Diaphorase (DPH)

rp216196

Storage: -20℃. Avoid freeze/thaw cycle. Store in the dark. Desiccated.

Introduction:

Application: For the R&D and large-scale preparation of β-hydroxybutyrate reagents.

Enzymatic Properties:

Source:	Microorganism	
Classification:	EC 1.6.5.2	
Molecular Weight:	28 kDa (SDS-PAGE)	
Isoelectric Point (pI):	5.2	
Km Value:	7.4 × 10^{-4} M (NADH), 6.0 × 10^{-2} M (NADPH)	
Inhibitors:	Fe^{3+}, Mn^{2+}, Cu^{2+}	
Optimal pH:	7.5	Fig.1
Optimal Temperature:	45℃	Fig.2
pH Stability:	4.5-9.0 (25℃, 16 h)	Fig.3
Thermal Stability:	Stable below 50℃ (pH 7.5, 30 min)	Fig.4
Storage Stability:	≥90% activity retained after 12 months of static storage at -25~-15℃	Fig.5
Protectants:	FMN, NAD(P)H	

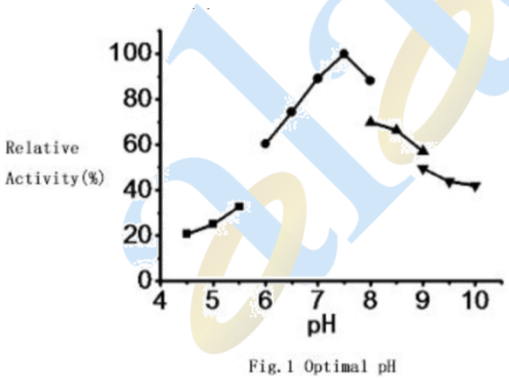

Fig.1 Optimal pH

Buffer solution: pH 4.5-5.5, acetate buffer; pH 6.0-8.0, Na-phosphate; pH 8.0-9.0, Tris-HCl; pH 9.0-10.0, Glycine-NaOH.

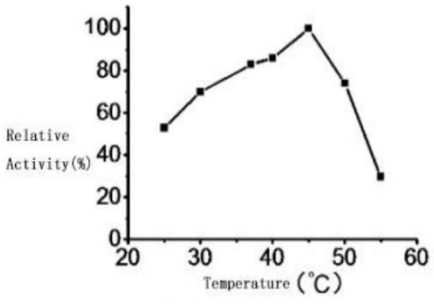

Fig.2 Optimal Temperature

Reaction in 200 mM Tris-HCl buffer pH 7.5.
Enzyme concentration: 1 mg/mL

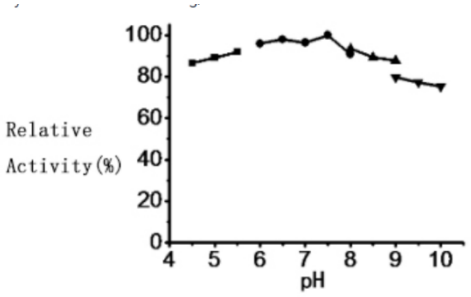

Fig.3 pH Stability

25℃, 16 h-treatment with 50 mM buffer solution: pH 4.5-5.5, acetate buffer; pH 6.0-8.0; Na-phosphate; pH 8.0-9.0, Tris-HCl; pH 9.0-10.0, Glycine-NaOH. Enzyme concentration: 1 mg/mL

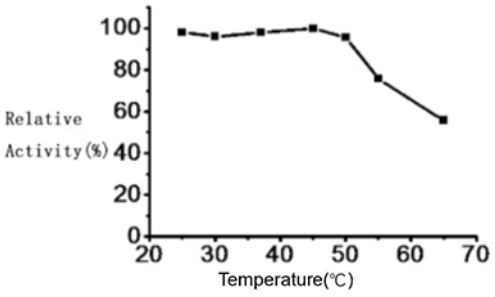

Fig.4 Thermal Stability

30 min-treatment with 200 mM Tris-HCl buffer, pH 7.5. Enzyme concentration: 1 mg/mL

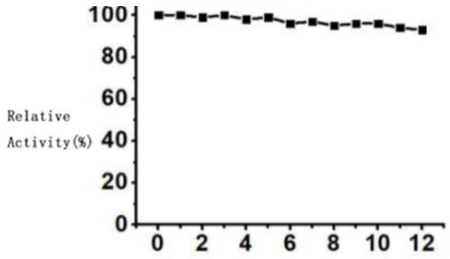

Fig.5 Storage Stability(-25~-15℃)

Activity Assay Method:

1. Principle:

$$NADH + H^+ + DCPIP \xrightarrow{\text{Diaphorase}} NAD^+ + Leucodye$$

The concentration of DCPIP (2,6-dichlorophenolindophenol) can be determined by ultraviolet-visible spectrophotometry at 600 nm.

2. Definition of Enzyme Activity:

One unit (U) of enzyme activity is defined as the amount of enzyme required to oxidize 1 μmol of DCPIP per minute under the specified reaction conditions.

3. Reagent Preparation:

Reagent I: 200 mM Tris-HCl buffer (pH 7.0).

Reagent II: 36 mM NADH solution (dissolved in water, freshly prepared, stored on ice).

Reagent III: 2.4 mM DCPIP solution (dissolved in water, stored at room temperature, freshly prepared).

Reagent IV: 200 mM Tris-HCl buffer (pH 7.5) containing 0.5% Tween 20.

Test Sample: Dilute the enzyme solution to 0.1-0.25 U/mL with Reagent IV.

4. Procedure:

4.1 Add 2.4 mL of water, 0.3 mL of Reagent I, and 0.1 mL of Reagent II to a 3 mL cuvette.

Preincubate at 37℃ for 4 minutes.

4.2 Add 0.1 mL of the test sample and 0.1 mL of Reagent III, then mix rapidly.

4.3 Measure the rate of absorbance change (ΔAs) at 600 nm within 1 minute.

4.4 Blank Control: Replace the enzyme solution with enzyme dilution buffer (Reagent IV) and follow the same steps to obtain the blank absorbance change (ΔAb).

ΔA = ΔAs - ΔAb

5. Activity Calculation:

$$\text{Volume activity}(U/ml) = \frac{\Delta A \times vt \times df}{20.9 \times Vs \times 1.0} = \Delta A \times 1.44 \times df$$

$$\text{Weight activity}(U/mg) = \text{Volume activity} \times 1/C$$

- Vt: Total volume of the reaction mixture (3.0 mL).
- Vs: Volume of the enzyme solution added (0.1 mL).
- 1.0: Light path length (cm).
- df: Dilution factor of the enzyme solution.
- C: Concentration of the enzyme solution (mg/mL).
- 20.9: Molar extinction coefficient of the chromophore (DCPIP) at 600 nm under standard conditions (cm²/μmol).