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Carboxypeptidase B Assay

Method

Activity is measured by the spectrophotometric method of Folk et al. (1960) where the reaction velocity is determined by an increase in absorbance at 254 nm resulting from the hydrolysis of hippuryl-L-arginine. One unit causes the hydrolysis of one micromole of hippuryl-L-arginine per minute at 25° C and pH 7.65 under the specified conditions.

Reagents

1）0.025 M Tris· HCl buffer, pH 7.65 containing 0.1 M sodium chloride.

2）0.001 M Hippuryl-L-arginine in 0.025 M Tris· HCl pH 7.65 containing 0.1 M sodium chloride.

Enzyme

Dilute stock solution with reagent grade water to a concentration of 1-5 units/ml.

Procedure

1）Set spectrophotometer at 254 nm and 25° C.

2）Pipette 2.9 ml of substrate into cuvette and incubate in spectrophotometer at 25° C for 3-4 minutes to reach temperature equilibration and establish blank rate, if any. Add 0.1 ml of diluted enzyme and record increase in A254 for 3-4 minutes. Determine Δ A254/minute from the initial linear portion of the curve.

Calculation

$$\frac{\text{Units}}{\text{mg}} = \frac{\frac{\Delta A_{254}}{\text{min}}}{\frac{0.349 \text{ x mg enzyme}}{\text{ml reaction mixture}}}$$

where 0.349 is the extinction coefficient of hippuric acid formed during the reaction