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: 100 tests

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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 vial (lyophilized)	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

Cell and bacteria- Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5 × 106 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.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately or aliquot & store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Plasma- Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g. within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- **Substrate:** 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.

- **Dye Reagent A:** Reconstitute the **Dye Reagent A** with **7 ml** of distilled water. 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**. 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:** Reconstitute the **Positive Control** with **100µl** of Assay Buffer. Allow the **Positive Control** keep on bench for few minutes. Make sure the **Positive Control** is dissolved completely and mixed thoroughly before use.

- **Enzyme:** Reconstitute the **Enzyme** with **1ml** of Assay Buffer. Allow the **Enzyme** keep on bench for few minutes. Make sure the **Enzyme** is dissolved completely and mixed thoroughly before use.

- **Standard:** Perform 2-fold serial dilutions of the top standards to make the standard curve.

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					

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, mg/ml;

W: the weight of sample, g;

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

$V_{Standard}$: the volume of the standard, 0.1 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.

b.) Calculation:

Formula:

a). According to the e protein concentration:

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

= **(OD_{Sample}- $OD_{Control}$) / [($OD_{Standard}$- OD_{Blank}) x $C_{Protein}$)]**

b). According to the weight:

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

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

c). According to the Cells or bacteria:

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

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

d). According to the volume:

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

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