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

PRINCIPLE:

$$\text{D-Alanine} + O_2 \xrightarrow[\text{Catalase}]{\text{D-Amino Acid Oxidase}} \text{Pyruvate} + NH_3$$

$$\text{Pyruvate} + \text{ß-NADH} \xrightarrow{\text{Lactic Acid Dehydrogenase}} \text{Lactate} + \text{ß-NAD}$$

Abbreviations used:
ß-NADH = ß-Nicotinamide Adenine Dinucleotide, Reduced Form
ß-NAD = ß-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 ß-Nicotinamide Adenine Dinucleotide, Reduced Form Solution (ß-NADH)
(Dissolve the contents of one 5 mg vial of ß-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 (EC 1.4.3.3)

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 NH_4OH.

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:

	Test	Blank
Reagent A (Buffer)	2.25	2.25
Reagent B (D-Alanine Solution)	0.50	0.50
Reagent C (ß-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:

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

6.22 = Millimolar extinction coefficient of ß-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 µmole of D-alanine to pyruvate per minute at pH 8.3 at 25°C, in the presence of catalase. (This is equivalent to an O_2 uptake of approximately 335 µl 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 ß-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 µmole of H_2O_2 per minute at pH 7.0 at 25°C, while the H_2O_2 concentration falls from 10.3 to 9.2 mM.

2. Lactic Dehydrogenase Unit Definition: One unit will reduce 1.0 µmole 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.