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Cystathionine β Synthase Activity Assay Kit (Fluorometric)

rev 05/20

(Catalog # K998-100; 100 assays; Store at -20°C)

I. Introduction:

Cystathionine β Synthase (EC 4.2.1.22, CβS) is a PLP-dependent enzyme that catalyzes the formation of H_2S and cystathionine when using cysteine and homocysteine as substrates. Known for its role in human sulfur metabolism, mutations in the gene encoding CβS can result in high concentrations of homocysteine in plasma. Elevated circulating concentrations of homocysteine result in genetic disorders including Homocysteinuria and Down syndrome. Specifically, Homocysteinuria is an autosomal recessive disease with clinical manifestations including mental retardation, thromboembolism and connective tissue defects. Hydrogen sulfide, CβS product, is an important gaseous mediator, like nitric oxide, that has significant effects on the immunological, neurological, cardiovascular and pulmonary systems of mammals. BioVision's Cystathionine β Synthase Assay Kit utilizes cysteine and homocysteine as substrates to produce H_2S. Hydrogen sulfide reacts with the azido-functional group of the fluorescent probe yielding a fluorescent amino group (Ex/Em = 368/460 nm). The assay is highly sensitive, has a simple easy-to-follow protocol, and can detect as low as 1.45 mU of CβS activity.

$$\text{Cysteine + Homocysteine} \xrightarrow{\text{CβS}} \text{Cystathionine + } H_2S \xrightarrow{\text{CβS Probe}} \text{Fluorescence (Ex/Em= 368/460 nm)}$$

II. Applications:

- Screening and characterization of Cystathionine β Synthase (CβS) activity in tissues or cells
- Identification/characterization of primary cells and cell lines to detect Cystathionine β Synthase (CβS) activity

III. Sample Type:

- Animal tissues: liver, pancreas, plasma, etc.
- Cell culture: adherent or suspension cells (i.e. HepG2 cells)

IV. Kit Contents:

Components	K998-100	Cap Code	Part Number
CβS Assay Buffer	25 ml	WM	K998-100-1
CβS Probe (in DMSO)	500 µl	Purple	K998-100-2
CβS Substrate	4.0 ml	Amber/NM	K998-100-3
Cofactor 1	500 µl	Amber	K998-100-4
Cofactor 2	500 µl	Orange	K998-100-5
Reducing Agent	1 vial	Yellow	K998-100-6
AMC Standard	100 µl	Amber/Yellow	K998-100-7
CβS Positive Control	50 µl	Green	K998-100-8

V. User Supplied Reagents and Equipment:

- Cell line for testing: cells with high levels of endogenous CβS or heterologous cells stably transfected with human CβS.
- Appropriate cell culture medium and 5% CO_2 cell culture incubator.
- Multi-well fluorescence microplate reader.
- 96-well white plates with flat bottom.

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **CβS Assay Buffer:** Equilibrate to room temperature before running the assay. Store at 4°C.
- **CβS Probe (in DMSO) and CβS Substrate:** Light sensitive. Aliquot and store at -20°C. Allow reagents to equilibrate to RT before use.
- **Cofactor 1 and Cofactor 2:** Aliquot and store at -20°C, stable for at least 4 freeze/thaw cycles.
- **Reducing Agent:** Reconstitute with 250 µl CβS Assay Buffer. Store at 4°C. Keep on ice during use. Stable for 4 freeze/thaw cycles.
- **AMC Standard (in DMSO, 1 mM):** Store at -20°C. Use within two months.
- **CβS Positive Control:** Aliquot and store at -80°C. Keep on ice while in use. Use within two months.

VII. CβS Activity Assay Protocol:

The procedure described below is for a 96-well microplate format but may be adapted to other formats by scaling the reagent volumes according to the desired microplate size.

1. **Sample Preparation:** Add 500 µl of CβS Assay Buffer to 10 mg of sample (wet weight or cell pellet). Homogenize on ice using a Dounce homogenizer (Cat. # 1998). Centrifuge at 10,000 X g, 4°C for 10 min. Collect the supernatant. Add 5-30 µl of supernatant into desired well(s) in a 96-well white microplate. If necessary, adjust the volume to 30 µl with CβS Assay Buffer. For positive control: add 3 µl into desired well(s) and adjust the final volume to 30 µl with CβS Assay Buffer.

   Notes:

   a. Cell and tissue lysate can be stored at -80°C for future experiments.
   b. For unknown samples, we suggest doing a pilot experiment & test several doses to ensure the readings are within the Standard Curve range.
   c. For samples having high background, prepare parallel well(s) containing the same amount of sample as in the test well. Adjust the volume to 30 µl with CβS Assay Buffer.

d. For No Enzyme Control (NEC): Add 30 µl CβS Assay Buffer. Add 170 µl of Master Mix (MM, **see Step 3**) to the well. Compare linear range of NEC with Background (BK) to determine which value should be subtracted from Positive Control or Sample in determining activity.

2. AMC Standard Curve: Dilute 1 mM CβS Standard to 10 µM working concentration: Add 2 µl of 1 mM AMC Standard to 198 µl with dH_2O. Mix well. Add 0, 5, 10, 15, 20, 25 and 30 µl of the diluted 10 µM AMC Standard into a series of wells in a 96-well plate. Adjust the volume to 30 µl/well with the dH_2O. Add 170 µl of Standard Reaction Mix (SC, see Step 3) to each well to generate 0, 50, 100, 150, 200, 250, and 300 pmol/well of AMC Standard.

