（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K811-M

Specification：48T(46 samples)/ 96T(94 samples)

Measuring instrument：Microplate reader (330-350 nm)

Detection range：0.005-20.0 U/L

Elabscience® Cystathionine-β-synthase (CBS) Activity Colorimetric Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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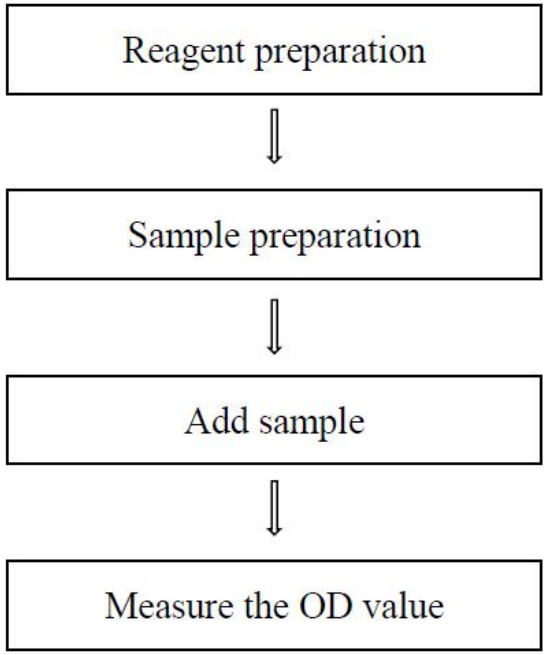
Assay summary



Intended use

This kit can be used to measure cystathionine-β-synthase (CBS) activity in animal tissue and cell samples.

Detection principle

Cystathionine-β-synthase (CBS) can participate in the production of hydrogen sulfide, a gas signaling molecule, and play an important role in cell metabolism. The principle of this kit is that CBS and enzyme catalyzed substrates can convert NADH into NAD^+ through enzyme-linked reaction. By detecting the change of NADH per unit time, the CBS enzyme activity in the sample can be calculated.

Kit components & storage

Item	Component	Size 1 (48T)	Size 2 (96T)	Storage
Reagent 1	Buffer Solution	12 mL × 1 vial	24 mL × 1 vial	-20℃, 12 months, shading light
Reagent 2	Substrate	Powder × 1 vial	Powder × 2 vials	-20℃, 12 months, shading light
Reagent 3	Enzyme Reagent	0.075 mL × 1 vial	0.15 mL × 1 vial	-20℃, 12 months, shading light
	UV-Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (330-350 nm, optimum wavelength: 340 nm)

Reagents:

Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4)

Reagent preparation

① Equilibrate all reagents to 25°C before use..

② The preparation of substrate solution：
Dissolve one vial of substrate with 1 mL of buffer solution, mix well to dissolve. Store at -20°C for 7 days.

③ The preparation of substrate working solution：
Before testing, please prepare sufficient substrate working solution according to the test wells. For example, prepare 4000 μL of substrate working solution (mix well 3600 μL of buffer solution and 400 μL of substrate solution). Store at 2-8℃ for 1 day protected from light.

④ The preparation of chromogenic working solution：
Before testing, please prepare sufficient chromogenic working solution according to the test wells. For example, prepare 2012 μL of chromogenic working solution (mix well 2000 μL of substrate working solution and 12 μL of enzyme reagent). The chromogenic working solution should be stored protected from light and used up within 30 min.

Sample preparation

① Sample preparation

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL normal saline (0.9% NaCl) with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

Cell (adherent or suspension) samples:

① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).

② Wash cells with PBS (0.01 M, pH 7.4).

③ Homogenize 1×10^6 cells in 200 μL normal saline (0.9% NaCl) with a ultrasonic cell disruptor at 4℃.

④ Centrifuge at 10000×g for 10 minutes at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
10% Mouse kidney tissue homogenization	1
10% Mouse brain tissue homogenization	1
10% Mouse heart tissue homogenization	1
10% Mouse liver tissue homogenization	1
10% Mouse lung tissue homogenization	1-5
1×10^6 293T cells	1

Note: The diluent is normal saline (0.9% NaCl). For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

During the use and preparation of the chromogenic working solution should be protected from light and used up within 30 min.

