LEADGENE
LEADGENE BIOMEDICAL, INC.

Product Information & Manual

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Glucose dehydrogenase (GLD) (NAD(P)-dependent)

Cat no. LDG0023RG

Product Overview

Specification

Appearance	White amorphous powder, lyophilized
Activity	300 U/ mg or more (containing approx. 10% of stabilizers)

Properties

Stability	Stable at −20℃ for at least one year
Molecular weight	30.3 kDa
Isoelectric point	5.36

Assay

1. Assay principle

β-D-Glucose + NAD^+ $\xrightarrow{\text{Glucose dehydrogenase}}$ D-Glucono-δ-lactone + NADH + H^+

2. Unit definition

One unit causes the formation of one micromole of NADH per minute under the conditions described below.

3. Reagents

A. Tris-HCl buffer, pH 8.0	0.1 M
B. D-Glucose solution	1.5 M
C. β-NAD^+ solution	80 mg/ mL
D. Enzyme diluent	50 mM K-phosphate buffer, pH 7.0 containing 0.1% BSA

4. Procedure

(1) Prepare the following **working solution** immediately before use and equilibrate at 37℃ for approximately 5 minutes (for 4 reactions).

Working solution

Tris-HCl buffer, pH 8.0 (Reagent A)	5.2 mL
D-Glucose solution (B)	0.6 mL
β-NAD^+ solution (C)	0.2 mL
Total	**6 mL**

(2) Pipette 1.5 mL of working solution into a tube.

(3) Add 0.025 mL of the enzyme solution* and mix by gentle inversion.

Concentration in a reaction	
Tris-HCl buffer	85.25 mM
D-Glucose	147.54 mM
NAD^+	3.66 mM

(4) Pipette the mixture into a cuvette (d=1.0 cm).

(5) Record the increase in optical density at 340 nm against water for 1 to 5 minutes in a spectrophotometer at room temperature and calculate the ΔOD per minute from the initial linear portion of the curve (ΔOD test). At the same time, measure the blank rate (ΔOD blank) by using the same method as the test except that the enzyme diluent is added instead of enzyme solution.

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* Dilute the enzyme in ice-cold enzyme diluent **(Reagent D)** to **0.8−1.2 U/ mL** and store on ice.

(6) Activity can be calculated by using the following formula:

Volume activity (U/ mL)＝

$$\frac{\Delta OD/\ min\ (\Delta OD\ test - \Delta OD\ blank) \times Vt \times df}{6.22 \times 1.0 \times Vs} = \Delta OD/\ min \times 9.807 \times df$$

Weight activity (U/ mg)＝ (U/ mL) × 1/C

Vt: Total volume (1.525 mL)

Vs: Sample volume (0.025 mL)

6.22: Millimolar extinction coefficient of NADH at 340 nm (㎖ /micromole)

1.0: Light path length (cm)

df: Dilution factor

C: Enzyme concentration in dissolution (mg/ mL)

<u>The effect of different conditions on Glucose dehydrogenase</u>

A.

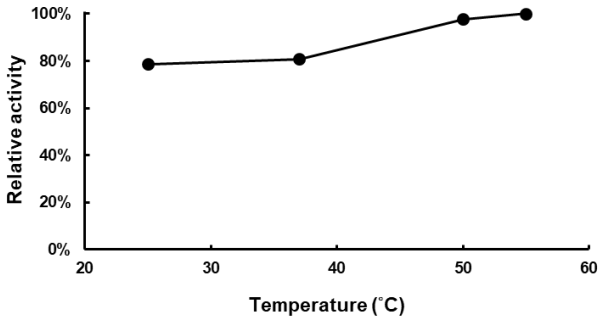

Figure A. Temperature activity of Glucose dehydrogenase. The enzyme reactions in 0.1 M Tris-HCl buffer, pH 8.0, were carried out under different temperature.

B.

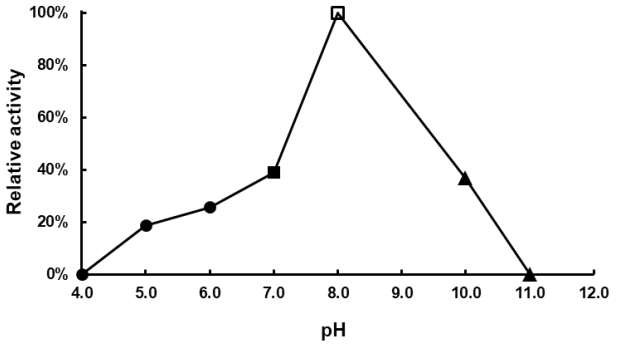

Figure B. pH activity of Glucose dehydrogenase. The buffer conditions with various pH values were used in the reaction at 37°C. pH 4.0-6.0, 0.1 M Sodium citrate buffer; pH 7.0, 0.1 M Potassium phosphate buffer; pH 8.0, 0.1 M Tris-HCl buffer; pH 10.0-11.0, 0.1 M Carbonate-bicarbonate buffer.

C.

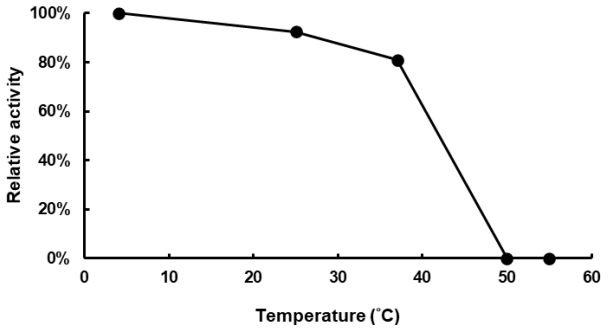

Figure C. Thermal stability of Glucose dehydrogenase. The enzyme powder was reconstituted by double-distilled water and treated with different temperature for 15 minutes. Final concentration: 23.5 U/ mL

D.

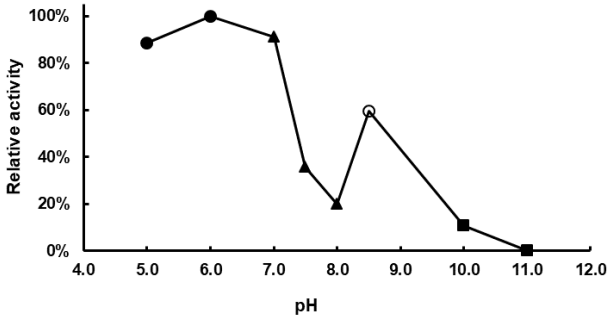

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Figure D. pH stability of Glucose dehydrogenase. The enzyme powder was reconstituted by double-distilled water and treated with different pH buffer condition for 20 hours. pH 4.0-6.0, 0.1 M Sodium citrate buffer; pH 7.0-8.0, 0.1 M Potassium phosphate buffer; pH 8.5, 0.1 M Tris-HCl buffer; pH 10.0-11.0, 0.1 M Carbonate-bicarbonate buffer.

Disclaimer

This product is for research use only and is not intended for diagnostic use.