Tribioscience

Acylase 1, Enzyme Activity

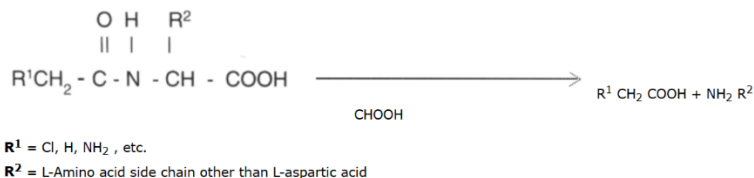| Catalog	Unit
TBP0051-500MG	500 mg
TBP0051-1G	1 g

Product Details

Form: Freeze-dried powder, salt free

Solubility: Distilled water or dilute buffer

Stability: Store at -20° C (-4° F)

Activity: 1000 U/mg protein

Protein: 90%

$R^1 = $ Cl, H, NH_2 , etc.
$R^2 = $ L-Amino acid side chain other than L-aspartic acid

Unit Definition

That amount of enzyme which catalyzes the hydrolysis of one micromole of N-Acetyl-L-Methionine per hour at 25° C, pH 7.0.

Assay Method

The assay is based on the reaction described by Mitz and Schulueter, Biochim. The enzyme catalysis is followed by measuring absorbance at 238 nm.

Applications

Acylase 1 (EC 3.5.1.14) is an extremely stable enzyme in the dry state. In solution, the enzyme is stable at high temperatures (70° C) at pH 7, but below pH 5 it is rapidly and irreversibly inactivated. The enzyme shows a high degree of optical specificity toward its substrates. For this reason, it has been used extensively for the resolution of racemic amino acids.

Reagents

1. 0.1 M Potassium phosphate buffer, pH 7.0.
2. 0.015 M N-Acetyl-L-Methionine (2.87 mg/ml). Dissolve in 0.01 M potassium phosphate buffer. Adjust pH to 7.0 with 2 M NaOH if necessary.
3. Acylase 1 (enzyme) solution. Dilute in 0.01 M potassium phosphate buffer, pH 7.0 to yield a concentration of 1000-2000 U/ml. Prepare fresh prior to assay.

Procedure

1. Set the spectrophotometer (equipped with strip chart recorder and temperature control) at 238 nm and 25° C.
2. In a cuvette place 2.9 ml of 0.015 M N-Acetyl-L-Methionine (substrate). Incubate cuvette in spectrophotometer at 25°C for 5 minutes.
3. Record absorbance at 238 nm (blank).
4. Initiate the reaction by adding 0.1 ml enzyme solution to the cuvette. Follow the reaction by measuring the absorbance at 238 nm for 5-8 minutes.
5. Calculate E238nm/min

Calculation

$$\text{Activity (U/mg)} = \frac{(\Delta E_{238nm/min})(\text{Total Vol.})(\text{Enz. Diln.})(60)}{(0.018)(\text{Enz. Vol.})(\text{mg Enz./ml})}$$

For research use only