Operating steps

① Sample well: Add 20 μL of sample to the wells.
Control well: Add 20 μL of normal saline (0.9% NaCl) to the wells.

② Add 180 μL of chromogenic working solution to each well.

③ Measure the OD value (A_1) of each well at 340 nm.

④ Incubate at 37°C for 10 min protected from light, measure the OD value (A_2) of each well at 340 nm.

Calculation

The sample:

Animal tissue and cell samples:

Definition: The amount of enzyme of in 1 g sample protein that hydrolyze 1 μmol NADH in 1 min at 37°C is defined as 1 unit.

$$\text{CBS activity (U/gprot)} = \frac{\Delta A_{sample}-\Delta A_{control}}{6220 \times 0.6} \times 0.2 \div 0.02 \div t \div C_{pr} \times f \times 10\text{^}6$$

[Note]

ΔA_{sample}: $\Delta A_{sample} = A_1 - A_2$.

ΔA_{blank}: $\Delta A_{blank} = A_1 - A_2$.

0.2: The volume of reaction system, mL.

0.02: The volume of sample added to the reaction system, mL.

6220: The molar extinction coefficient of NADH, L/mol/cm.

0.6: Optical path, cm.

t: Reaction time, 10 min.

f: Dilution factor of sample before test.

C_{pr}: Concentration of protein in sample, gprot/L.

10^6: 1 mol = 10^6 μmol.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three mouse kidney tissue samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	2.50	10.00	15.00
%CV	2.5	3.2	4.9

Inter-assay Precision

Three mouse kidney tissue samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	2.50	10.00	15.00
%CV	5.2	10.1	8.7

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 98.3%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	2.5	10	15
Observed Conc. (U/L)	2.3	10.5	14.7
Recovery rate (%)	92	105	98

Sensitivity

The analytical sensitivity of the assay is 0.005 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Appendix II Example Analysis

Example analysis:

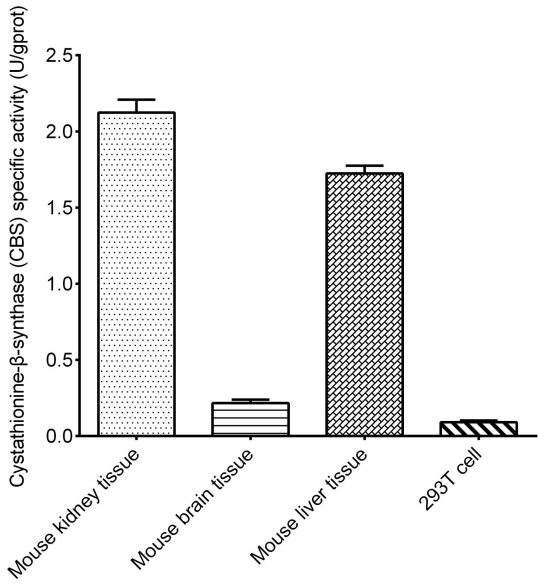
Take 20 μL of 10% mouse kidney tissue homogenization and carry the assay according to the operation steps. The results are as follows:

The A_1 of the sample is 0.891, the A_2 of the sample is 0.714, ΔA_{sample} = 0.891 - 0.714 = 0.177. The A_1 of the control is 0.861, the A_2 of the control is 0.847, $\Delta A_{control}$ = 0.861 - 0.847 = 0.014, the concentration of protein is 19.54 gprot/L and the calculation result is:

CBS activity(U/gprot) = (0.177-0.014) ÷ 6220 ÷ 0.6 × 0.2 ÷ 10 ÷0.02 × 10^6 ÷ 19.54

= 2.24 U/gprot

Detect 10% mouse kidney tissue homogenization (the concentration of protein is 19.459 gprot/L), 10% mouse brain tissue homogenization (the concentration of protein is 14.23 gprot/L), 10% mouse liver homogenization (the concentration of protein is 19.45 gprot/L) and 1×10^6 293T cells (the concentration of protein is 0.31 gprot/L), according to the protocol, the result is as follows:



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.