Cystathionine β-Synthase (TBP0223)

Catalog	Unit Size
TBP0223-500u	500u
TBP0223-2ku	2ku

Description

Cystathionine β-Synthase (CBS) is a key enzyme in the transsulfuration pathway that catalyzes the conversion of homocysteine and serine into cystathionine in a pyridoxal-5′-phosphate (PLP)–dependent manner. It plays an essential role in homocysteine metabolism, cysteine biosynthesis, and the production of the gasotransmitter hydrogen sulfide (H_2S). Dysregulation of CBS is associated with homocystinuria, cardiovascular disorders, and neurological diseases, making it an important target in metabolic research and drug discovery.

Cystathionine-β-synthase (EC 4.2.1.22, from Microorganism)

L-Homocysteine + L-Serine ⟶ L-Cystathionine + H_2O

Specifications

Appearance:	Yellow amorphous powder, lyophilized	
Protein purity:	≥90%	
Activity	≥8 U/mg solid	
Glucose-6-phosphate dehydrogenase:	≤0.01%	
Lactate dehydrogenase:	≤0.01%	
EC number:	4.2.1.22 (Recombinant from microorganism)	
Molecular weight:	51 kDa	
Isoelectric point:	5.4	
Michaelis constants:	8.0×10^{-4} M (L-Serine), 1.6×10^{-4} M (L-Homocysteine)	
Inhibitors:	Not inhibited by NaN_3	
Optimum Ph:	8	Fig. 1
Optimum temperature:	40 ℃	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	

Applications

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

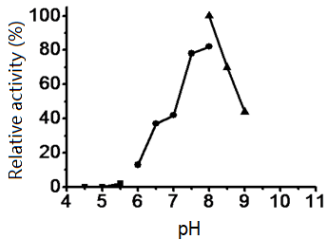

Fig. 1 Optimum pH

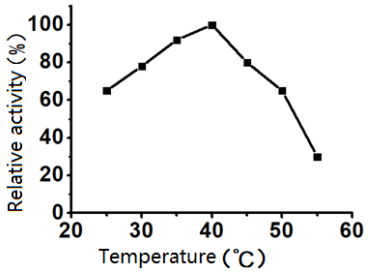

Fig. 2 Optimum temperature

Buffer solution: pH 5.0-6.0, Acetate; pH 6.0-7.5, K-phosphate; pH 7.5-9.0, Tris-HCl; pH 9.0-10.0, Glycine-NaOH.
Enzyme concentration: 1 mg/mL

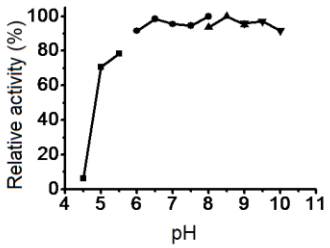

Fig. 3 pH Stability

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

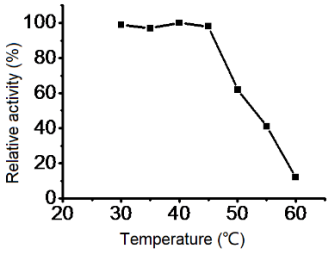

Fig. 4 Thermal stability

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

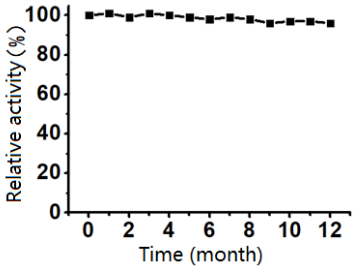

Fig.5 Storage stability (-25～-15 ℃)

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

Assay principle

$$\text{L-Homocysteine + L-Serine} \xrightarrow{\text{CBS}} \text{L-Cystathionine + } H_2O$$

$$\text{L-Cystathionine} \xrightarrow{\text{CBL}} \text{L-Homocysteine + Pyruvate + } NH_3$$

$$\text{Pyruvate + NADH} \xrightarrow{\text{LDH}} \text{Lactate + } NAD^+$$

The consumption of NADH is measured at 340nm by spectrophotometry.

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.

Reagents preparation

Reagent I: 50 mM pH 8.0 PBS, contains 10 mM L-Serine, 2 mM DL-Homocysteine, 0.38 mM S-adenosyl-methionine, 0.25 mM PLP, 1 mM DTT, 5 U/mL CBL, 0.2 mM NADH, 5 U/mL LDH.
Enzyme diluent: 50 mM pH 8.0 PBS 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 ΔAs at 340 nm in 1 minute in a spectrophotometer thermostated at 37℃.

At the same time, measure the blank rate ΔAb 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

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

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

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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