Unit definition

That amount of enzyme which catalyzes the hydrolysis of one micromole L-leucinamide per minute at 25°C at pH 8.5.

Assay method

The assay method is based on the one described by Binkley and Torres (Binkley, F. and Torres, C., Arch. Biochem. Biophys., 86, 201, 1960). The rate of decrease in absorbance at 238 nm, resulting from the hydrolysis of L-leucinamide, is proportional to the catalytic activity of the enzyme.

Rgagents

0.5 M Tris-HCl buffer, pH 8.5.

0.025 M Maganese chloride.

0.0625 M Magnesium chloride.

0.06 M L-leucinamide-HCl (11 mg/ml) in buffer.

Leucine aminopeptidase (enzyme) solution. Dissolve in buffer to a concentration of 1000-2000 U/ml.

Procedure

1、Set spectrophotometer (equipped with strip chart recorder and temperature control) at 238 nm and 25°C.

2、Enzyme treatment. In a test tube pipette the following:

Manganese chloride 0.4 ml

Tris buffer 2.5 ml

Enzyme (LAP) 0.1 ml

Incubate the mixture at 40°C for about 60 minutes. An aliquot from this treated enzyme mixture will be used in the assay system.

3、In a quartz cuvette, place the following in the amounts indicated:

Tris buffer 0.20 ml

Magnesium chloride 0.20 ml

L-leucinamide-HCl 2.50 ml

Place cuvette in spectrophotometer at 25°C for 5 minutes. Record absorbance at 238 nm (blank).

4、To initiate the reaction, add 0.10 ml of treated enzyme (step 2) to the cuvette. Follow the reaction by recording the decrease in absorbance at 238 nm for 5-8 minutes.

5、 5、Calculate E238nm/min

Calculation

$$\text{Activity (U/mg)} = \frac{(\Delta E_{238nm/min})(\text{Total Vol.})(\text{Enz. Diln.})}{(0.011)(\text{Enz. Vol.})(\text{mg Enz./ml})}$$