

beta-Glucuronidase, Enzyme Activity

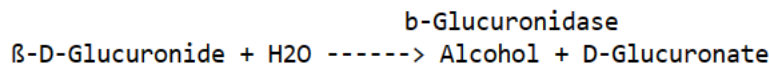
Catalog	Unit
TBP0058-500KU	500,000 U
TBP0058-1000KU	1,000,000 U

Unit Definition

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

Applications

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



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 β-glucuronides and also the transfer of glucuronyl radicals to acceptor alcohols. β-Glucuronidase is a structural protein of the endoplasmic reticulum and its occurrence in the lysosomes 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 (OD_{test}). 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 (OD_{blank}).
5. Measure the absorbance of the test (OD_{test}) and blank (OD_{blank}) at 405nm against H₂O.

Calculation

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

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