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蛋白酶S检测方法

1 原理

Enzyme activity is determined by the casein digestion assay described by Drapeau (1976). Since substrate grade caseins can vary from lot to lot and according to the manner produced, the standardization of casein digestion assays has been difficult to achieve. The use of a reference enzyme preparation is suggested. One unit is that amount of enzyme which releases acid soluble fragments equivalent to 0.001 A₂₈₀ per minute at 37°C and pH 7.8 under the specified conditions.

2 试剂:

- 1% Casein in 0.05 M Tris·PO₄ buffer, pH 7.8. (Dissolve 1 gram "Hammersten" casein in 50 ml 0.01 N NaOH with gentle heating and stirring. Add 40 ml reagent grade water and 5.0 ml 1.0 M Tris. Adjust pH to 7.8 with H₃PO₄ and q.s. to 100 ml.)
- 10% Trichloroacetic acid (TCA)

3 酶:

Dissolve at 1 mg/ml in reagent grade water.

4 操作规程:



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Equilibrate a series of tubes with 5.0 ml of 1% casein at 37°C for 5 minutes.

At zero time add 10 ul or 20 ul of enzyme. Mix. Include a reagent blank.

Exactly ten minutes after adding sample, stop reaction by adding 5.0 ml

TCA. Mix. Allow tubes to stand ten minutes and then filter. Read A₂₈₀ of the filtrate.

5 计算

$$\frac{\text{Units}}{\text{mg}} = \frac{(A_{280} - A_{280} \text{ Reagent Blank})}{10 \text{ min} \times \text{mg enzyme in reaction}}$$