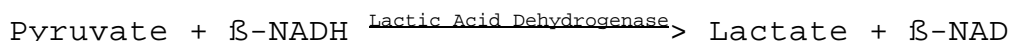
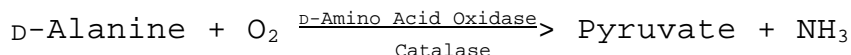


**Enzymatic Assay of D-AMINO ACID OXIDASE
(EC 1.4.3.3)**

PRINCIPLE:



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, Prod. No. T-1503. Adjust to pH 8.3 at 25°C with 1 M HCl. Bubble oxygen through the buffer for 5 minutes immediately before use.)
- B. 224 mM D-Alanine Solution
(Prepare 5 ml in deionized water using D-Alanine, Prod. No. A-7377.)
- C. 6.4 mM β -Nicotinamide Adenine Dinucleotide, Reduced Form Solution (β -NADH)
(Dissolve the contents of one 5 mg vial of β -Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Stock No. 340-105, in the appropriate volume of Reagent A. **PREPARE FRESH.**)
- D. Catalase Enzyme Solution
(Immediately before use, prepare a solution containing 600 units/ml in cold deionized water using Catalase, Stock No. C-100.)

**Enzymatic Assay of D-AMINO ACID OXIDASE
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REAGENTS: (continued)

- E. Lactic Dehydrogenase Enzyme Solution (LDH)
(Immediately before use, prepare a solution containing 400 units/ml in cold deionized water using L-Lactic Dehydrogenase, Prod. No. L-2500.)
- F. 3.6 M Ammonium Sulfate Solution, pH 6.5 at 25°C (Enz Dil)
(Prepare 25 ml in deionized water using Ammonium Sulfate, Sigma Prod. No. A-4915. Adjust to pH 6.5 at 25°C with 5 M NH₄OH.)
- G. D-Amino Acid Oxidase Enzyme Solution (D-AAO)
(Immediately before use, prepare a solution containing 0.4 - 0.8 unit/ml of D-Amino Acid Oxidase in cold Reagent F.)

PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	2.25	2.25
Reagent B (D-Alanine Solution)	0.50	0.50
Reagent C (β-NADH)	0.050	0.050
Reagent D (Catalase)	0.050	0.050
Reagent E (LDH)	0.050	0.050

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

Deionized Water	-----	0.10
Reagent G (D-AAO)	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:

$$\text{Units/mg enzyme} = \frac{r_{A_{340\text{nm}}/\text{min Test}} - r_{A_{340\text{nm}}/\text{min Blank}}}{(6.22) (\text{mg enzyme/ml RM})}$$

6.22 = Millimolar extinction coefficient of β-NADH at 340

nm
RM = Reaction Mix

**Enzymatic Assay of D-AMINO ACID OXIDASE
(EC 1.4.3.3)**

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. (This is equivalent to an O₂ uptake of approximately 335 μ l in 30 minutes.)

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 153 mM Tris, 37 mM D-alanine, 0.11 mM β -NADH, 30 units catalase, 20 units lactic acid dehydrogenase, 120 mM ammonium sulfate, and 0.04 - 0.08 unit D-amino acid oxidase.

REFERENCES:

Bergmeyer, H.U. (1974) *Methods of Enzymatic Analysis*, Vol. 1, 2nd edition, 431.

NOTES:

1. 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.
2. 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. All product and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.

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