Note: For enhanced sensitivity, dilute the 10 µM AMC Standard 2-fold (add 50 µl 10 µM AMC plus 50 µl H_2O) to create a 5 µM AMC Standard. Add 0, 2, 4, 6, 8, 10 µl of 5 µM AMC Standard Curve into a series of wells. Adjust volume to 30 µl/well with dH_2O. Add 170 µl of Standard Curve Reaction Mix (SC, see **Step 3**) to each well to generate 0, 10, 20, 30, 40, and 50 pmol/well of AMC Standard.

3. Reaction Mix: Dilute Reducing Agent before use, by adding 17 µl of stock solution to 483 µl CβS Assay Buffer to create a working solution. Mix enough reagents to prepare Master Mix (MM), Background Mix (BK), Standard Curve Mix (SC) and No Enzyme Control (NEC) for the number of assays and standards to be performed. For each well, prepare 170 µl MM, BK or SC, containing:

	MM	BK	SC
CβS Assay Buffer	115	155	157
CβS Probe (in DMSO)	2	2	--
CβS Substrate	40	--	--
Cofactor 1	2	2	2
Cofactor 2	1	1	1
Working Reducing Agent	10	10	10

Add 170 µl of each Reaction Mix as follows: 170 µl MM to the wells containing Samples, NEC and positive control. 170 µl of BK to the wells containing sample background(s) and 170 µl SC to the AMC Standard Curve wells. Mix well.

Note: Do not store the Working Reducing Agent. Always prepare fresh dilution prior to the assay.

4. Measurement: Measure fluorescence immediately at Ex/Em= 368/460 nm in kinetic mode for 40-60 min. at 37°C.

Note: Incubation time depends on CβS Activity in the samples. We recommend measuring fluorescence in kinetic mode, and choosing two time points (t1 and t2) in the linear range to calculate the CβS activity of the samples. We recommend running the assay for at least 40-60 minutes in the kinetic mode. The standard curve can be read in end point mode (i.e. at the end of incubation time).

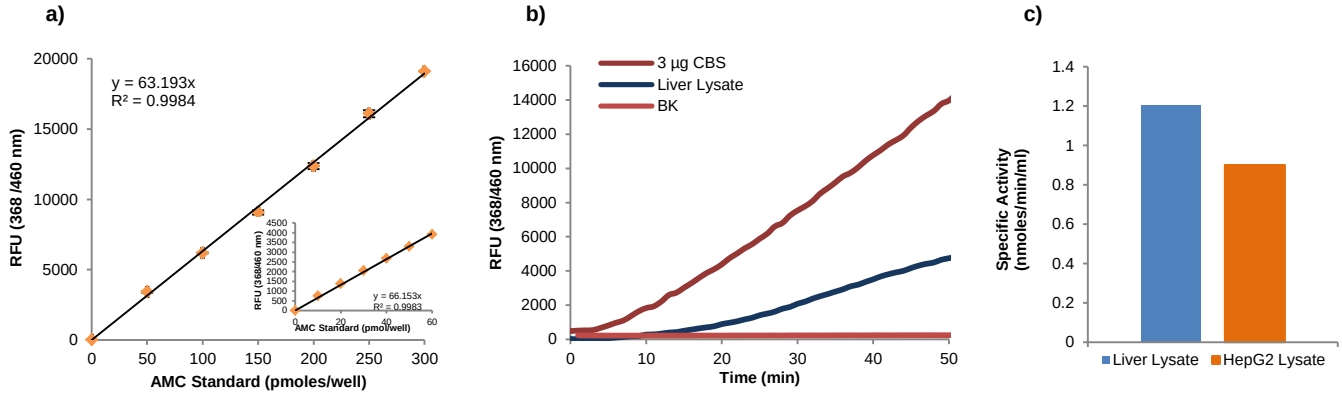
Note: The enzymatic product (H_2S) reacts with the CβS probe to yield fluorescence. This may cause a lag phase to appear in the CβS Activity Progress Curve.

5. Calculation: Plot the Standard Curve. If sample Background Control (BK) reading is higher than NEC, subtract that value from the sample readings, otherwise subtract NEC value from sample value: $\Delta RFU = \Delta RFU_S - \Delta RFU_{BK/NEC}$. Apply ΔRFU to Standard Curve to get B pmol of AMC generated by CβS during the reaction time ($\Delta t = t_2 - t_1$).

Sample's CβS Activity = B/(Δt X V) x D = nmole/min/ml = U/ml

Where: **B=** AMC amount from Standard Curve (pmol).
Δt= reaction time (min.).
V = sample volume added into the reaction well (ml).
D = Dilution Factor

Unit Definition: One unit of CβS activity is the amount of enzyme that generates 1.0 nmol of AMC per min. at pH 8.0 at 37℃.
CβS Activity can also be expressed as U/mg protein.



Figures: (a) AMC Standard Curve, (b) CβS activity in Liver Lysate (20 µg) & Positive Control (1.5 µg) (c) CβS specific activity in HepG2 cell lysate and Liver lysate. Assays were performed following the kit protocol.

VIII. Related Products:

Cysteine Assay Kit (Fluorometric) (K558)
AHCY Inhibitor Screening Kit (Fluorometric) (K326)
Adenosylhomocysteinase (AHCY) Activity Fluorometric Assay Kit (K807)

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