α-GLUCOSIDASE (α-Glu)

[EC 3.2.1.20]

from *Bacillus stearothermophilus*

\[ \text{α-D-Glucoside + H}_2\text{O} \rightleftharpoons \text{D-Glucose + Alcohol} \]

**SPECIFICATION**

State : Lyophilized
Specific activity : more than 40 U/mg protein
Contaminants : (as α-Glu activity = 100 %)
  - Phosphoglucomutase < 0.01 %
  - NADH oxidase < 0.01 %
  - Alcohol dehydrogenase < 0.01 %

**PROPERTIES**

Molecular weight : ca. 50,000
Optimum pH : 6.0 - 7.0 (Fig. 1)
pH stability : 5.0 - 11.0 (Fig. 2)
Isoelectric point :
Thermal stability : No detectable decrease in activity up to 60 °C. (Fig. 3, 4)
Michaelis constants : (50 mM Potassium phosphate buffer, pH 6.3, at 30 °C)
  - p-Nitrophenyl-α-glucopyranoside (PNPG) 0.73 mM
  - Maltose 1.3 mM
  - Phenyl-α-glucopyranoside 2.4 mM
Substrate specificity : PNPG 100 %
  - Maltose 177 %
  - Phenyl-α-glucopyranoside 59 %

**STORAGE**

Stable at -20 °C for at least one year

**APPLICATION**

The enzyme is useful for diagnostic reagent, for example, α-amylase determination.
ASSAY

Principle
The change in absorbance is measured at 400 nm according to the following reaction.

\[ \text{p-Nitrophenyl-\(\alpha\)-glucopyranoside (PNPG)} \xrightarrow{\alpha\text{-Glu}} \text{p-Nitrophenol (PNP) + Glucose} \]

Unit Definition
One unit of activity is defined as the amount of \(\alpha\)-Glu that forms 1 \(\mu\)mol of PNP per minute at 30 °C.

Solutions
I Buffer solution; 100 mM Potassium phosphate buffer, pH 6.3
II PNPG solution; 20 mM (0.603 g PNPG/100 mL distilled water) (Stable for two weeks if stored at 0 - 5 °C)
III \(\text{Na}_2\text{CO}_3\) solution; 0.2 M (2.12 g \(\text{Na}_2\text{CO}_3\)/100 mL distilled water)

Preparation of Enzyme Solution
Dissolve the lyophilized enzyme with distilled water and dilute to 0.006 to 0.022 U/mL with 10 mM Potassium phosphate buffer containing 1 mg/mL BSA, pH 7.5.

Procedure
1. Prepare the following reaction mixture and pipette 1.5 mL of reaction mixture into a test tube.
   - Solution I 10.0mL
   - Solution II 5.0mL
2. Incubate at 30 °C for 5 minutes.
3. Add 0.5 mL of the enzyme solution and mix.
4. Incubate at 30 °C for exactly 15 minutes.
5. After incubation, add 2.0 mL of Solution III and mix.
6. Read absorbance at 400 nm (Abs•test).
   - At the same time, prepare the blank with 1.5 mL of the reaction mixture, and add 2.0 mL of Solution III after incubation at 30 °C for 15 minutes, followed by addition of the enzyme solution (Abs•blank).

Calculation

Volume activity (U/mL) = \(\frac{4.0}{(\text{Abs•test}) - (\text{Abs•blank})} \times \frac{4.0}{18.1 \times 15 \times 0.5} \times \text{d.f.} \)

Specific activity (U/mg protein) = \(\frac{\text{Volume activity (U/mL)}}{\text{Protein concentration (mg/mL)}}\)

\text{d.f.} \quad ; \text{dilution factor}
18.1 ; \text{millimolar extinction coefficient of PNP (cm}^2/\text{\(\mu\)mol})
\text{*Protein concentration} \quad ; \text{determined by Bradford's method}
Fig. 1  pH profile

△ acetate, ○ phosphate, □ Gly-NaOH

Fig. 2  pH stability

treated for 24 hr at 4 °C in the following buffer solution (0.1 M);
△ acetate, ○ phosphate, □ TEA-NaOH, ■ Gly-NaOH

Fig. 3  Thermal stability

treated for 15 min in 0.1M potassium phosphate buffer, pH 8.0

Fig. 4  Thermal stability

treated in 0.1M potassium phosphate buffer, pH 8.0
○ 60 °C, ● 65°C