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Product Information & Manual

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Glycerol Dehydrogenase (GlyDH)

Cat no. LDG0020RG

Product Overview

Description

Glycerol dehydrogenase catalyzes the oxidation of glycerol to dihydroxyacetone (DHA), using NAD^+ as a cofactor that is subsequently reduced to NADH. It plays a key role in glycerol metabolism, contributing to pathways such as gluconeogenesis and lipid metabolism. In industry, glycerol dehydrogenase is widely used to produce DHA for cosmetic and pharmaceutical applications.

Expression system

Escherichia coli

Specification

Appearance	Light brown amorphous powder, lyophilized
Activity	40 U/mg or more (containing approx. 10% of stabilizers)

Properties

Stability	Stable at −20℃ for at least one year
Isoelectric point	5.27

Reconstitution

It is recommended to weigh and reconstitute 30 mg of lyophilized powder in 157 µL double-distilled water directly and incubate the solution for at least 20 mins to ensure sufficient re-dissolved.

Applications

1. Enzymatic determination of glycerol
2. Research of energy metabolism [1]
3. Glycerol metabolism of pathogenic bacteria [1]

Assay

1. Assay principle

Glycerol + NAD^+ $\xrightarrow{\text{Glycerol dehydrogenase}}$ Dihydroxyacetone + NADH + H^+

2. Unit definition

One unit causes the formation of one micromole of NADH per minute under the following conditions：0.1 M Carbonate buffer pH 11, 0.1 M Glycerol, 1 mM NAD+ and 33 mM Ammonium sulfate.

3. Reagents

A. Carbonate-bicarbonate buffer, pH 11.0	0.2 M (Prepare by mixing 0.2 M K_2CO_3 and 0.2 M $NaHCO_3$ to reach pH 11.0)
B. Glycerol solution	0.3 M
C. Ammonium sulfate solution	1.0 M
D. NAD^+ solution	10 mM [Weigh 133 mg of NAD^+ (MW = 663.43) and dissolve in 18 mL of H_2O. Adjust the pH to 7.0 with 0.5 N KOH and fill up to 20.0 mL with H_2O] (Should be prepared freshly)
E. Enzyme diluent	20 mM K-phosphate buffer pH 7.5

4. Procedure

(1) Prepare the following **Working Solution** immediately before use (for 4 reactions).

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Working Solution

Carbonate-bicarbonate buffer, pH 11.0 (Reagent A)	3 mL
Glycerol solution (Reagent B)	2.2 mL
Ammonium sulfate solution (Reagent C)	0.2 mL
NAD^+ solution (Reagent D)	0.6 mL
Total	**6 mL**

Be sure the pH in the range (pH 10.0–10.5). If not, adjust the pH to 10.5 with 1.0 N KOH or 1.0 N HCl, and store on ice in a brownish bottle.

(2) Pipette 1.45 mL of the Working Solution into a tube and equilibrate at 25℃ for about 5 minutes.

(3) Add 0.05 mL of the enzyme solution* and mix by gentle inversion.

Concentration in a reaction	
Carbonate-bicarbonate buffer	0.1 M
Glycerol	0.1 M
NAD^+	1 mM
Ammonium sulfate solution	33 mM

(4) Add 1 mL of 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.

* Dilute the enzyme in ice-cold Enzyme diluent (**Reagent E**) to **0.10–0.25 U/ mL** and store on ice.

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

Volume activity (U/mL) =

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

$$= \Delta OD/min \times 4.82 \times df$$

Weight activity (U/mg) = (U/mL)×1/C

Vt: Total volume (1.5 mL)

Vs: Sample volume (0.05 mL)

6.22 : Millimolar extinction coefficient of NADH (cm^2 /micromole)

1.0: Light path length (cm)

df: Dilution factor

C: Enzyme concentration in dissolution (mg/mL)

Reference

1. *Cedric Blötz. et al.* Glycerol metabolism and its implication in virulence in *Mycoplasma. FEMS Microbiology Reviews* (2017).

The effect of different conditions on Glycerol dehydrogenase

A.

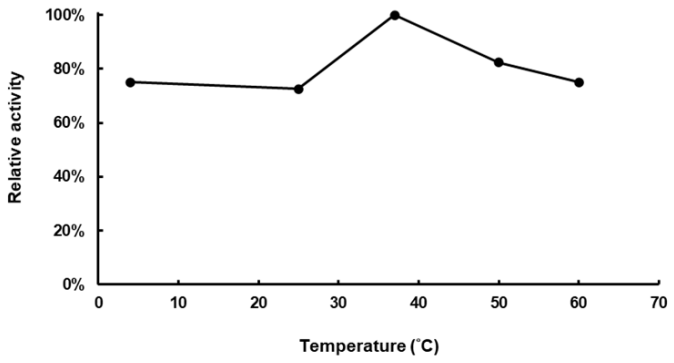

Figure A. Temperature activity of Glycerol dehydrogenase. The enzyme reactions in 0.1 M Carbonate-bicarbonate buffer, pH 11.0, were carried out under different temperatures.

