GLUCOSE DEHYDROGENASE (GlcDH2)

[EC 1.1.1.47]

from recombinant *E. coli*

\[
\text{D-Glucose + NAD(P)^+} \leftrightarrow \text{D-Glucono-\(\delta\)-lactone + NAD(P)H + H}^+ 
\]

SPECIFICATION

State : Lyophilized
Specific activity : more than 900 U/mg protein
Contaminants : (as GlcDH2 activity = 100 %)
  NADH oxidase < 0.01 %

PROPERTIES

Molecular weight : ca. 126,000
Subunit molecular weight : ca. 31,500
Optimum pH : 8.5 (Fig. 1)
pH stability : 5.0 - 10.0 (with 3M NaCl) (Fig. 2)
Thermal stability : No significant decrease in activity up to 70 °C.
  (with 3M NaCl and 0.1% BSA) (Fig. 3, 4)
Michaelis constants:
  D-Glucose : 3.7 mM
  NAD^+ : 0.06 mM
  NADP^+ : 0.02 mM
Substrate specificity (100mM):
  D-Glucose : 100 %
  D-Maltose : 1.1 %
  D-Galactose : 0.1 %
  D-Xylose : 3.0 %
  D-Fructose : 0.3 %
  D-Mannose : 4.8 %
  D-Arabinose : 0 %
  Trehalose : 0 %
  D-Lactose : 1.3 %
  D-Sucrose : 0 %
  2-Deoxy-D-Glucose : 100 %
  D-Glucose-1-Phosphate : 0 %
  D-Glucose-6-Phosphate : 0 %
  D-Sorbitol : 0 %

STORAGE

Stable at -20 °C for at least one year

APPLICATION

This enzyme is useful for determination of glucose.
ASSAY

Principle
The change in absorbance is measured at 340 nm according to the following reaction.

\[
\text{D-Glucose} + \text{NAD}^+ \xrightarrow{\text{GlcDH2}} \text{D-Glucono-\(\delta\)-lactone} + \text{NADH} + \text{H}^+
\]

Unit Definition
One unit of activity is defined as the amount of GlcDH2 that forms 1 \(\mu\text{mol}\) of NADH per minute at 37 °C.

Solutions
I. Buffer solution; 100 mM Tris-HCl, pH8.5 (at 25°C)
II. NAD\(^+\) solution; 100 mM (0.663 g NAD\(^+\) free acid/10 mL distilled water)
III. D-Glucose solution; 1 M (1.802 g glucose (anhyd.)/10 mL distilled water)
IV. NaCl solution; 5 M (2.92 g NaCl/10 mL distilled water)

Preparation of Enzyme Solution
Dissolve the lyophilized enzyme with distilled water and dilute to 5 to 15 U/mL with 20 mM potassium phosphate buffer containing 1 mg/mL BSA and 2 M NaCl, pH 6.5.

Procedure
1. Prepare the following reaction mixture and pipette 2.70 mL of reaction mixture into a cuvette.
   Solution I 17.22mL
   Solution II 0.50mL
   Solution III 2.00mL
   Solution IV 0.28mL
2. Incubate at 37 °C for about 3 minutes.
3. Add 0.015 mL of enzyme solution into the cuvette and mix.
4. Read absorbance change at 340 nm per minute (\(\Delta\text{Abs}_{340}\)) in the linear portion of curve.

Calculation

\[
\text{Volume activity (U/mL)} = \frac{(\Delta\text{Abs}_{340}) \times (2.70 + 0.015)}{6.22 \times 0.015} \times \text{d.f.}
\]

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

\*Protein concentration; determined by the absorbance at 280 nm (Abs280), where 1 Abs280 = 1 mg/mL

REFERENCE
Fig. 1  pH profile

O phosphate,  ● Tris-HCl,  ▲ glycine

Fig. 2  pH stability

treated for 24 hr at 4°C in the following buffer solution (0.1 M) containing 3M NaCl:  △ acetate,  O phosphate,  ● Tris-HCl,  ▲ glycine

Fig. 3  Thermal stability

treated for 15 min in 0.1M phosphate buffer, pH 6.5, containing 3M NaCl and 0.1% BSA

Fig. 4  Thermal stability

treated for in 0.1M phosphate buffer, pH 6.5, containing 3M NaCl and 0.1% BSA,  ○ 60°C,  □ 70°C,  ● 80°C