Active Porcine D-Amino Acid Oxidase

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

SPECIFICATION

Species	Pig
Source	Porcine Kidney
Description	D-Amino acid oxidase catalyzes the oxidation of D-aminoacids as shown below:RCHNH2COOH + O2 + H2O----->RCOCOOH+ NH3 + H2O2. The D isomers of alanine,methionine, valine, isoleucine, phenylalanine and proline serve as goodsubstrates while the L isomers do not react at all. The enzyme is aflavoprotein. D-amino acid oxidase from porcine kidney has been extensivelystudied. It has a monomeric molecular weight of 38,000-39,000. D-Amino acidoxidase has several possible applications such as the determination ofD-amino acids, the separation of natural L-amino acid isomers from a racemicmixture and in the preparation of keto acids. The usefulness and applicationof D-amino acid oxidase can be significantly increased if it is available inan immobilized form.
Form	Freeze-driedpowder
Solubility	Distilledwater or dilute buffer
Stability	Storeat -20° C
Activity	15-20U/mg protein
Protein	90%(biuret)

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Unit Definition	The amount of enzyme that will deaminate by oxidationone micromole of D-alanine to pyruvate per minute at pH 8.3, at 37°C in thepresence 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 tothe 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 MD-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). Lactatedehydrogenase (LDH) (200 U/ml) in buffer. Prepare fresh. (6). FAD (Prepare1mg/ml solution). (7). D-Amino acid oxidase solution. Dilute in buffer to givea concentration of 0.1-0.5 U/ml. Must be prepared fresh prior to assay.
Procedure	(1). Setspectrophotometer (equipped with a strip chart recorder and temperaturecontrol) at 340 nm and 37°C. (2). Bubble oxygen through the buffer for 5-10min. to saturate it with oxygen. (3). In a cuvette, pipette the followingreagents in the amounts indicated: Tris buffer (oxygenated) 2.00 ml;D-Alanine 0.50 ml; NADH 0.10 ml; Catalase 0.10 ml ; LDH 0.10ml; FAD 0.10 ml. Incubate in spectrophotometer at 37°C for 5 min. toattain temperature equilibration. Record absorbance at 340 nm (blank). (4).Initiate the reaction by adding 0.1 ml D-amino acid oxidase (enzyme) to thecuvette. Follow the reaction by recording the decrease in the absorbance at340 nm for 5-8 min. (5). Calculate ΔE340nm/min.

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