B.

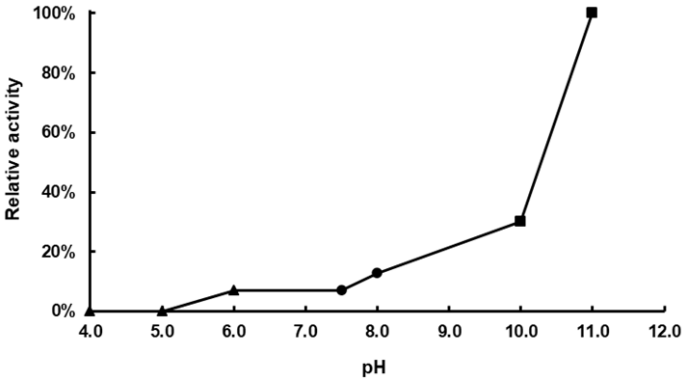

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Figure B. pH activity of Glycerol dehydrogenase. The buffer conditions with various pH values were used in the reaction at 25°C. pH 4.0-6.0, 0.1 M Sodium citrate buffer; pH 7.5-8.0, 0.1 M Potassium phosphate buffer; pH 10.0-11.0, 0.1 M Carbonate-bicarbonate buffer.

C.

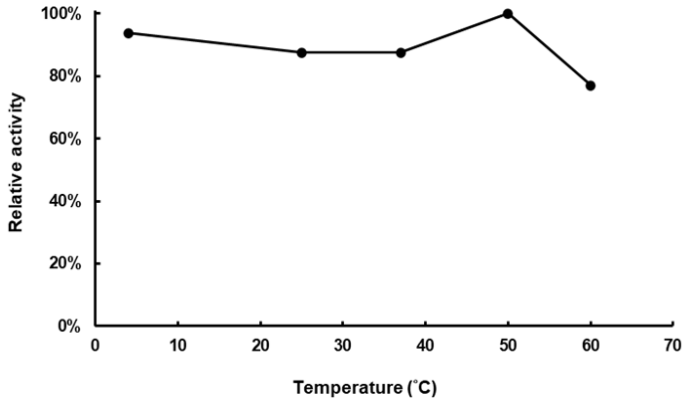

Figure C. Thermal stability of Glycerol dehydrogenase. The enzyme powder was reconstituted by double-distilled water and treated with different temperatures for 15 minutes. Final concentration: 16 U/mL

D.

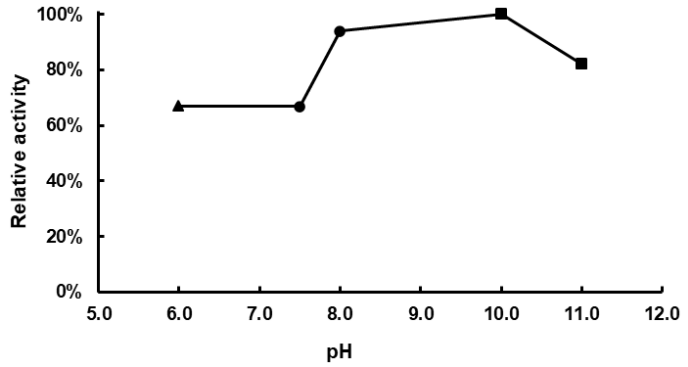

Figure D. pH stability of Glycerol dehydrogenase. The enzyme powder was reconstituted by double-distilled water and treated with different pH buffer condition for 20 hours at 25°C. pH 6.0, 0.1 M sodium citrate buffer; pH 7.5-8.0, 0.1 M potassium phosphate buffer; pH 10.0-11.0, 0.1 M carbonate-bicarbonate buffer.

E.

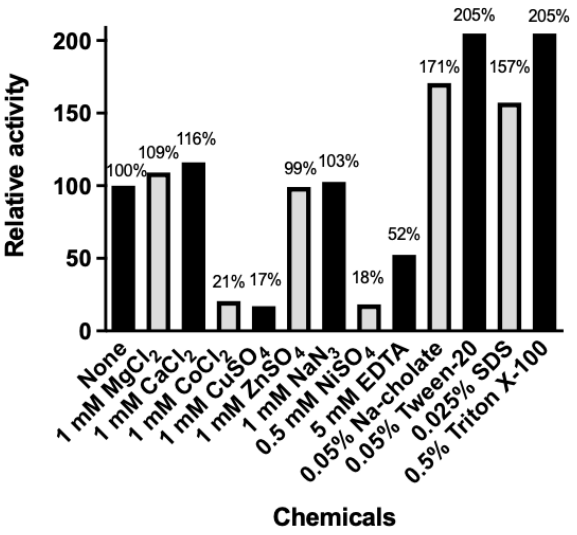

Figure E. The effects of various chemicals on Glycerol dehydrogenase. The enzyme was incubated with the chemicals at 25°C for 1 hour.

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