Tribioscience

Carbonic Anhydrase, Enzyme Activity

Catalog	Unit	
TBP0059-1MG	1 mg	
TBP0059-5MG	5 mg	

Product Details

Form: Freeze-dried powder

Solubility: Distilled water or dilute buffer

Stability: Store at -20° C (-4° F)

Activity: 2000 U/mg protein

Protein: 10 mg/ml

Applications

Carbonic anhydrase (carbonate dehydratase; EC 4.2.1.1) catalyzes the following reaction:

CA CO2 + H2O -----> H2CO3

The enzyme is widespread in nature. In animals it plays an important role in respiration by facilitating the transport of carbon dioxide. In plants, carbonic anhydrases are involved in the photosynthetic fixation of CO2.

Mammalian erythrocytes contain two distinct forms of carbonic anhydrase distinguished by differences in their catalytic activities. The enzyme requires zinc for its activity and it has a molecular weight of 30,000.

Reagents

1. 0.05 M Tris-HCl buffer, pH 7.6.

2. 0.003 M p-Nitrophenyl acetate. Dissolve 13.6 mg p-nitrophenyl acetate in 1 ml acetone and dilute with water to a volume of 25 ml. Prepare fresh daily and store in dark.

3. Carbonic anhydrase (enzyme) solution. Prepare in Tris buffer to yield a final concentration of 1-2 U/ml. Prepare fresh prior to assay.

Procedure

1. Set spectrophotometer (equipped with a strip chart recorder and temperature control) at 348 nm and 25°C.

2. In a cuvette, pipette the following reagents:

Tris-HCl buffer 1.9 ml

p-nitrophenyl acetate 1.0 ml

Incubate in spectrophotometer at 25°C for 5 min.

3. Record blank rate at 348 nm, if any.

4. Initiate the reaction by adding 0.1 ml enzyme solution (1-2 U/ml) to the cuvette. Record the absorbance at 348 nm for 5 min.

5. Calculate (delta)E348 nm/min

Calculation

Activity (U/mg) =
$$\frac{(\Delta E_{348nm/min})(\text{Total Vol.})(\text{Enz. Diln.})}{(5.0)(\text{Enz. Vol.})(\text{mg Enz./ml})}$$

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