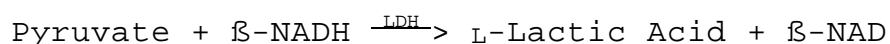
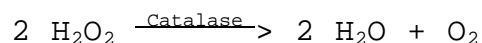
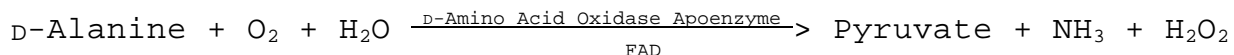


**Enzymatic Assay of D-AMINO ACID OXIDASE APOENZYME  
(Reactivation Assay)<sup>1</sup>**

**PRINCIPLE:**



Abbreviations used:

FAD = Flavin Adenine Dinucleotide

$\beta$ -NADH =  $\beta$ -Nicotinamide Adenine Dinucleotide, Reduced Form

$\beta$ -NAD =  $\beta$ -Nicotinamide Adenine, Dinucleotide, Oxidized Form

**CONDITIONS:** T = 25°C, pH = 8.3, A<sub>340nm</sub>, 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  $\beta$ -Nicotinamide Adenine Dinucleotide, Reduced Form ( $\beta$ -NADH)  
(Dissolve the contents of one 5 mg vial of  $\beta$ -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.)

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**REAGENTS:** (continued)

- 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.)
- 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.)
- G. 50 mM Pyrophosphate Buffer, pH 8.5 at 25°C (PPi)  
(Prepare 5 ml in deionized water using Pyrophosphate, Tetrasodium, Decahydrate, Sigma Prod. No. P-9146. Adjust to pH 8.5 at 25°C with 1 M HCl.)
- H. 60 mM Flavin Adenine Dinucleotide Solution (FAD)  
(Prepare 1 ml in Reagent G using Flavin Adenine Dinucleotide, Disodium Salt, Sigma Prod. No. F-6625.)

**PROCEDURE:**

Reactivation of D-Amino Acid Oxidase Apoenzyme: Combine 1 ml of Reagent F (D-AAO) with 0.1 ml of Reagent H (FAD). Incubate at 25°C for 30 - 45 minutes.

Saturate Reagent A (Buffer) with O<sub>2</sub> 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 <sub>2</sub> 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<sub>340nm</sub> until constant, using a suitably thermostatted spectrophotometer. Then add:

Reactivated D-Amino Oxidase Apoenzyme	0.10	-----
Deionized Water	-----	0.10

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**PROCEDURE:** (continued)

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

df = Dilution factor

6.22 = Millimolar extinction coefficient of  $\beta$ -NADH  
at 340 nm

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

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

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

**UNIT DEFINITION:**

One unit will oxidatively deaminate 1.0  $\mu$ 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  $\beta$ -nicotinamide adenine dinucleotide, reduced form, 30 units catalase, 20 units L-lactic dehydrogenase, 0.2 mM flavin adenine dinucleotide, 0.2 mM pyrophosphate, 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

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**NOTES:**

1. This assay is used to measure the D-Amino Acid Oxidase activity of D-Amino Acid Oxidase Apoenzyme, after it has been reactivated by incubating it with flavin adenine dinucleotide.
2. Catalase Unit Definition: One unit will decompose 1.0  $\mu$ mole of H<sub>2</sub>O<sub>2</sub> per minute at pH 7.0 at 25°C, while the H<sub>2</sub>O<sub>2</sub> concentration falls from 10.3 to 9.2 mM. The rate of disappearance of H<sub>2</sub>O<sub>2</sub> is followed by observing the rate of decrease in absorbance at 240 nm.
3. L-Lactic Dehydrogenase Unit Definition: One unit will reduce 1.0  $\mu$ mole of pyruvate to L-lactate per minute at pH 7.5 at 37°C.
4. This assay is based on the cited reference.
5. 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.**