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Product Information

Arginase Inhibitor Screening Kit

Catalog Number **MAK328**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Arginase (L-arginine ureohydrolase) is present in mammals and plants. In humans, arginase is expressed predominantly in the liver, and to lesser degrees in breast, kidney, testes, salivary glands, epidermis, and erythrocytes. Arginase catalyzes the conversion of arginine to ornithine and urea, important for protection against NH_3 toxicity and for cell growth and repair. Excessive arginase activity has been linked to cardiovascular diseases and contributes to vascular structural problems and neural toxicity. Studies show that arginase inhibitors have been proven to be beneficial in cardiovascular and nervous system diseases.

Simple, direct, and automation-ready procedures for measuring arginase inhibition are highly desirable in research and drug discovery. The Arginase Inhibitor Screening Kit provides a sensitive and convenient method to screen for arginase inhibitors. The method utilizes a chromogen that forms a colored complex specifically with the urea by-product of the arginase reaction. The intensity of the color is directly proportional to the arginase activity in the sample. Percent inhibition of a test compound can be determined by comparing the color intensity of the reaction preincubated with the test compound with the color intensity of an untreated control reaction.

This kit can be readily automated on HTS liquid handling systems and is suitable for inhibitor screening and evaluation of arginase inhibitors.

Components

The kit is sufficient for 100 colorimetric assays in 96 well plates.

Arginine Buffer (pH 9.5) 1 mL
Catalog Number MAK328A

Mn Solution 300 µL
Catalog Number MAK328B

Reagent A 12 mL
Catalog Number MAK328C

Reagent B 12 mL
Catalog Number MAK328D

Reagents and Equipment Required but Not Provided.

- Purified Arginase I
- If desired, a control inhibitor: ABH hydrochloride (Catalog Number SML1466)
- Pipetting devices and accessories (e.g., multichannel pipettor)
- 96 well flat bottom plate. It is recommended to use clear plates for colorimetric assays
- Spectrophotometric multiwell plate reader

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Kit is shipped at room temperature. Store the Arginine Buffer at –20 °C. Store all other components at 2–8 °C.

Procedure

This assay is based on an enzyme-catalyzed kinetic reaction. To ensure identical incubation time, addition of Substrate Buffer and Urea Reagent should be quick and mixing should be brief but thorough. Use of a multichannel pipettor is recommended.
Note: Neither arginase nor a control inhibitor is included in the kit.

Reagent Preparation
Bring all reagents to room temperature prior to assay. The Substrate Buffer and Urea Reagent should be prepared freshly and used within 2 hours.

Substrate Buffer
For each well of reaction, prepare Substrate Buffer by mixing into a clean tube:
4 µL of Arginine Buffer
2 µL of Mn Solution

Urea Reagent
For each well of reaction, prepare Urea Reagent by mixing into a clean tube:
105 µL of Reagent A
105 µL of Reagent B

Sample Preparation
The following protocol is optimized for human arginase I. If another species is being analyzed, it is recommended to experimentally determine the K_M value and then adjust the volume of Arginine Buffer in the Substrate Buffer so that the final concentration of the substrate in the 50 µL reaction is near the K_M.

Enzyme Preparation - Dilute the purified arginase to 0.0012 units/µL using ultrapure water.

Inhibitor Solution - Dissolve the test compounds (i.e., inhibitors) in solvent of choice. It is prudent to first test the tolerance of the solvent for the enzyme of choice.

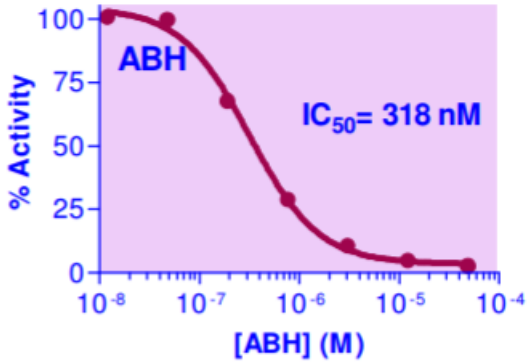
Arginase Reaction Preparation
1. Transfer 40 µL of arginase into separate wells.
2. Reserve two wells with arginase for the Blank (No Substrate) and Control (No Inhibitor).
3. To the Control and Blank wells, add 5 µL of the solvent used to dissolve the test compounds. For example, if the test compounds are dissolved in 100% (v/v) DMSO, add 5 µL of 100% (v/v) DMSO to these wells.
4. To the remainder of the wells containing arginase, add 5 µL of the test compounds. Tap plate and mix.
5. Incubate the plate for 15 minutes at 25 °C.
6. Add 5 µL Substrate Buffer into all sample wells except the Blank well. Add 5 µL of ultrapure water into the Blank well. Tap plate and mix. Incubate the plate for 30 minutes at 25 °C.
7. Add 200 µL of Urea Reagent to all wells. Tap the plate to mix.
   Note: Urea Reagent stops the arginase reaction.
8. Incubate the plate for 60 minutes at room temperature.
9. Measure the absorbance at 430 nm (A_{430}).

Results
Arginase inhibition for a test compound is calculated as follows:

$$\% \text{ Inhibition} = (1 - \Delta A_{\text{Test Cpd}} / \Delta A_{\text{No Inhibitor}}) \times 100\%$$

where:
$\Delta A_{\text{Test Cpd}}$ = the A_{430} value of a test compound minus the A_{430} value of the Blank well (No Substrate) at 60 minutes
$\Delta A_{\text{No Inhibitor}}$ = the A_{430} value of the Control well (No Inhibitor) minus the A_{430} value of the Blank well (No Substrate) at 60 minutes

Figure 1.
ABH titration



Human Arginase I was incubated with various concentrations of ABH in 100% (v/v) DMSO (final concentration10% (v/v) DMSO in 50 µL reaction).

References
1. Segal, R. et al., Chronic oral administration of the arginase inhibitor 2 (S)-amino-6-boronohexanoic acid (ABH) improves erectile function in aged rats. Journal of Andrology, **33.6**, 1169-1175 (2012).
2. Di Costanzo, L. et al., Crystal structure of human arginase I at 1.29-Å resolution and exploration of inhibition in the immune response. Proc.Natl. Acad. Sci. USA, **102.37**, 13058-13063 (2005).
3. Pham, T.N. et al., Arginase inhibitors: from chlorogenic acid to cinnamides. Planta Med., **82**, S1 (2016).

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