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Amino Acid Oxidase, L- Assay

Method

The reaction velocity is determined in a peroxidase coupled system by measuring the increase in A_{436} resulting from the oxidation of L-leucine. One unit oxidizes one micromole of L-leucine per minute at 25° C and pH 7.6 under the specified conditions.

Reagents

- 0.2 M Triethanolamine buffer pH 7.6 containing 0.1% L-leucine and 0.0065% o-dianisidine
- 1.0% Peroxidase: Dissolve Peroxidase (HPOD) at 10 mg/ml in water.

Enzyme

Dilute enzyme in reagent grade water to 0.05-0.2 units per milliliter.

Procedure

Adjust spectrophotometer to 436 nm and 25°C.

Pipette into cuvettes 0.01 ml of 10 mg/ml peroxidase and 2.9 ml of 0.2 M triethanolamine-leucine-o-dianisidine mixture.

Incubate in spectrophotometer at 25°C for 4-5 minutes to achieve temperature equilibration and record blank rate, if any. Add 0.1 ml of appropriately diluted enzyme and record increase in absorbance at 436 nm for 4-5 minutes. Calculate ΔA_{436} from the initial linear portion of the slope. Subtract blank rate if present.