

Enzymatic Assay of D-AMINO ACID OXIDASE APOENZYME

PRINCIPLE:

D-Alanine + O₂ + H₂O $\xrightarrow{\text{D-Amino Acid Oxidase Apoenzyme}}$ Pyruvate + NH₃ + H₂O₂

2 H₂O₂ $\xrightarrow{\text{Catalase}}$ 2 H₂O + O₂

Pyruvate + β-NADH $\xrightarrow{\text{LDH}}$ L-Lactic Acid + β-NAD

Abbreviations used:

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

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

CONDITIONS: T = 25°C, pH = 8.3, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 200 mM Tris HCl Buffer, pH 8.3 at 25°C
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 8.3 at 25°C with 1 M HCl.)
- B. 224 mM D-Alanine Solution (D-Ala)
(Prepare 5 ml in deionized water using D-Alanine, Sigma Prod. No. A-7377.)
- C. 6.4 mM β-Nicotinamide Adenine Dinucleotide, Reduced Form (β-NADH)
(Dissolve the contents of one 5 mg vial of β-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Stock No. 340-105, in the appropriate volume of Reagent A. **PREPARE FRESH.**)
- D. Catalase Enzyme Solution (Catalase)
(Immediately before use, prepare a solution containing 600 units/ml of Catalase, Sigma Stock No. C-100, in cold deionized water.)
- E. L-Lactic Dehydrogenase Enzyme Solution (LDH)
(Immediately before use, prepare a solution containing 400 units/ml of L-Lactic Dehydrogenase, Sigma

Prod. No. L-2500, in cold deionized water.

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REAGENTS: (continued)

- F. D-Amino Acid Oxidase Apoenzyme (D-AAO)
(Immediately before use, prepare a solution containing 1 mg/ml of D-Amino Acid Oxidase Apoenzyme in cold deionized water.)

PROCEDURE:

Saturate Reagent A (Buffer) with O₂ by bubbling oxygen gas through Reagent A (Buffer) for 5 minutes immediately before use.

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

	<u>Test</u>	<u>Blank</u>
Reagent A (O ₂ Saturated Buffer)	2.25	2.25
Reagent B (D-Ala)	0.50	0.50
Reagent C (β-NADH)	0.05	0.05
Reagent D (Catalase)	0.05	0.05
Reagent E (LDH)	0.05	0.05

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

Reagent F (D-AAO)	0.10	-----
Deionized Water	-----	0.10

Immediately mix by inversion and record the decrease in A_{340nm} for approximately 5 minutes. Obtain the r A_{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 = Total volume (in milliliters) of the assay

df = Dilution factor

6.22 = Millimolar extinction coefficient of β-NADH
at 340 nm

0.1 = Volume (in milliliter) of enzyme used in assay

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

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CALCULATIONS: (continued)

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

UNIT DEFINITION:

One unit will oxidatively deaminate 1.0 μ mole of D-alanine to pyruvate per minute at pH 8.3 at 25°C, in the presence of catalase.

FINAL ASSAY CONCENTRATIONS:

In a 3.00 ml reaction mix, the final concentrations are 153 mM Tris, 37 mM D-alanine, 0.11 mM β -nicotinamide adenine dinucleotide, reduced form, 30 units catalase, 20 units L-lactic dehydrogenase, and 0.1 mg D-amino acid oxidase apoenzyme.

REFERENCE:

Bergmeyer, H.U., Gawehn, K., and Grassl, M. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H.U. ed.) 2nd ed., Volume I, 431-432, Academic Press, New York, NY

Massey, V. and Curti, B. (1966) *Journal of Biological Chemistry* **241**, 3417-3423

NOTES:

1. This assay is based on the cited references.
2. L-Lactic Dehydrogenase Unit Definition: One unit will reduce 1.0 μ mole of pyruvate to L-lactate per minute at pH 7.5 at 37°C.
3. Catalase Unit Definition: One unit will decompose 1.0 μ mole of H₂O₂ per minute at pH 7.0 at 25°C while the H₂O₂ concentration falls from 10.3 to 9.2 mM. The rate of disappearance of H₂O₂ is followed by observing the rate of decrease in absorbance at 240 nm.
4. 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.