AHCY / Adenosylhomocysteinase Activity Assay Kit

ARG83568 AHCY / Adenosylhomocysteinase Activity Assay Kit can be used to measure AHCY / Adenosylhomocysteinase in Tissue extracts, Cell lysate, Cell culture media, other biological fluids

Catalog number: ARG83568

Package: 96 wells

TABLE OF CONTENTS

MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: 9F.-7, No. 12, Taiyuan 2nd St., Zhubei City,

Hsinchu County 302082, Taiwan

Phone: +886 (3) 622 1320

Fax: +886 (3) 553 0266

Email: info@arigobio.com

PRINCIPLE OF THE ASSAY

ARG83568 AHCY / Adenosylhomocysteinase Activity 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.

MATERIALS PROVIDED & STORAGE INFORMATION

Store Substrate, Positive Control and Enzyme at -20 °C, all other component at 2-8°C. Use the kit before expiration date.

Component	Quantity	Storage
Microplate	1 X 96-well plate	
Standard (1 µmol/ml)	1 ml	4 °C
Substrate	1 vial (lyophilized)	-20 °C
Reaction Buffer	10 ml	4 °C
Positive Control	1 vial (lyophilized)	-20 °C
Enzyme	1 vial (lyophilized)	-20 °C
Assay Buffer	4x 30 ml	4 °C
Dye Reagent A	1 vial (lyophilized)	4 °C
Dye Reagent B	1 vial (lyophilized)	4 °C
Dye Reagent B Diluent	3 ml	4 °C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 620 nm
- Pipettes and pipette tips
- Deionized or distilled water

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store Substrate, Positive Control and Enzyme at -20 °C, all other component at 2-8°C. Use the kit before expiration date.
- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design

Cell and bacteria- 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.

Tissue - 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.

Liquid samples- Detect directly or dilute with Assay Buffer.

REAGENT PREPARATION

- **Substrate:** Briefly centrifuge prior to opening. Reconstitute the **Substrate** with **1 ml** of Reaction Buffer. Warm to 40-50 °C with water bath to dissolve. Make sure the **Substrate** is dissolved completely and mixed thoroughly before use. Store at 4 °C for 1-2 days or -20°C for 1-2 months after reconstitution.
- **Dye Reagent A:** Reconstitute the **Dye Reagent A** with **7 ml** of distilled water. Keep in dark for immediate use after reconstitution. Allow the **Dye Reagent A** keep on bench for few minutes. Make sure the **Dye Reagent A** is dissolved completely and mixed thoroughly before use.
- **Dye Reagent B:** Add **3 ml** of Dye Reagent B Diluent into **Dye Reagent B**. Keep in dark and store at 4 °C for 1-2 weeks after reconstitution. Allow the **Dye Reagent B** keep on bench for few minutes. Make sure the **Dye Reagent B** is dissolved completely and mixed thoroughly before use.
- **Positive Control:** Briefly centrifuge prior to opening. Reconstitute the **Positive Control** with **100 µl** of Assay Buffer. Aliquot & store at -80 °C and use within 1 month. Allow the **Positive Control** keep on bench for few minutes. Make sure the **Positive Control** is dissolved completely and mixed thoroughly before use.
- **Enzyme:** Briefly centrifuge prior to opening. Reconstitute the **Enzyme** with **1ml** of Assay Buffer. Aliquot & store at -80 °C and use within 1 month. Allow the **Enzyme** keep on bench for few minutes. Make sure the **Enzyme** is dissolved completely and mixed thoroughly before use.
- **Standard:** Briefly centrifuge prior to opening. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard

curve. The concentration of standard curve could be **1 µmol/ml; 0.5 µmol/ml; 0.25 µmol/ml; 0.125 µmol/ml; 0.0625 µmol/ml; 0.0312 µmol/ml; 0.0156 µmol/ml**.

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

ASSAY PROCEDURE

Standards and samples should be assayed in at least duplicates.

1. Add **70 µl Reaction Buffer** into Sample, Control, Positive Control wells.
2. Add **10 µl Sample, Distilled water, Positive Control** into Respective wells
3. Add **10 µl Enzyme** into Sample, Control and Positive Control wells.
4. Add **10 µl Substrate** into Sample, Control and Positive Control wells.
5. Add **100 µl Standard** into Standard wells
6. Add **100 µl Distilled water** into Blank wells
7. Add **70 µl** Dye Reagent A into all wells.
8. Add **30 µl** Dye Reagent B into all wells.
9. Mix well, incubate at **RT** for **10 min.** Read the OD at 620nm.

Summary of AHCY / Adenosylhomocysteinase Activity Assay Kit Procedure

Reagent	Sample	Control	Positive Control	Standard	Blank
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 Reagent A	70 µl	70 µl	70 µl	70 µl	70 µl
Dye Reagent B	30 µl	30 µl	30 µl	30 µl	30 µl
Mix well incubate for 10 min at RT. Read the OD at 620 nm					

Note:

1. Reagents must be added sequentially and should not be premixed prior to addition.
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 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.
3. 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.

CALCULATION OF RESULTS

Calculate the average absorbance values for each set of samples, standard and blank.

a.) Definition:

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

$C_{Standard}$: the concentration of standard, 1 µmol/ml;

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

W: the weight of total sample, g;

N: the quantity of total cell or bacteria sample, $N \times 10^4$;

$V_{Standard}$: the volume of the standard in assay procedure, 0.1 ml;

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

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

T: the reaction time, 10 minutes.

b.) Calculation:

1. According to the slope of the standard curve

AHCY (U/mL) =

$[((OD_{Sample} – OD_{Control})$- Intercept) / Slope x T] x$((V_{Standard} / V_{sample})$ x n

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

Formula:

a). According to the protein concentration:

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

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

b). According to the weight:

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

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

c). According to the Cells or bacteria:

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

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

d). According to the volume:

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

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

3. Detection range:

The detection range is from 0.01 µmol/ml – 1 µmol/ml