# Adenosylhomocysteinase Activity FluorometricMicroplate Assay Kit User Manual

**Catalog # CAK8002**

(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 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 mlx 4 | 4 °C |
| Reaction Buffer | 20 mlx 1 | 4 °C |
| Substrate | Powderx 1 | -20 °C |
| Enzyme | Powderx 1 | -20 °C |
| Probe | Powder x 1 | -20 °C, keep in dark |
| Probe Diluent | 1 mlx 1 | 4 °C |
| Standard | Powderx 1 | 4 °C |
| Positive Control | Powder x 1 | -20 °C |
| Plate Adhesive Strips | 3 Strips | |
| Technical Manual | 1 Manual | |

## 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. REAGENT PREPARATION

**Standard**:Briefly centrifuge prior to opening.Dissolvein1 ml distilled waterto generate 15 mmol/L of standardstock solution, store at 4 °C for 1 day or -20°C for 3daysafter reconstitution.Then dilute to 150 µmol/Lstandard top solutionby adding 10 µlstock solution into 990 µldistilled water. Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve. The concentration of standard curve could be 150/75/37.5/18.75/9.375/4.688/2.344/1.172µmol/L.

**Substrate**: Briefly centrifuge prior to opening. Add 1 mlReaction Buffer before use, warm at 40-50 °Cwater bath to dissolve.Store at -20 °C and use within 1 month.

**Enzyme**: Briefly centrifuge prior to opening. Add 1 ml Reaction Buffer to dissolve before use.Aliquot & store at -20 °C and use within 1 month.

**Probe:** Warm Probe Diluent to RT prior to use to melt frozen Probe Diluent. Briefly centrifuge prior to opening. Add 1 ml Probe Diluent to dissolve. Store at -20 °C, protect from light and moisture. Use within 1 month.

**Positive Control**: Briefly centrifuge prior to opening. add 1 ml Assay Buffer to dissolve before use.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 | 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:**

*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.

## VII. CALCULATION

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

1. According to the slope of the standard curve

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

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

2.1 According to the protein concentration of sample

$$C = \frac{(C_{Standard} \times V_{Standard}) \times (RFU_{Sample} - RFU_{Control})}{(RFU_{Standard} - RFU_{Blank}) \times C_{Protein} \times V_{Sample}} (\mu mol/mg)$$

2.2According to the quantity of cells or bacteria

$$C = \frac{(C_{Standard} \times V_{Standard}) \times (RFU_{Sample} - RFU_{Control})}{(RFU_{Standard} - RFU_{Blank}) \times N \times (V_{Sample} / V_{Assay})} (\mu mol/10^4)$$

2.3 According to the weight of sample

$$C = \frac{(C_{Standard} \times V_{Standard}) \times (RFU_{Sample} - RFU_{Blank})}{(RFU_{Standard} - RFU_{Blank}) \times W \times (V_{Sample} / V_{Assay})} (\mu mol/g)$$

2.4According to the volume of sample

$$C = \frac{(C_{Standard} \times V_{Standard}) \times (RFU_{Sample} - RFU_{Blank})}{(RFU_{Standard} - RFU_{Blank}) \times V_{Sample}} (\mu mol/ml)$$

