

Active Porcine D-Amino Acid Oxidase

Cat. No. CED055 **Lot. No.** (See product label)

SPECIFICATION

Product Overview

Species	Pig
Source	Porcine Kidney
Description	D-Amino acid oxidase catalyzes the oxidation of D-aminoacids as shown below: $\text{RCHNH}_2\text{COOH} + \text{O}_2 + \text{H}_2\text{O} \rightarrow \text{RCOCOOH} + \text{NH}_3 + \text{H}_2\text{O}_2$. The D isomers of alanine, methionine, valine, isoleucine, phenylalanine and proline serve as good substrates while the L isomers do not react at all. The enzyme is a flavoprotein. D-amino acid oxidase from porcine kidney has been extensively studied. It has a monomeric molecular weight of 38,000-39,000. D-Amino acid oxidase has several possible applications such as the determination of D-amino acids, the separation of natural L-amino acid isomers from a racemic mixture and in the preparation of keto acids. The usefulness and application of D-amino acid oxidase can be significantly increased if it is available in an immobilized form.
Form	Freeze-dried powder
Solubility	Distilled water or dilute buffer
Stability	Store at -20° C
Activity	15-20U/mg protein

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Protein	90%(biuret)
Unit Definition	The amount of enzyme that will deaminate by oxidation one micromole of D-alanine to pyruvate per minute at pH 8.3, at 37°C in the presence of catalase.
Assay Method	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 (Prepare 1mg/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 $\Delta E_{340\text{nm}}/\text{min}$.