Tribioscience

# Trypsin, Enzyme Activity

| Catalog | Unit |
|---|---|
| TBP0088-10G | 10 g |
| TBP0088-50G | 50 g |

## Product Details

Form: Freeze-dried

Solubility: Soluble in distilled water or dilute buffer

Stability: -20° C; -4° F

Activity: 5000 U/mg material

Protein: 90-95%

## Unit Definition

That amount of enzyme which causes an increase in absorbance of 0.003 per minute at 25ºC and 253 nm, pH 7.6, on the substrate benzoyl-L-arginine ethyl ester (BAEE).

## Assay Method

The ester linkage of N-benzoyl-L-arginine ethyl ester is hydrolyzed thus resulting in an increase in absorbance at 253 nm.

## Applications

Trypsin (EC 3.4.21.4) is an important proteolytic enzyme which is widely distributed in animals and in some bacteria. Trypsinogen is the inactive precursor of trypsin, which is secreted by the exocrine cells of the pancreas and then released into the lumen of the small intestine. Trypsinogen is converted to the active trypsin by enterokinase from the intestinal mucosa and by trypsin itself.

Trypsin is an endopeptidase and it hydrolyzes peptide bonds in which the carbonyl group is contributed by the basic amino acids lysine or arginine. The optimum pH for trypsin is in the range of 7-9. Calcium ions contribute to increasing the stability of trypsin, while several proteinlike substances inhibit the activity of trypsin. These trypsin inhibitors have been isolated from soybean, barley, mung bean and pancreas.

## Reagents

1. 0.001 N HCl.
2. 0.067 M Potassium phosphate buffer, pH 7.6.
3. 0.00025 M BAEE (8.6 mg/100 ml Reagent 2).
4. Trypsin (enzyme) solution. Dilute 5 mg enzyme/ml 0.001 N cold HCl just prior to assay to yield a concentration of about 40 U/ml.

## Procedure

1. Set spectrophotometer (equipped with strip chart recorder and temperature control) at 253 nm and 25ºC.
2. Into a quartz cuvette pipette 3.0 ml of BAEE substrate.
3. Incubate cuvette in spectrophotometer at 25ºC for 5 min. to equilibrate.
4. Initiate the reaction by adding 0.2 ml of the enzyme solution to the cuvette. Mix and record the reaction at 253 nm for 5 min.

## Calculation

$$\text{Activity (U/mg)} = \frac{(\Delta E_{253nm/min})(\text{Enz. Diln.})}{(0.003)(\text{Enz. Vol.})(\text{mg Enz./ml})}$$

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