Tribioscience

beta-Glucuronidase, Enzyme Activity

Catalog	Unit
TBP0058-500KU	500,000 U
TBP0058-1000KU	1,000,000 U

Unit Definition

The amount of enzyme which catalyzes the formation of one micromole of p-Nitrophenol per minute at 37°C.

Applications

ß-Glucuronidase (EC 3.2.1.31) catalyzes the following reaction:

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                                        b-Glucuronidase
ß-D-Glucuronide + H2O ------> Alcohol + D-Glucuronate
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It has been found in tissue extracts of mammals and other vertebrates, digestive juices of snails, molluscs, locusts, bacteria and plants. The enzyme catalyzes the hydrolysis of §-glucuronides and also the transfer of glucuronly radicals to acceptor alcohols. §-Glucuronidase is a structural protein of the endoplasmic reticulum and its occurance in the lysozomes is due to changes in the endoplasmic reticulum.

Reagents

1. 0.1 M Acetate buffer, pH 4.5.
2. 0.1M p-Nitrophenyl glucuronide.
3. Enzyme solution in distilled water.
4. 0.5 N NaOH.

Procedure

1. Set up spectrophotometer at 405 nm and 37°C.
2. Place the following reagents in a test tube and equilibrate in the water bath at 37°C for about 15 minutes:

0.1 M Acetate buffer, pH 4.5	1.0 ml
p-Nitrophenyl glucuronide	0.03 ml

3. Add 0.03 ml of the enzyme solution and mix.
4. After the test tubes have incubated for exactly 10 minutes at 37°C, add 2.0 ml of 0.5 N NaOH solution to stop the reaction (ODtest). At the same time, prepare the blank by first mixing the reagent mixture with 2.0 ml of NaOH solution after the 10 minute incubation period at 37¡C, and then adding the enzyme solution (ODblank).
5. Measure the absorbance of the test (ODtest) and blank (ODblank) at 405nm against H2O.

Calculation

$$\text{Activity (U/mg)} = \frac{\Delta OD(OD_{test} - OD_{blank})(\text{Total Vol.})(\text{Enz. Diln.})}{(18.5)(\text{Reaction Time})(\text{Enz. Vol.})(\text{mg Enz./ml})}$$

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