

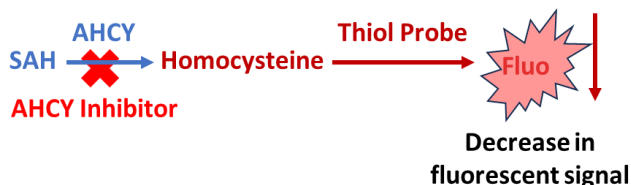
Adenosyl homocysteine / AHCY Inhibitor Screening Assay (Fluorometric, TBS2099, 100 Assays, Store at -20 °C)

DESCRIPTION

Adenosyl Homocysteine (AHCY) or S-adenosylhomocysteine hydrolase (SAHH) is an enzyme that catalyzes the reversible hydrolysis of S-Adenosyl Homocysteine (SAH) to adenosine and homocysteine. 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 induces 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 inhibitor screening 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.

Fig. 1: Principle of AHCY Inhibitor Screening Assay



APPLICATIONS

Screening of AHCY inhibitor.

KIT CONTENTS FOR 100 TESTS

| Name | Size (100 tests) |
|-----------------------------------|------------------|
| AHCY Substrate SAH | 100 µL |
| AHCY Assay Buffer | 12 mL |
| Thiol Probe (80X) | 60 µL |
| AHCY Enzyme Mix | 2500 µL |
| 3-Deazaneplanocin A (DZNep, 2 mM) | 20 µL |

Storage conditions: Store the Reagent at -20° (-80°C for AHCY Enzyme Mix), protected from light. Shelf life: 12 months.

PROCEDURES

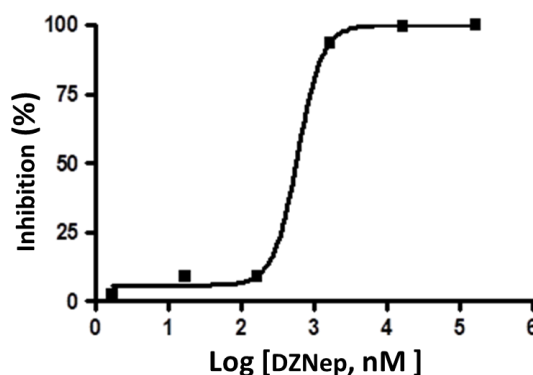
1. Equilibrate all the kit components until room temperature before starting the experiment.
2. Use a black microplate for the assay. Set up the assay as shown as Table 1.
3. Prepare the screening compound, inhibitor, and blank control: Dissolve the test compounds in an appropriate solvent, further dilute the compound in the AHCY assay buffer. The effect of the solvent on the AHCY activity should be considered by including a solvent control in the assay. Add 10 µL of the test compound, or the diluted DZNep (*Note: the dilution is optimized by client*) to the indicated wells in black

Table.1: Setup Assay:

| | Test Inhibitor | Inhibitor Control | Enzyme Control | Blank Control | Solvent Control |
|-----------------|----------------|-------------------|----------------|---------------|-----------------|
| Test Inhibitor | 10 µL | | | | |
| Diluted DZNep | | 10 µL | | | |
| Assay Buffer | | | 10 µL | 35 µL | |
| Solvent Control | | | | | 10 |

4. Prepare SAH working solution: Mix 2420 µL assay buffer with 80 µL of AHCY Substrate SAH to make SAH working solution of 2500 µL. It is enough for 100 assays. *Note; please adjust the volume as you need and store the unused portion at -20°C.*
5. Add 25 µL of SAH solution to the wells, except the blank control.
6. Add 25 µL of the AHCY Enzyme mix to the wells.
7. Incubate at 37°C for 60 minutes with gentle shaking and protect from light.
8. Prepare the Thio Probe working solution: Add 50 µL of Thiol Probe Stock (80X) into 3950 µL of DMSO to make the probe working solution.
9. Add 40 µL of the probe working solution to the wells.
10. Incubate at 37°C for 10 minutes with gentle shaking and protect from light.
11. Read the plate at excitation and emission wavelength at Ex/Em = 400 nm/465 nm respectively.
12. Calculation:
Subtract the Blank Control reading from all readings (Compound, Enzyme Control and Inhibitor Control). Set the ΔRFU of Enzyme Control (EC) as 100% and calculate the % inhibition.
% of Inhibition = 100* [ΔRFU(EC)-ΔRFU(TC)] / ΔRFU(EC)
Here EC=Enzyme Control; TC= Test Compound.
13. Inhibition of AHCY activity by DZNep displays in Fig.2, with IC₅₀ of 580 nM.

Fig. 2. Inhibition of AHCY activity by DZNep



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microplate. Add 35 μ L Assay Buffer into blank wells.

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 Activity Fluorometric Assay (TBS2056)
G6PDH Activity Colorimetric Assay (TBS2102)
ATP Colorimetric/Fluorometric Assay Kit (TBS2010)
ADP Colorimetric/Fluorometric Assay Kit (TBS2020)
Caspase-3 Colorimetric Assay kit (TBS2030)

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