上海源叶生物科技有限公司
Shanghai yuanye Bio-Technology Co., Ltd
电话：021-61312973　　传真：021-55068248
网址：www.shyuanye.com
邮箱：shyysw@sina.com

# beta-Glucosidase

(§-D-Glucoside glucohydrolase; EC 3.2.1.21)

ß-Glucosidase catalyzes the following reaction:

b-Glucosidase
b-D-Glucoside + H2O ----------> D-Glucose + Alcohol

## ASSAY

The method of assay is based on the reaction of p-Nitrophenol, which is formed during the reaction, is determined spectrophotometrically at 400nm.

## REAGENTS

1. 0.1 M Acetate buffer, pH 5.0.
2. 0.02 M p-Nitrophenyl-§-D-glucopyranoside (PNPG) solution. Dissolve 603 mg PNPG/100 ml H2O (stable for two weeks if stored at -5°C).
3. 0.2 M Na2CO3 solution. Dissolve 21.2 gm Na2CO3/1000 ml H2O.
4. 0.2% Bovine serum albumin (BSA) solution. Dissolve 0.2 gm of BSA in 100 ml of 0.01 M phosphate buffer, pH 7.0.
5. §-Glucosidase (enzyme) solution (0.006-0.022 U/ml). Dissolve the enzyme preparation in ice-cold 0.05 M Tris-HCl buffer, pH 7.8 (about 1 mg/ml) and dilute to 0.006-0.022 U/ml with 0.2% BSA, immediately before assay.

## PROCEDURE

1. Set up a water bath at 37°C and a spectrophotometer at 400nm.
2. Place the following reagents in a test tube and equilibrate in the water bath at 37°C for about 15 minutes:

| | |
|---|---|
| Acetate buffer, pH 5.0 | 1.0 ml |
| PNPG solution | 0.5 ml |

3. Add 0.5 ml of the enzyme solution and mix.
4. After the test tubes have incubated for exactly 15 minutes at 37°C, add 2.0 ml of Na2CO3 solution to stop the reaction (ODtest). At the same time, prepare the blank by first mixing the reagent mixture with 2.0 ml of Na2CO3 solution after the 15 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 400nm against H2O.

上海源叶生物科技有限公司
Shanghai yuanye Bio-Technology Co., Ltd
电话：021-61312973　　传真：021-55068248
网址：www.shyuanye.com
邮箱：shyysw@sina.com

CALCULATION

$$\text{Activity (U/mg)} = \frac{\Delta OD(OD_{test} - OD_{blank})(\text{Total Vol.})(\text{Enz. Diln.})}{(18.1)(\text{Reaction Time})(\text{Enz. Vol.})(\text{mg Enz./ml})}$$