Adenosylhomocysteinase (AHCY) Activity Fluorometric Assay Kit

7/14

(Catalog # K807-100; 100 assays; Store at -20°C)

I. Introduction:

Adenosylhomocysteinase (AHCY) (EC 3.3.1.1) or S-adenosylhomocysteine hydrolase (SAHH); is an enzyme that catalyzes the reversible hydrolysis of S-Adenosyl Homocysteine (SAH) to adenosine and homocysteine. AHCY regulates the intracellular SAH concentration which in turn regulates S-adenosyl methionine (SAM)-dependent methyltransferases. Down-regulation of AHCY has been associated with certain forms of cancer and Huntington's disease, while in Wilson's disease; the enzyme is inhibited by the accumulated copper. Mutations in the AHCY gene cause SAHH deficiency disease. BioVision's AHCY Activity Assay kit can kinetically measure AHCY activity by detecting adenosine generation resulting from the hydrolysis of SAH. Adenosine is detected via a multi-step reaction, resulting in the generation of an intermediate that reacts with the OxiRed™ Probe. The fluorescent product is measured at Ex/Em = 535/587 nm. Limit of quantification (L.O.Q) is 1 µU recombinant human AHCY.

II. Application:

- Detection of AHCY activity

III. Sample Type:

- Purified recombinant protein
- Cell and tissue lysate

IV. Kit Contents:

Components	K807-100	Cap Code	Part Number
AHCY Assay Buffer	25 ml	WM	K807-100-1
Homogenization Buffer	60 ml	NM	K807-100-2
OxiRed™ Probe	200 µl	Red	K807-100-3A
SAH Substrate	100 µl	Brown	K807-100-4
Enzyme Mix I	Lyophilized	Blue	K807-100-5
Enzyme Mix II	Lyophilized	Clear	K807-100-6
Adenosine Standard (10 mM)	100 µl	Yellow	K807-100-7
AHCY Enzyme (Positive Control)	10 µl	Green	K807-100-8

V. User Supplied Reagents and Equipment:

- 96-well plate with flat bottom. White plate is preferred for this assay.
- Fluorescence microplate reader
- Protease Inhibitor Cocktail (Cat. # K271 or its equivalent)

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.

- **AHCY Assay Buffer:** Bring to 37°C before use. Store at -20°C or 4°C
- **Enzyme Mix I and Enzyme Mix II:** Reconstitute with 210 µl Homogenization Buffer and mix gently by pipetting. Briefly centrifuge to collect the contents in the bottom of the tube. Aliquot and store at -20°C. Avoid repeated freeze/thaw.
- **AHCY Enzyme:** Store at -20°C. Avoid repeated freeze/thaw.

VII. AHCY Activity Assay Protocol:

1. **Sample Preparation:** Rinse tissue and transfer ~100 mg of fresh or frozen tissue (stored at -80°C) to a prechilled tube. Add 300 µl cold Homogenization Buffer containing protease inhibitor cocktail (not provided) and thoroughly homogenize tissue on ice. Transfer the tissue homogenate to a cold microfuge tube. To prepare cell extract, add 150-300 µl cold Homogenization Buffer containing protease inhibitor cocktail (not provided) to 1-5 x 10^6 fresh or frozen cells and pipette several times to disrupt the cells. Transfer cell homogenate including cell debris to a cold microfuge tube and agitate on a rotary shaker at 4°C for at least 15 min. Centrifuge the tissue or cell homogenate at 16,000 X g for 10 min. at 4°C. Transfer the clarified supernatant to a fresh pre-chilled tube & store on ice. Use lysates immediately to assay AHCY activity.

   Note: Lysates can be aliquoted and snap frozen in liquid nitrogen before storing at -20°C. Avoid freeze/thaw.

2. **Adenosine Standard:** Dilute Adenosine Standard to 1 mM by adding 10 µl of 10 mM Adenosine Standard to 90 µl AHCY Assay Buffer. Further dilute the Adenosine Standard to 10 µM by adding 10 µl of 1 mM Adenosine to 990 µl AHCY Assay Buffer. Add 0, 2, 4, 6, and 8 µl of diluted 10 µM Adenosine Standard into a series of wells in 96-well plate to generate 0, 20, 40, 60, and 80 pmol/well Adenosine Standard. Adjust the volume to 50 µl/well with AHCY Assay Buffer.

3. **AHCY Activity Assay:** Add 2-50 µl (5-25 µg protein) of cell/tissue homogenate or purified protein into 96-well plate. Dilute AHCY enzyme (Positive Control) as needed, 1:10 in AHCY Assay Buffer just before use. Use 1-4 µl of diluted AHCY enzyme for the assay. Make up the volume of samples and Positive Control to 50 µl/well with AHCY Assay Buffer.

