Tribioscience

Tyrosinase, Enzyme Activity

Catalog	Unit
TBP0090-10KU	10 KU
TBP0090-50KU	50 KU

Product Details

Form: Freeze-dried

Solubility: Soluble in distilled water or dilute buffer

Stability: -20° C; -4° F

Activity: 2000 U/mg solid

Protein: 90%

Catalog No.: 115A2000

Unit Definition

That amount of enzyme which causes an increase in extinction at 280 nm of 0.001 per minute at pH 6.5 and 25¡C.

Assay Method

Tyrosinase catalyzes the oxidation of tyrosine to O-quinone which results in an increase in the absorbance at 280 nm.

Applications

Tyrosinase (polyphenol oxidase, catecholase) (EC 1.14.18.1) is a copper-containing oxidase which is widely distributed in plants, animals and man. It oxidizes tyrosine to the pigment melanin in mammals and causes the cut surfaces of many fruits and vegetables to darken (browning reactions). The enzyme from mushrooms has a molecular weight of 128,000. Hearing, et al, report the occurrence of four isozymes of tyrosinase in tissues of mice with malignant melanoma. This observation may have some clinical significance.

Reagents

1. 0.5 M Potassium phosphate buffer, pH 6.5.
2. 0.001 M L-Tyrosine (0.181 mg/ml) in buffer.
3. Tyrosinase (enzyme) solution. Prepare in distilled water to yield a final concentration of 200-400 U/ml. The enzyme solution must be prepared fresh immediately prior to assay.

Procedure

1. Set spectrophotometer (equipped with strip chart recorder and temperature control) at 25°C and 280 nm.
2. Into a quartz cuvette, pipette the following:
   Phosphate buffer 1.90 ml
   Tyrosine 1.00 ml
3. Oxygenate this reaction mixture by bubbling oxygen into the cuvette through a capillary tube for 5 minutes.
4. Transfer cuvette to spectrophotometer and incubate for 5 minutes at 25°C to attain temperature equilibration. Record absorbance at 280 nm to establish the blank rate, if any.
5. Initiate the reaction by adding 0.1 ml of tyrosinase solution (200-400 U/ml) to the cuvette and record absorbance at 280 nm for 10 minutes.
6. Determine μE280nm/min from the linear portion of the curve.

Calculation

$$\text{Activity (U/mg)} = \frac{(\Delta E_{280nm/min})(\text{Total Vol.})(\text{Enz. Diln.})}{(0.001)(\text{Enz. Vol.})(\text{mg Enz./ml})}$$

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