

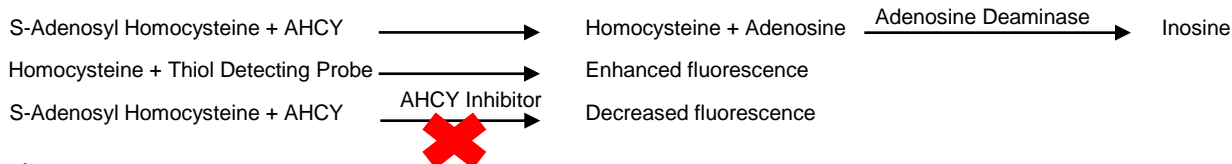
AHCY Inhibitor Screening Kit (Fluorometric)

2/15

(Catalog # K326-100; 100 assays; Store kit at -20°C)

I. Introduction:

Adenosyl homocysteinase (AHCY) (EC 3.3.1.1) or S-adenosyl homocysteine hydrolase (SAHH); is an enzyme that catalyzes the reversible hydrolysis of S-Adenosyl Homocysteine (SAH) to adenosine and homocysteine. Inhibition of AHCY results in the accumulation of SAH, a product inhibitor of S-adenosyl methionine (SAM)-dependent methyltransferases. AHCY is an important target as an antiviral and anticancer drug. Several characterized SAH inhibitors inhibit some DNA viruses (e.g. Pox viruses) and some negative stranded RNA viruses (e.g.: Marburg virus, Ebola virus & rabies). In BioVision's AHCY Inhibitor Screening kit the homocysteine generated from the breakdown of SAH is measured using a Thiol Detecting Reagent resulting in enhanced fluorescence which is measured at Ex/Em = 392/482 nm. The AHCY reaction is reversible and hence Adenosine Deaminase is included in the reaction to ensure that the reaction proceeds towards hydrolysis of SAH. In the presence of AHCY inhibitor, there is a decrease in fluorescence of the Thiol Detecting Reagent.



II. Application:

- Screening/characterizing/studying AHCY inhibitors

III. Kit Contents:

Components	K326-100	Cap Code	Part Number
AHCY Assay Buffer	25 ml	WM	K326-100-1
AHCY Reconstitution Buffer	0.25 ml	Clear	K326-100-2
AHCY Enzyme	1 Vial	Red	K326-100-3
Adenosine Deaminase	1 Vial	Green	K326-100-4
3-Deazaneplanocin A (10 µM) (in DMSO)	10 µl	Blue	K326-100-5
AHCY Substrate (in DMSO)	100 µl	Brown	K326-100-6
Thiol Detecting Reagent (in DMSO)	200 µl	Violet	K326-100-7

IV. User Supplied Reagents and Equipments:

- 96-well plate with flat bottom. White plates are preferred for this assay.
- Fluorescence microplate reader
- DMSO
- Isopropyl alcohol (chilled at -20°C)

V. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Avoid repeated freeze/thaw for all non-buffer components. Briefly centrifuge small vials before opening. Read entire protocol before performing the assay.

- **AHCY Assay Buffer:** Store at 4°C or -20°C. Warm to 37°C before use.
- **AHCY Enzyme:** Reconstitute with 220 µl AHCY Reconstitution Buffer, keep on ice, and mix gently by pipetting. Aliquot and store at -80°C. Avoid repeated freeze/thaw. After opening, use within two months.
- **Adenosine Deaminase:** Reconstitute with 110 µl deionized water. Avoid repeated freeze/thaw. Aliquot and store at -80°C. After opening, use within two months.
- **3-Deazaneplanocin A (10 µM):** Store at -20°C. After opening, use within two months.
- **AHCY Substrate:** Store at -20°C. Thaw and mix gently by pipetting before use. After opening, use within two months.
- **Thiol detecting Reagent:** Store at -20°C. Thaw before use. After opening, use within two months.

VI. AHCY Inhibitor Screening Protocol:

1. **Enzyme Solution Preparation:** Mix enough reagents for the number of assays to be performed. For each well, prepare 25 µl AHCY Enzyme Solution:

AHCY Assay Buffer	23 µl
AHCY Enzyme	2 µl

Mix & add 25 µl of the AHCY Enzyme Solution into desired wells.