   Notes:

   a. For unknown samples, we suggest testing several doses to ensure the readings are within the Standard Curve range.

   b. For samples having adenosine background, prepare parallel sample well(s) as sample background control(s).

4. Reaction Mix: Prepare enough reagents for the number of assays to be performed. Make 50 µl of Reaction Mix and Background Control Mix containing:

	Reaction Mix	Background Control Mix
AHCY Assay Buffer	44.5 µl	45.5 µl
Enzyme Mix I	2 µl	2 µl
Enzyme Mix II	2 µl	2 µl
OxiRed™ Probe	0.5 µl	0.5 µl
SAH Substrate	1 µl	-----

Add 50 µl of Reaction Mix into each Sample and Positive Control wells and 50 µl of Background Control mix to Standards and Sample Background Control well(s). Mix well.

5. Measurement: Measure fluorescence (Ex/Em = 535/587 nm) in kinetic mode for at least 30 min. at 37°C. Choose two time points (T_1 & T_2) in linear range (can be as short as 2 min.) of plot and obtain corresponding RFU for sample (R_{S1} and R_{S2}) and sample Background Control (R_{BG1} and R_{BG2}). Read the Adenosine Standard Curve along with the samples.

6. Calculations: Subtract 0 Standard reading from all Standard and Positive Control readings. Plot the Adenosine Standard Curve. Subtract sample Background Control reading from sample reading. Apply the ΔRFU to the Standard Curve to get B pmoles of Adenosine generated by the sample during the reaction time ($\Delta T = T_2 - T_1$).

$$\text{Sample's AHCY Activity} = \frac{\mathbf{B}}{\Delta T \times \mu g\ of\ protein} = \text{pmol/min/µg} = \text{µU/µg}$$

Where: **B** is Adenosine amount from Standard Curve (pmol).
ΔT is the reaction time (min.)
µg of protein is the amount of protein/well in µg

Sample AHCY Activity can also be expressed as mU/mg (nmoles/min adenosine generated per mg) of protein.

Unit Definition: One unit of AHCY activity is the amount of enzyme that hydrolyzes the substrate to yield 1.0 µmol of adenosine/min. at 37°C.

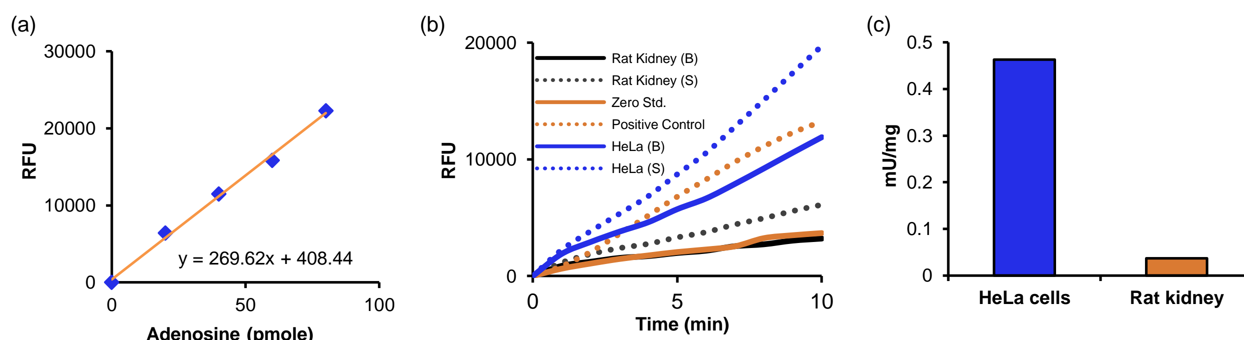

Figure: (a) Adenosine Standard Curve, (b) AHCY activity in rat kidney lysate (24 µg), HeLa cell lysate (7.6 µg) and Positive Control; S: Sample, B: Background (c) AHCY specific activity in HeLa cell lysate (4 µg) and rat kidney lysate (28 µg). Assays were performed following the kit protocol.

VIII. RELATED PRODUCTS:

Xanthine Oxidase Colorimetric/Fluorometric Assay Kit (K710)
Xanthine/Hypoxanthine Colorimetric/Fluorometric Assay Kit (K685)
ADP Colorimetric/Fluorometric Assay Kit (K355)
ATP Colorimetric/Fluorometric Assay Kit (354)
Inosine Fluorometric Assay Kit (K712)
Uric Acid Colorimetric/Fluorometric Assay Kit (K608)
ADP Colorimetric Assay Kit II (K356)

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