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

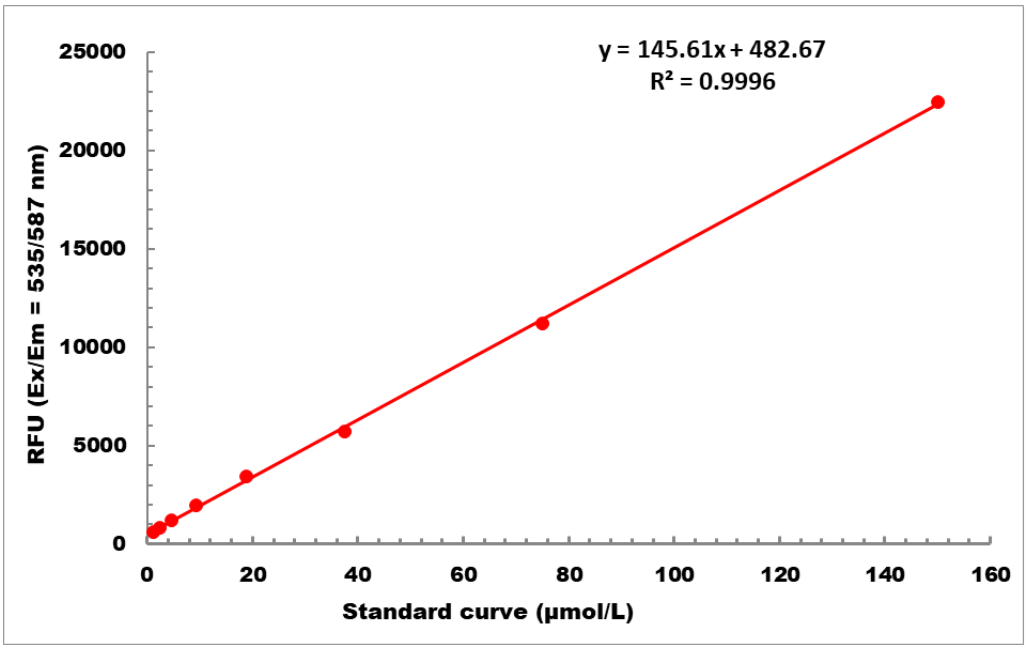
$V_{Sample}$: the volume of sample in assay procedure, mL

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

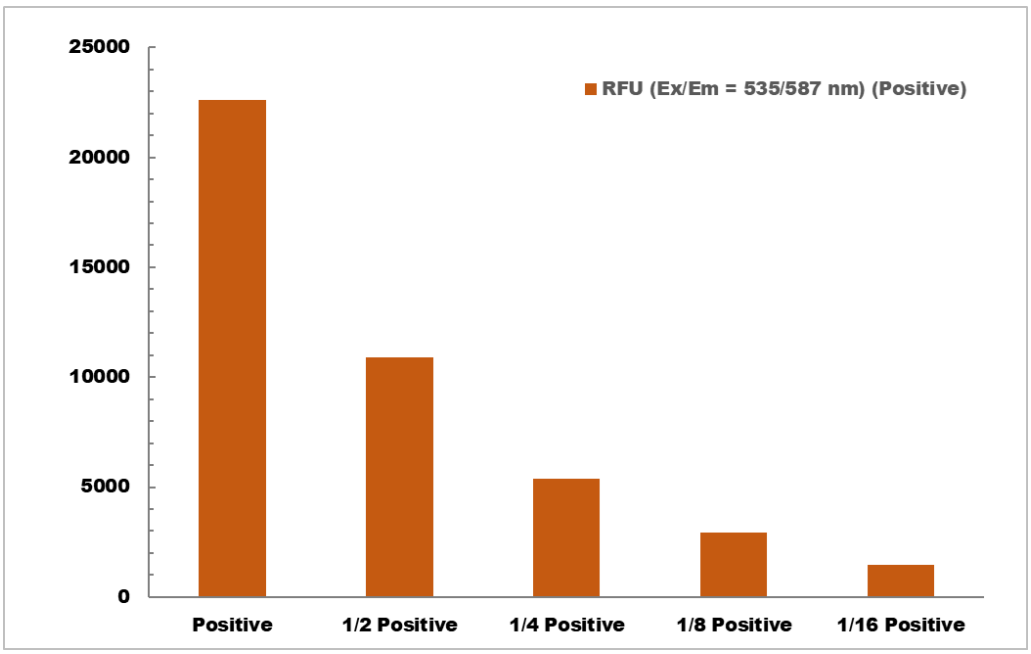
T: the reaction time, minute

## VIII. TYPICAL DATA

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




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




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