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D-Amino Acid Oxidase (DAAO)

(D-Amino acid: oxygen oxidoreductase (deaminating); EC 1.4.3.3)

D-Amino acid oxidase catalyzes the oxidation of D-amino acids as shown below:

$$RCHNH_2COOH + O_2 + H_2O \xrightarrow{D\text{-}AAO} RCOCOOH + NH_3 + H_2O_2$$

ASSAY

The assay is based on the method described by Bergmeyer (Methods of Enzymatic Analysis, Bergmeyer, H.U. ed. Vol 1, 431, 1974, Academic Press, New York). The decrease in the absorbance at 340 nm, due to the oxidation of NADH, is a measure of D-amino acid oxidase activity.

REAGENTS

1. 0.2 M Tris-HCl buffer, pH 8.3.
2. 0.02 M D-Alanine (17.8 mg/ml) in buffer.
3. 0.008 M NADH disodium salt (5 mg/ml) in buffer.
4. Catalase (200 U/ml) in buffer. Prepare fresh.
5. Lactate dehydrogenase (LDH) (200 U/ml) in buffer. Prepare fresh.
6. FAD (Prepare1 mg/ml solution)
7. D-Amino acid oxidase solution. Dilute in buffer to give a concentration of 0.1-0.5 U/ml. Must be prepared fresh prior to assay.

PROCEDURE

1. Set spectrophotometer (equipped with a strip chart recorder and temperature control) at 340 nm and 37°C.
2. Bubble oxygen through the buffer for 5-10 min. to saturate it with oxygen.
3. In a cuvette, pipette the following reagents in the amounts indicated:
   Tris buffer (oxygenated) 2.00 ml
   D-Alanine 0.50 ml
   NADH 0.10 ml
   Catalase 0.10 ml
   LDH 0.10 ml
   FAD 0.10 ml

   Incubate in spectrophotometer at 37°C for 5 min. to attain temperature equilibration. Record absorbance at 340 nm (blank).

4. Initiate the reaction by adding 0.1 ml D-amino acid oxidase (enzyme) to the cuvette. Follow the reaction by recording the decrease in the absorbance at 340 nm for 5-8 min.
5. Calculate ΔE340nm/min

CALCULATION

$$\text{Activity (U/mg)} = \frac{(\Delta E_{340nm/min})(\text{Total Vol.})(\text{Enz. Diln.})}{(6.22)(\text{Enz. Vol.})(\text{mg Enz./ml})}$$