Tribioscience

Carboxypeptidase B, Enzyme Activity

Catalog	Unit
TBP0060-1MG	1 mg
TBP0060-5MG	5 mg

Product Details

Form: Freeze-dried powder

Solubility: Distilled water or dilute buffer

Stability: Store at -20° C (-4° F)

Activity: 50 U/mg protein

Protein: 80%

Unit Definition

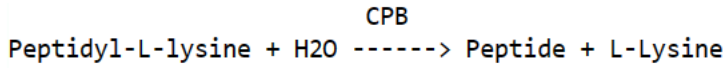
That amount of enzyme which hydrolyzes 1 micromole of hippuryl-L-arginine per minute at 25°C and pH 7.65.

Assay Method

Carboxypeptidase B activity is determined from the increase in absorbance due to the hydrolysis of hippuryl-L-arginine at 254 nm.

Applications

Carboxypeptidase B, (CPB) (EC 3.4.12.3) like carboxypeptidase A, is a pancreatic exopeptidase. Unlike carboxypeptidase A, however, carboxypeptidase B catalyzes the hydrolysis of the peptide bonds involving basic amino acids lysine, arginine and ornithine. This hydrolysis occurs at the C-terminal bond in these polypeptides.



Carboxypeptidase B shows minimal activity towards Carboxypeptidase A substrates. It is a metalloenzyme containing zinc. Porcine carboxypeptidase B has a molecular weight of 34,300.

Reagents

1. 25 mM Tris/HCl buffer, pH 7.65 (containing 0.1 M NaCl).
2. 1 mM Hippuryl-L-arginine in Tris buffer, pH 7.65.
3. Enzyme solution: dilute enzyme with double distilled water to a concentration of 1-5 U/ml. (mg/ml = E278nm x 0.476).

Procedure

1. Set spectrophotometer (equipped with strip chart recorder and temperature control) at 254 nm and 25°C.
2. Into quartz cuvettes pipette 2.9 ml Hippuryl-L-arginine substrate. Incubate in spectrophotometer for 5 min. to equilibrate and to establish a blank rate, if any.
3. Add 0.1 ml. of the enzyme solution to the test cuvette, mix, and record the rate of absorbance at 254 nm for 5 min.
4. Calculate the (delta)E254nm per minute from the initial linear portion of the curve.

Calculation

$$\text{Activity (U/mg)} = \frac{(\Delta E_{254nm/min})(\text{Total Vol.})(\text{Enz. Diln.})}{(0.349)(\text{mg Enz./ml})}$$

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Tribioscience, Inc.; 365 San Aleso Ave, Sunnyvale, CA 94085 Phone: 408-498-0197
info@tribioscience.com; www.tribiosciences.com