GLUCOSE-6-PHOSPHATE DEHYDROGENASE (ZM-G6PDH)

[EC 1. 1. 1. 49]

from *Zymomonas mobilis*

D-Glucose 6-phosphate + NAD(P)* → D-Gluconolactone 6-phosphate + NAD(P)H + H*

**SPECIFICATION**

State: Lyophilized
Specific activity: more than 250 U/mg protein
Contaminants: (as ZM-G6PDH activity = 100 %)
- Glucokinase < 0.02 %
- Phosphoglucomutase < 0.01 %
- 6-Phosphogluconate dehydrogenase < 0.02 %
- Hexose-6-phosphate isomerase < 0.01 %
- Glutathione reductase < 0.01 %

**PROPERTIES**

- Molecular weight: ca. 208,000
- Subunit molecular weight: ca. 52,000
- Optimum pH: 8.0 (Fig. 1)
- pH stability: 5.0 - 10.0 (Fig. 2)
- Thermal stability: No detectable decrease in activity up to 50 °C. (Fig. 3, 4)
- Michaelis constants: (30mM Tris-HCl buffer, pH 8.0, at 30 °C)
  - Glucose 6-phosphate: 0.14 mM
  - NADP*: 0.02 mM
  - NAD*: 0.14 mM
- Substrate specificity: NADP* 70 %
  - NAD* 100 %

**STORAGE**

Stable at -20 °C for at least one year

**APPLICATION**

The enzyme is useful for diagnostic reagent, for example, glucose determination or CK determination, and for the specific determination of glucose.
ASSAY

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

\[
\text{Glucose 6-phosphate} + \text{NAD}^+ \xrightarrow{\text{ZM-G6PDH}} \text{Gluconolactone 6-phosphate} + \text{NADH} + \text{H}^+
\]

Unit Definition
One unit of activity is defined as the amount of ZM-G6PDH that forms 1 μmol of NADH per minute at 30 °C.

Solutions
I. Buffer solution; 50 mM Tris-HCl, pH 8.0
II. NAD\(^+\) solution; 100 mM (0.663 g NAD\(^+\) free acid/10 mL distilled water)
III. Glucose 6-phosphate (G6P) solution; 33 mM (0.112 g G6P disodium salt 2H\(_2\)O/10mL distilled water)

Preparation of Enzyme Solution
Dissolve the lyophilized enzyme with distilled water and dilute to 5 to 10 U/mL with 50mM potassium phosphate buffer containing 1 mg/mL BSA, pH 7.0.

Procedure
1. Prepare the following reaction mixture and pipette 3.00 mL of reaction mixture into a cuvette.
   - Solution I 26.40mL
   - Solution II 0.90mL
   - Solution III 2.70mL
2. Incubate at 30 °C for about 3 minutes.
3. Add 0.01 mL of enzyme solution into the cuvette and mix.
4. Read absorbance change at 340nm per minute (ΔAbs\(_{340}\)) in the linear portion of curve.

Calculation
\[
\text{Volume activity (U/mL)} = \frac{(\Delta \text{Abs}_{340}) \times (3.00 + 0.01)}{6.22 \times 0.01} \times \text{d.f.}
\]
\[
\text{Specific activity (U/mg protein)} = \frac{\text{Volume activity (U/mL)}}{\text{Protein concentration (mg/mL)}^*}
\]

d.f. ; dilution factor
6.22 ; millimolar extinction coefficient of NADH (cm\(^2\)/μmol)
*Protein concentration ; determined by Bradford's method

REFERENCE
Fig. 1  pH profile

Fig. 2  pH stability

Fig. 3  Thermal stability

Fig. 4  Thermal stability