2. **Screen compounds, Inhibitor Control, Enzyme Control & Blank Control Preparations:** Dissolve candidate inhibitors at 1000X highest final test concentration into an appropriate solvent. Dilute to 4X the desired test concentration with AHCY Assay Buffer. Add 25 µl diluted test inhibitor or Assay Buffer into AHCY Enzyme solution wells as sample screen [S] or Enzyme Control [EC] (no inhibitor) respectively. Add 1 µl of 3-Deazaneplanocin A and 24 µl AHCY Assay Buffer into one of the enzyme containing wells as Inhibitor Control. For Background Control (no substrate), add 25 µl AHCY Assay Buffer into one of the wells. Mix well, and incubate the plate for 5 min. at 37°C.

Notes:

- (a) High solvent concentration might affect the enzymatic activity. Prepare parallel well(s) as Solvent Control to test the effect of the solvent on enzyme activity.

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(b) Thiol Detecting Reagent reacts with the thiol groups in the enzymes and in the homocysteine. Hence a Background Control (BC) containing AHCY and Adenosine Deaminase should be used.

3. **Substrate preparation:** Make 50 μ l of Reaction Mix and Background Control Mix:

	Reaction Mix	Background Control Mix
AHCY Assay Buffer	48 μ l	49 μ l
AHCY Substrate	1 μ l	—
Adenosine Deaminase	1 μ l	1 μ l

Add 50 μ l of Background Control Mix to the Background Control well and 50 μ l/well of Reaction Mix to all other wells. Mix & incubate at 37°C for 15 min.

Stop the reaction by adding 50 μ l of pre-chilled isopropyl alcohol (not provided) into each well. Mix and keep on ice for five min. For each well, prepare 50 μ l of Thiol Detecting Reagent working solution by adding 2 μ l Thiol Detecting Reagent into 48 μ l of DMSO (not provided) just before use. Make as much as needed. Add 50 μ l of Thiol Detecting Reagent working solution into each well, mix and incubate at room temperature for 5 min. (Do not incubate more than 5 min.).

4. **Measurement:** Read fluorescence in end point mode at Ex/Em = 392/482 nm.

5. **Calculation:** Subtract the Background Control reading from all readings to obtain Δ RFU for each reading. Set the Δ RFU of Enzyme Control [EC] as 100%, and calculate % Inhibition or % Relative Activity of the test inhibitors as follows:

$$\% \text{ Inhibition} = \frac{\Delta \text{RFU of EC} - \Delta \text{RFU of S}}{\Delta \text{RFU of EC}} \times 100$$

$$\% \text{ Relative Activity} = \frac{\Delta \text{RFU of S}}{\Delta \text{RFU of EC}} \times 100$$

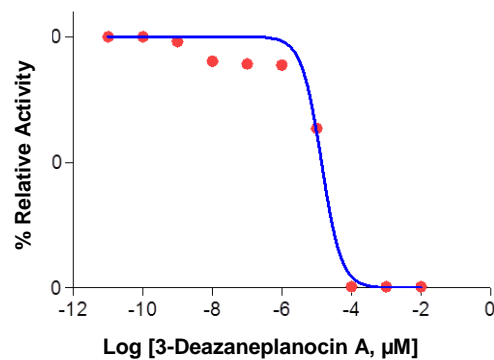


Figure: Inhibition of AHCY Enzyme activity by 3-Deazaneplanocin A. IC_{50} of 3-Deazaneplanocin A was determined to be 0.137 nM. Assays are performed following the kit protocol.

VII. Related Products:

AHCY Activity Fluorometric Assay Kit (K807)
 NNMT Inhibitor Screening Kit (K822)
 Xanthine Oxidase Colorimetric/Fluorometric Assay Kit (K710)
 Xanthine/Hypoxanthine Colorimetric/Fluorometric Assay Kit (K685)
 ADP Colorimetric/Fluorometric Assay Kit (K355)
 ATP Colorimetric/Fluorometric Assay Kit (354)
 Uric Acid Colorimetric/Fluorometric Assay Kit (K608)
 S-Adenosylmethionine Antibody (Clone # 118-6) (6940)
 S-Adenosylmethionine Antibody (Clone # 118-18) (6942)
 S-Adenosylmethionine Antibody (6944)

Active AHCY, human recombinant (7527)
 Nicotinamide N-Methyltransferase, (7261)
 Human Recombinant ADA (6393)
 3-Deazaneplanocin A (2060)
 3-Deazaneplanocin A hydrochloride (2444)
 Inosine Fluorometric Assay Kit (K712)
 ADP Colorimetric Assay Kit II (K356)
 S-Adenosylmethionine Antibody (Clone # 84-3) (6941)
 S-Adenosylmethionine Antibody (Clone # 84-19) (6943)
 S-Adenosylhomocysteine Antibody (Clone # 301-10) (6945)

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