Adenosylhomocysteinase Activity Fluorometric Microplate Assay Kit User Manual

Catalog # CAK8002

(Version 1.1A)

Detection and Quantification of Adenosylhomocysteinase Activity in Tissue extracts, Cell lysate, Cell culture media, Other biological fluids Samples.

For research use only. Not for diagnostic or therapeutic procedures.

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.

Adenosylhomocysteinase Activity Microplate Assay Kit provides a simple and sensitive method for monitoring adenosylhomocysteinase activity in various samples. In this assay, AHCY activity is detected by 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 probe, which can be detected fluorometrically (Ex/Em 535/587).

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Black Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	20 ml x 1	4 °C
Substrate	Powder x 1	-20 °C
Enzyme	Powder x 1	-20 °C
Probe	Powder x 1	-20 °C, keep in dark
Probe Diluent	1 ml x 1	4 °C
Standard	Powder x 1	4 °C
Positive Control	Powder x 1	-20 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Substrate: add 1 ml Reaction Buffer before use, warm at 40-50 °C water bath to dissolve. Store at -20 °C. Use within one month.

Enzyme: add 1 ml Reaction Buffer to dissolve before use. Aliquot & store at -20 °C. Use within one month.

Probe: Warm Probe Diluent to RT prior to use to melt frozen Probe Diluent; then add 1 ml Probe Diluent to dissolve. Store at -20 °C, protect from light and moisture. Use within one month.

Standard: add 1 ml distilled water to dissolve before use; then add 0.01 ml into 0.99 ml distilled water, mix, the concentration will be 150 μmol/L. Store at -20 °C. Use within one month.

Positive Control: add 1 ml Assay Buffer to dissolve before use. Store at -80 °C. Use within one month.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Fluorescence microplate reader to read fluorescence at Ex/Em = 535/587
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

IV. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 10000g 4 °C for 20 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 10000g 4 °C for 20 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For liquid samples

Detect directly or dilute with Assay Buffer.

V. ASSAY PROCEDURE

Warm the solution to room temperature before use.

Add following reagents into the microplate:

Reagent	Sample	Control	Standard	Blank	Positive Control
Reaction Buffer	160 µl	160 µl	170 µl	170 µl	160 µl
Sample	10 µl	--	--	--	--
Distilled water	--	10 µl	--	10 µl	--
Standard	--	--	10 µl	--	--
Positive Control	--	--	--	--	10 µl
Substrate	10 µl	10 µl	--	--	10 µl
Probe	10 µl	10 µl	10 µl	10 µl	10 µl
Enzyme	10 µl	10 µl	10 µl	10 µl	10 µl
Mix, put it in the oven, 37 °C for 10 minutes, protected from light, record fluorescence measured at Ex/Em = 535/587 nm.					

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

Unit Definition: One unit of Adenosylhomocysteinase activity is defined as the enzyme produce 1 μmol adenosine per min at 37° C.

1. According to the protein concentration of sample

$$AHCY\ (U/mg) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (C_{Protein} \times V_{Sample}) / T$$

$$= 0.015 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$AHCY\ (U/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W/ V_{Assay}) / T$$

$$= 0.015 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cells or bacteria

$$AHCY\ (U/10^4) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times N/ V_{Assay}) / T$$

$$= 0.015 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / N$$

4. According to the volume of sample

$$AHCY\ (U/mg) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} / T$$

$$= 0.015 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank})$$

$C_{Standard}$: the concentration of standard, 150 μmol/L = 0.15 μmol/ml;

$C_{Protein}$: the protein concentration, mg/ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria, N × 10^4;

$V_{Standard}$: the volume of the standard, 0.01 ml;

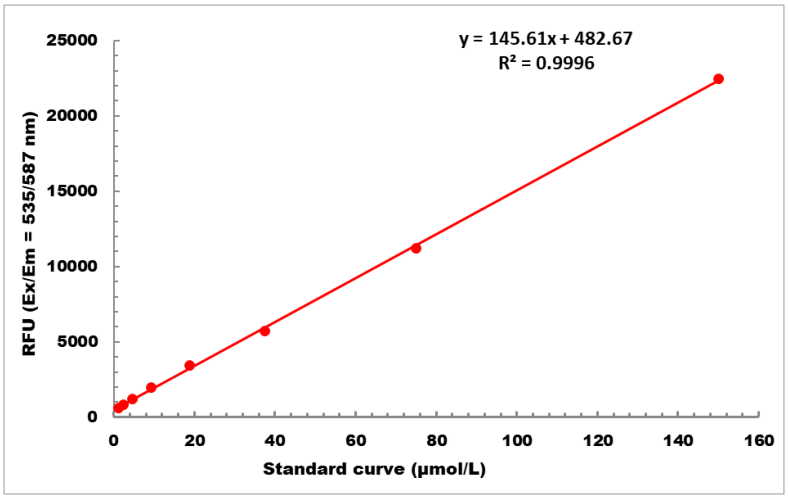
V_{Sample}: the volume of sample, 0.01 ml;

V_{Assay}: the volume of Assay buffer, 1 ml;

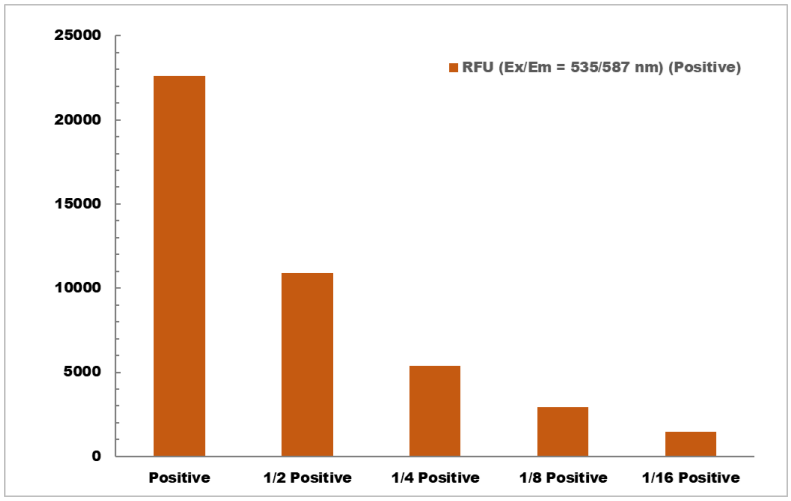
T: the reaction time, 10 minutes.

VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 1 µmol/L - 150 µmol/L



Positive Control reaction in 96-well plate assay with decreasing the concentration

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES