Adenosylhomocysteinase Activity Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1314

(Version 1.2A)

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 ActivityColorimetric Microplate Assay Kit provides a simple and sensitive method for monitoring adenosylhomocysteinase activity in various samples. In this assay, AHCY hydrolyses SAH and produces adenosine. Adenosine deaminase catalyzes conversion of adenosine into inosine and ammonia, which reacts with a developer to form a colored product that absorbs maximally at 620 nm.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 mlx 4	4 °C
Reaction Buffer	10 mlx 1	4 °C
Substrate	Powderx 1	-20 °C
Enzyme	Powderx 1	-20 °C
Dye ReagentI	Powderx 1	4 °C
Dye ReagentII	Powderx 1	4 °C
Dye Reagent II Diluent	3 mlx 1	4 °C
Standard (1mmol/L)	1 mlx 1	4 °C
Positive Control	Powder x 1	-20 °C
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 620 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

IV. REAGENT PREPARATION

Standard:Briefly centrifuge prior to opening.Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve. The concentration of standard curve could be 1/0.5/0.25/0.125/0.0625/ 0.0312/0.0156mmol/L.

Substrate: Briefly centrifuge prior to opening. Add 1 mlReaction Buffer before use, warm at 40-50 °Cwater bath to dissolve.Store at 4 °C for 1-2 days or -20°C for 1-2months after reconstitution.

Dye Reagent I: Add 7 ml distilled water to dissolve before use. Keep in dark for immediate use after reconstitution.

Dye Reagent II: Add 3 ml Dye Reagent II DiluentintoDye Reagent II,mix before use.Keep in dark and store at 4 °C for 1-2 weeks after reconstitution.

Enzyme: Briefly centrifuge prior to opening. Add 1mlAssay Buffer before use, mix.Aliquot & store at -80 °C and use within 1 month.

Positive Control: Briefly centrifuge prior to opening. Add 0.1mlAssay Buffer before use, mix.Aliquot & store at -80 °C and use within 1 month.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. SAMPLE PREPARATION

1.For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 mlAssay 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 20minutes, 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 mlAssay Buffer on ice, centrifuged at 10000g 4°C for 20minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3.For liquid samples

Detect directlyor dilute with Assay Buffer.

VI. ASSAY PROCEDURE

Warm thesolution to room temperature before use.

Add following reagents intothe microplate:

Reagent*	Sample**	Control	Standard	Blank	Positive Control
Reaction Buffer	70 µl	70 µl	--	--	70 µl
Sample	10 µl	--	--	--	--
Distilled water	--	10 µl	--	--	--
Positive Control	--	--	--	--	10 µl
Enzyme	10 µl	10 µl	--	--	10 µl
Substrate	10 µl	10 µl	--	--	10 µl
Standard	--	--	100 µl	--	--
Distilled water	--	--	--	100 µl	--
Dye ReagentI	70 µl	70 µl	70 µl	70 µl	70 µl
Dye Reagent II	30 µl	30 µl	30 µl	30 µl	30 µl
Mix,incubate at RT for 10 mins, record absorbance measured at 620 nm.					

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**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 samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

Unit Definition: One unit of Adenosylhomocysteinase activity is defined as the enzyme produce 1 μmolammonia per minat 37° C.

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(OD_{Sample}-OD_{Control}) - \text{Intercept}}{\text{Slope}\times T} \times \frac{V_{Standard}}{V_{Sample}} \times n(U/mL)$$

2. According to one point of the standard OD value and concentration

2.1 According to the protein concentration of sample

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

2.2According to the quantity of cells or bacteria

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

2.3 According to the weight of sample

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

2.4 According to the volume of sample

$$\text{Activity}=\frac{(C_{Standard}\times V_{Standard}) \times (OD_{Sample}-OD_{Control})}{(OD_{Standard}-OD_{Blank}) \times V_{Sample}\times T}(U/mL)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

$C_{Protein}$: the protein concentration of sample, mg/mL

W: the weight of total sample, g

N: the quantity of total cell or bacteria sample, 10^4

$C_{Standard}$: the concentration of standard, μmol/mL

$V_{Standard}$: the volume of standard in assay procedure, mL

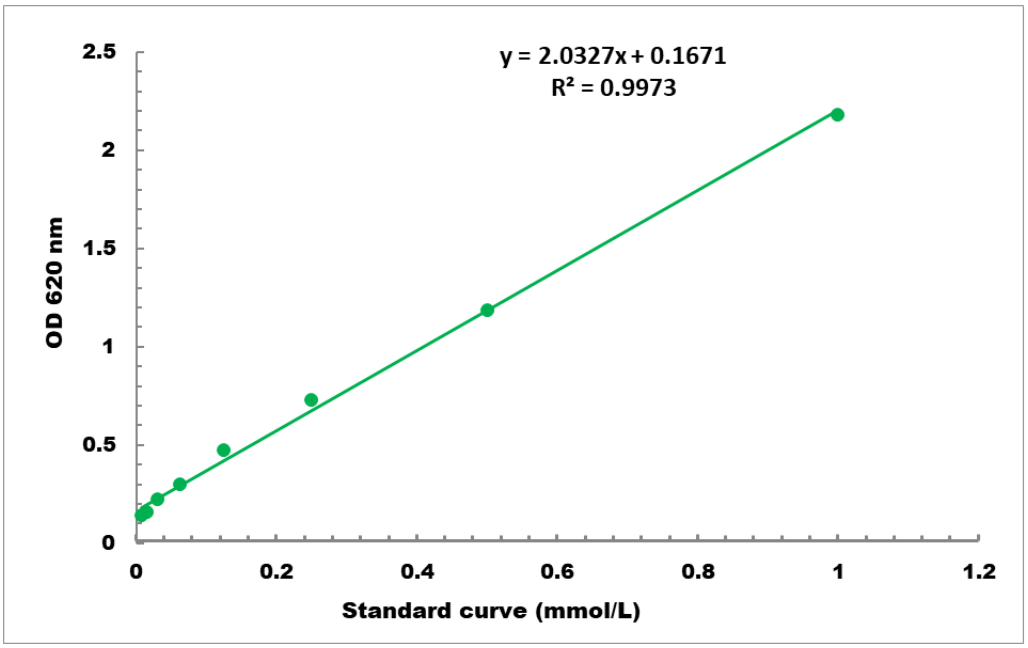
V_{Sample}: the volume of sample in assay procedure, mL

V_{Assay}: the volume of Assay Buffer in sample preparation, mL

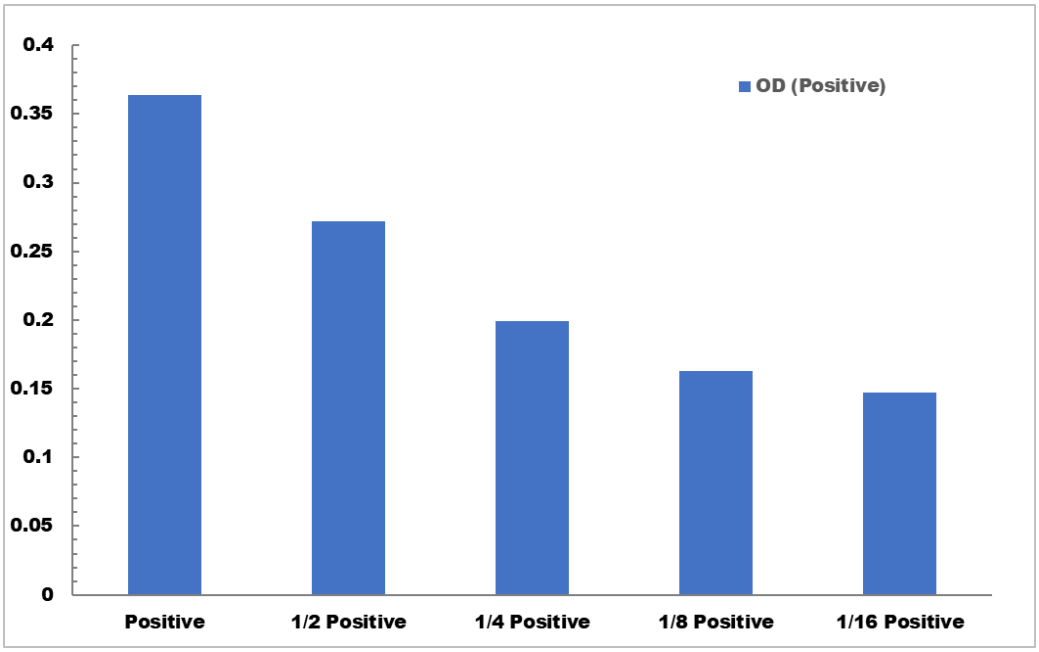
T: the reaction time, minute

VIII. TYPICAL DATA

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



Detection Range: 0.01mmol/L -1mmol/L



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