

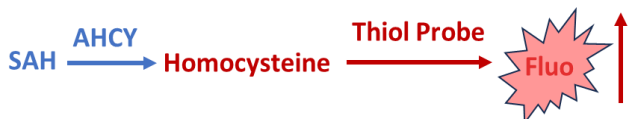
**Adenosyl homocysteinase / AHCY Activity Fluorometric Assay (TBS2056, 100 Assays, Store at -20 °C)**

**DESCRIPTION**

Adenosylhomocysteinase (AHCY) or S-adenosylhomocysteine hydrolase (SAHH) is an enzyme that catalyzes the reversible hydrolysis of S-Adenosyl Homocysteine (SAH) to adenosine and homocysteine (Hcy). In mammals, AHCY is the only enzyme capable of performing this reaction. SAH is the by-product and a potent inhibitor of methyltransferase activity. AHCY deletion is embryonic lethal in many organisms (from plants to mammals). In humans, AHCY deficiency is associated with an incurable rare recessive disorder in methionine metabolism.

Tribioscience’s AHCY Activity Assay utilizes SAH as the substrate to form homocysteine and adenosine. The free thiol group of the homocysteine can be detected with a thiol probe generating an enhanced fluorescent signal at Ex/Em = 400/465 nm. The assay principle is displayed in Figure 1. The kit provides the easiest and most accurate approach to measuring AHCY activity in a variety of samples.

**Fig. 1: Principle of AHCY Assay**



**APPLICATIONS**

Measure AHCY activity in a variety of samples.

**KIT CONTENTS FOR 100 TESTS:**

Name	Size (100 tests)
AHCY Substrate SAH	100 µL
AHCY Assay Buffer	12 mL
Thiol Probe (100X)	60 µL
AHCY Enzyme (positive control)	60 µL
Homocysteine (400 µM)	80 µL

**Storage conditions:** Store the Reagent at -20° (-80°C for AHCY Enzyme positive control), protected from light. Shelf life: 6 months.

**PROCEDURES**

1. Equilibrate all the kit components until room temperature before starting the experiment.
2. Use a black microplate for the assay.
3. Prepare the Homocysteine (Hcy) standard: Add 30 µL of Homocysteine stock to 270 µL of assay buffer, and then do a 2-fold serial dilution as the Table 1.

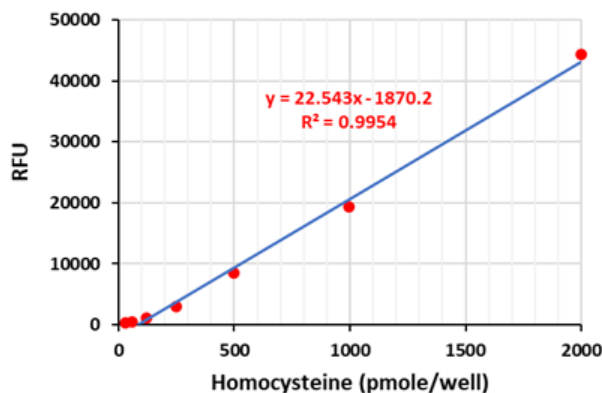
**Table 1: Homocysteine Standard Preparation**

Tube #	Vol. (µL) Hcy Standard	Vol. (µL) Assay Buffer	Hcy concentration (µM)	Hcy (pmol/Well)
1	30	270	40	2000
2	150	150	20	1000
3	150	150	10	500
4	150	150	5	250
5	150	150	2.5	125
6	150	150	1.25	62.5
7	150	150	0.625	31.25
8	0	150	0	0

4. Prepare SAH substrate working solution: Add 80 µL AHCY Substrate SAH stock into 2420 µL assay buffer to make SAH substrate working solution of 2500 µL. This solution is enough for 100 assays, please adjust the volume as you need and store the unused portion at -20°C.
5. Add 25 µL of test sample, or the AHCY positive control to the SAH substrate wells in duplicate manner.
6. Add 25 µL of SAH working solution to the sample or positive control wells, mix well by gentle tapping.
7. Incubate at 37°C for 60 minutes with gentle shaking and protect from light.
8. Preparation of Thiol Probe working solution: Add 50 µL of Thiol Probe (100X) into 4950 µL of DMSO to make probe working solution.
9. Add 50 µL of Hcy standards to indicated wells in duplicate manner.
10. Add 50 µL of the Thiol Probe working solution to the samples, Hcy standards, or AHCY positive control.
11. Incubate at 37°C for 10 minutes with gentle shaking and protect from light.
12. Read the plate at excitation and emission wavelength at Ex/Em = 400 nm/465 nm respectively.

The typical Hcy standard curve is shown in Fig. 2.

**Fig. 2 Homocysteine Standard Curve**



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13. Calculate the AHCY activity by the Hcy standard curve as follows:

$$Y = A * X + B$$

$$\text{The free thiol product (pmol): } X = (Y - B) / A$$

$$\text{The AHCY Activity (pmol/min/}\mu\text{g):}$$

$$= (X * DF) / (T * S)$$

**Y=RFU; A=Slope; B= constant value; X= Free Thiol product (pmol); DF=dilution factor; T=incubate Time (min); S=sample amount ( $\mu\text{g}$ )**

### RELATIVE PRODUCTS

Resazurin Cell Viability Kit (TBS2001)

CCK-8 Cell Viability Assay (TBS2022)

GOT Activity Assay (TBS2013)

Thiol Fluorometric Assay (TBS2026)

GSH Assay (TBS2028)

Homocysteine Fluorometric Assay (TBS2091)

NNMT Inhibitor Screening Assay (TBS2097)

NNMT Activity Fluorometric Assay (TBS2098)

AHCY Inhibitor Screening Assay (TBS2099)

G6PDH Activity Colorimetric Assay (TBS2102)

ATP Colorimetric/Fluorometric Assay Kit (TBS2010)

ADP Colorimetric/Fluorometric Assay Kit (TBS2020)

Caspase-3 Colorimetric Assay kit (TBS2030)

**This product is for research use only.**