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ab241043 Cystathionine beta Synthase Assay Kit

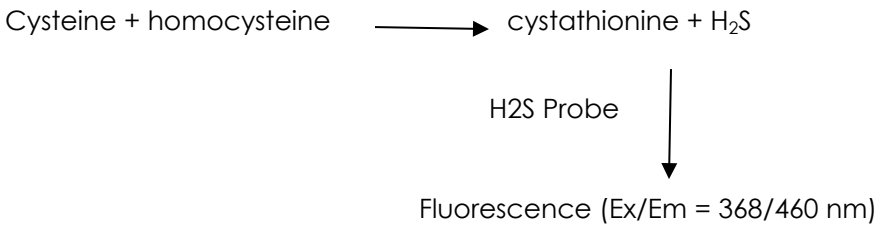
For the measurement of Cystathionine beta Synthase activity in cells and tissues.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

Cystathionine beta Synthase Assay Kit (ab241043) utilizes cysteine and homocysteine as substrates to produce H2S. 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.



Prepare tissue / cell lysate sample.

⇩

Prepare Standards and Positive Control. Prepare Master Mix, Background Mix and Standard Curve Mix.

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Add Sample, No Enzyme Control and Positive Control to Master Mix. Add Background Controls to Background Mix. Add Standard Curve samples to Standard Curve Mix.

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

2. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
CβS Assay Buffer	25 mL	-20°C	4°C
H2S Probe	0.5 mL	-20°C	-20°C
CβS Substrate	4 mL	-20°C	-20°C
Cofactor 1	0.5 mL	-20°C	-20°C
SAM Cofactor	500 µL	-20°C	-20°C
Reducing Agent I	1 vial	-20°C	4°C
AMC Standard	100 µL	-20°C	-20°C
CβS Positive Control	50 µL	-80°C	-80°C

3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- 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% CO2 cell culture incubator.
- Multi-well fluorescence microplate reader capable of reading at Ex/Em = 368/460 nm.
- 96-well white plates with flat bottom.
- Dounce homogenizer.
- We recommend Protease Inhibitor Cocktail II (ab201116) [AEBSF, aprotinin, E-64, EDTA, leupeptin] as general use cocktail.

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

5.1 CβS Assay Buffer

Ready to use as supplied. Equilibrate to room temperature before running the assay. Store at 4°C.

5.2 H2S Probe

Ready to use as supplied. Light sensitive. Aliquot and store at -20°C. Allow reagents to equilibrate to room temperature before use.

5.3 CβS Substrate

Ready to use as supplied. Light sensitive. Aliquot and store at -20°C. Allow reagents to equilibrate to room temperature before use.

5.4 Cofactor 1

Ready to use as supplied. Aliquot and store at -20°C, stable for at least 4 freeze/thaw cycles.

5.5 SAM Cofactor

Ready to use as supplied. Aliquot and store at -20°C, stable for at least 4 freeze/thaw cycles.

5.6 Reducing Agent I

Reconstitute with 250 µl CβS Assay Buffer. Store at 4°C. Keep on ice during use. Stable for 4 freeze/thaw cycles.

5.7 AMC Standard

Ready to use as supplied. Store at -20°C. Use within two months.

5.8 CβS Positive Control

Ready to use as supplied. Aliquot and store at -80°C. Keep on ice while in use. Use within two months.

6. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

1. Dilute 1 mM AMC Standard to 10 µM working concentration: Add 2 µL of 1 mM AMC Standard to 198 µL with dH_2O. Mix well.
2. 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.
3. Add 170 µL of Standard Reaction Mix (SC, see Step 8.2) to each well to generate 0, 50, 100, 150, 200, 250, and 300 pmol/well of AMC Standard.

Standard #	10 µM AMC Standard (µL)	dH_2O (µL)	Standard Reaction Mix (µL)	Final volume standard in well (µL)	AMC Standard in well (pmol/well)
1	0	30	170	200	0
2	5	25	170	200	50
3	10	20	170	200	100
4	15	15	170	200	150
5	20	10	170	200	200
6	25	5	170	200	250
7	30	0	170	200	300

ΔNote: 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 8.2) to each well to generate 0, 10, 20, 30, 40, and 50 pmol/well of AMC Standard.

7. Sample Preparation

General sample information:

We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.

We recommend that you use fresh samples for the most reproducible assay.

We recommend Protease Inhibitor Cocktail II (ab201116) [AEBSF, aprotinin, E-64, EDTA, leupeptin] as general use cocktail.

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.

7.1 Cells or tissue:

1. Add 500 µ of CβS Assay Buffer to 10 mg of sample (wet weight or cell pellet).
2. Homogenize on ice using a Dounce homogenizer.
3. Centrifuge at 10,000 X *g* at 4°C for 10 minutes. Collect the supernatant.
4. 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.

7.2 Positive Control:

For positive control: add 3 µL into desired well(s) and adjust the final volume to 30 µL with CβS Assay Buffer.

ΔNote: Cell and tissue lysate can be stored at -80°C for future experiments.

ΔNote: 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.

8. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
- Assay all standards, controls and samples in duplicate.

8.1 Reaction wells set up:

- Standard wells = 30 µL standard dilutions.
- Sample wells = 5-30 µL samples (adjust volume to 30 µL/well with CβS Assay Buffer).
- Sample Background Control wells = 5-30 µL samples (use the same volume as for the sample wells)(adjust volume to 30 µL/well with CβS Assay Buffer).

8.2 CβS Reaction mixes:

1. Dilute Reducing Agent I before use, by adding 17 µL of stock solution to 483 µL CβS Assay Buffer to create a working solution.
2. 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.

ΔNote: For No Enzyme Control (NEC): Add 30 µl CβS Assay Buffer. Add 170 µl of Master Mix 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.

3. For each well, prepare 170 µL MM, BK or SC, containing:

Component	Master Mix(µL)	Background Mix (µL)	Standard Curve Mix (µL)
CβS Assay Buffer	115	155	157
H2S Probe	2	2	-
CβS Substrate	40	-	-
Cofactor 1	2	2	2
SAM Cofactor	1	1	1
Working Reducing Agent I	10	10	10

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

4. Add 170 µL of Master Mix into each Sample, No Enzyme Control and Positive Control well.
5. Add 170 µL of Background Reaction Mix into the Background Control sample wells.
6. Add 170 µL of Standard Curve Mix into the AMC Standard Curve wells.

8.3 Measurement:

Measure fluorescence immediately at Ex/Em= 368/460 nm in kinetic mode for 40-60 minutes at 37°C. The Standard Curve may be read in end-point mode (i.e. At the end of the incucation period).

ΔNote: Incubation time depends on CβS Activity in the samples. We recommend measuring fluorescence in kinetic mode, and choosing two time points (t_1 and t_2) 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.

ΔNote: The enzymatic product (H_2S) reacts with the H2S Probe to yield fluorescence. This may cause a lag phase to appear in the CβS Activity Progress Curve.

9. Data Analysis

Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.

1. Average the duplicate reading for each standard, control and sample.
2. Subtract 0 Standard reading from all Standard readings. Plot the Standard Curve.
3. If sample Background Control (BK) reading is higher than NEC, subtract that value from the sample readings, otherwise subtract NEC value from sample value: $\Delta RFU = \Delta RFU_S - \Delta RFU_{BK/NEC}$.
4. Apply ΔRFU to Standard Curve to get B pmol of AMC generated by CβS during the reaction time ($\Delta t = t_2 - t_1$).

$$\text{C}\beta\text{S Activity (A)} = \frac{B}{(\Delta t * V)} * D$$

Where:

A = CβS Activity (pmol/minute/mL = U/mL)

B = amount of AMC produced in the sample well calculated from standard curve (pmol).

V = sample volume added in the sample wells (mL).

D = sample dilution factor.

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

10. Typical Data

Data provided **for demonstration purposes only.**

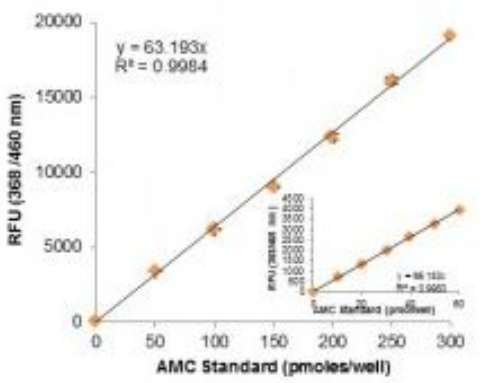

Figure 1. AMC Standard Curve.

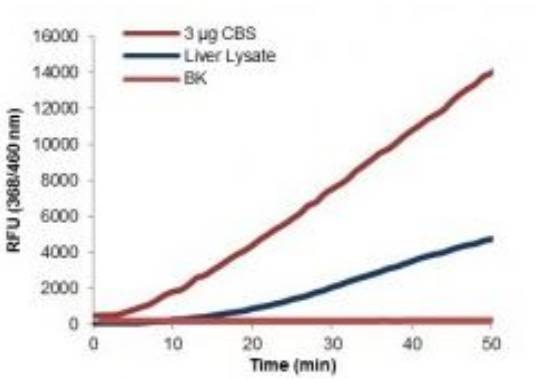

Figure 2. CβS activity in Liver Lysate (20 µg) & Positive Control (1.5 µg).

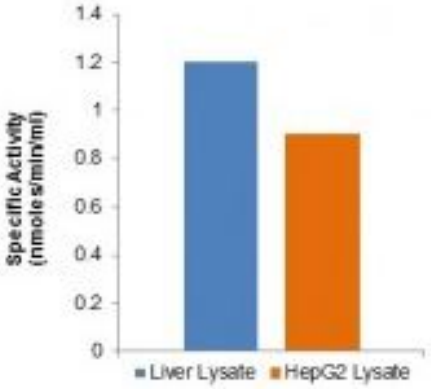

Figure 3. CβS specific activity in HepG2 cell lysate and liver lysate.

11. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)