Tribioscience

Cystathionine-β-lyase (CBL) enzyme (TBP0224)

Catalog	Unit Size
TBP0224-500u	500u
TBP0224-2ku	2ku

Description

Cystathionine-β-Lyase (CβL) is a pyridoxal phosphate (PLP)-dependent enzyme that catalyzes the cleavage of L-cystathionine into L-cysteine, α-ketobutyrate, and ammonia. This enzyme plays a key role in sulfur amino acid metabolism, particularly in the biosynthesis of cysteine from homocysteine.

Cystathionine-β-lyase (EC 4.4.1.8, from Microorganism)

L-Cystathionine ⟶ L-Homocysteine + Pyruvate + NH_3

Specifications

Appearance:	Yellow amorphous powder, lyophilized	
Purity:	≥90.0%	
Enzyme powder specific activity:	≥30.0 U/mg	
Glucose-6-phosphate dehydrogenase:	≤0.01%	
Lactate dehydrogenase:	≤0.01%	
EC number:	4.4.1.8 (Recombinant from microorganism)	
Molecular weight:	54 kDa	
Isoelectric point:	6	
Michaelis constants:	1.6×10^{-3} M (L-Cystathionine)	
Inhibitors:	Not inhibited by NaN_3	
Optimum pH:	8	Fig. 1
Optimum temperature:	50 ℃	Fig. 2
pH stability:	pH 6.0-10.0 (25 ℃, 16 h)	Fig. 3
Thermal stability:	Below 45℃ (pH 8.0, 30 min)	Fig. 4
Storage stability:	At least one year at -25 ~ -15 ℃	Fig. 5
Stabilizers:	Triton X-100, glycerol	
Unit definition:	One unit (U) is defined as the amount of enzyme which consumes 1 μmol of NADH per min under the conditions described below.	

Applications

This enzyme is useful for enzymatic determination of L-homocysteine when coupled with CBS and LDH in clinical analysis.

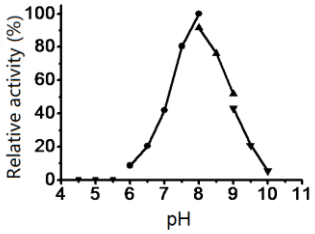

Fig. 1 Optimum pH

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.
Enzyme concentration: 1 mg/mL

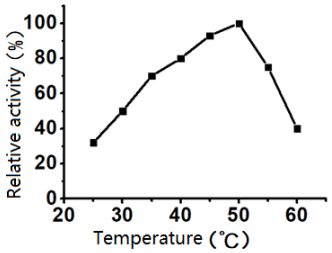

Fig. 2 Optimum temperature

Reaction in 50 mM Tris-HCl buffer, pH 8.0.
Enzyme concentration: 1 mg/mL

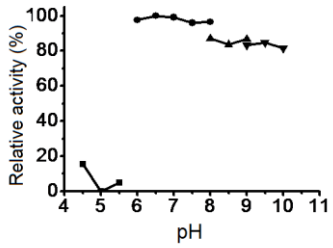

Fig. 3 pH Stability

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

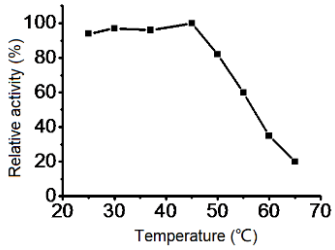

Fig 4 Thermal stability

30 min-treatment with 50 mM Tris-HCl buffer, pH 8.0.
Enzyme concentration: 1 mg/mL

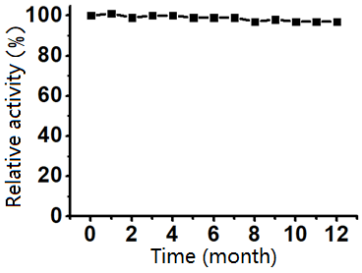

Fig.5 Storage stability (-25～-15 ℃)

Assay principle

L-Cystathionine $\xrightarrow{\text{CBL}}$ L-Homocysteine + Pyruvate + NH_3
Pyruvate + NADH $\xrightarrow{\text{LDH}}$ Lactate + NAD^+
The consumption of NADH is measured at 340nm by spectrophotometry.

Reagents preparation

Reagent I: 100 mM pH 8.0 Tris-HCl buffer, contains 3.5 mM DL-Cystathionine，0.15 mM NADH, 0.05 mM PLP, 10 U/mL LDH.
Enzyme diluent: 50 mM pH 8.0 Tris-HCl buffer.
Sample: The enzyme was diluted to 0.8-2.4 U/mL with the enzyme diluent.

Procedure

1. Add 1 mL Reagent I to the 1 mL cuvette and preheat it at 37℃ for 5 min.
2. Add 0.02 mL enzyme solution to the cuvette and mix.
3. Record the ΔA_s at 340 nm in 1 minute in a spectrophotometer thermostated at 37℃.

At the same time, measure the blank rate ΔA_b by using the same method as the test except that the enzyme diluent is added instead of the enzyme solution.
$\Delta A = \Delta A_s - \Delta A_b$

Calculation

$$\textbf{Volume activity } (\textbf{U/mL}) = \frac{\Delta A \times 1.02 \times df}{6.22 \times 0.02 \times 1.0} = \Delta A \times 8.20 \times df$$

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

1.02: Total volume (mL)
0.02: enzyme volume (mL)
1.0: Light path length (cm)
df: dilution factor
6.22: Millimolar extinction coefficient of NADH under 340nm ($cm^2/\mu mol$)

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