nicotinamide Adenine Dinucleotide, Oxidized Form

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

CONDITIONS: T = 25°C, pH = 10.0, $A_{340\text{nm}}$, 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 β -Nicotinamide Adenine Dinucleotide, Oxidized Form Solution (β -NAD)
(Dissolve the contents of one 20 mg vial of β -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:

	<u>Test</u>	<u>Blank</u>
Reagent C (Buffer)	2.70	2.70
Reagent D (L-ALA)	0.10	0.10
Reagent E (β -NAD) 0.10	0.10	

Mix by inversion and equilibrate to 25°C. Monitor the $A_{340\text{nm}}$ 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 $A_{340\text{nm}}$ for approximately 5 minutes. Obtain the $r A_{340\text{nm}}/\text{minute}$ using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

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

3 = 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 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 β -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.