ARGINASE (L-arginine ureohydrolase EC 3.5.3.1) 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 NH₃ toxicity and for cell growth and repair. Excessive arginase activity has been linked to cardiovascular diseases, also contribute to vascular structural problems and neural toxicity. Studies show that arginase inhibitors have been proved 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. BioAssay Systems’ 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 urea produced in the arginase reaction. The intensity of the color is directly proportional to the concentration of the substrate in the 50 µL reaction is near the K_m of the Blank well (no substrate) at 60 min and ∆OD_50 values the OD of the Control well (no inhibitor) minus the OD_50 of the Blank well (no substrate) at 60 min.

**APPLICATIONS**

HTS for inhibitor screening and evaluation of arginase inhibitors.

**KIT CONTENTS (100 tests in 96-well plates)**

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine Buffer (pH 9.5)</td>
<td>1 mL</td>
</tr>
<tr>
<td>Reagent A</td>
<td>12 mL</td>
</tr>
<tr>
<td>Storage conditions</td>
<td>Kit is shipped at room temperature. Store the Arginine Buffer at -20°C, and other components at 2-8°C. Shelf life: 12 months after receipt.</td>
</tr>
<tr>
<td>Precautions</td>
<td>Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.</td>
</tr>
</tbody>
</table>

**PROCEDURES**

Bring all reagents to room temperature prior to assay. The Substrate Buffer and Urea Reagent should be prepared freshly and used within 2 hours. This assay is based on an enzyme-catalyzed kinetic reaction. To ensure identical incubation time, addition of Working Reagent should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended. Note: Neither the enzyme arginase nor a control inhibitor is included in the kit.

**Sample Preparation:** Dissolve the test compounds in solvent of choice. It is prudent to first test the tolerance of the solvent for the enzyme of choice. The following protocol is optimized for human arginase I. Dilute purified arginase to 0.0012 U/µL using dH₂O. If another species is being analyzed, we recommend that you experimentally determine the K_m 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.

**Arginase Reaction Preparation:**
1. Transfer 40 µL of arginase into separate wells.
2. Reserve two wells with arginase for the Blank and Control.
3. To the Control and Blank wells, add 5 µL of solvent that the test compounds are dissolved in. For example, if the test compounds are dissolved in 100 v/v% DMSO, add 5 µL 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. Prepare sufficient Substrate Buffer (SB) by combining, for each well, 4 µL of Arginine Buffer and 2 µL of the Mn Solution. Add 5 µL SB into all sample wells except the Blank well. Add 5 µL dH₂O into the Blank well. Tap plate and mix. Incubate the plate for 30 minutes at 25°C.
7. **Urea Determination:** Prepare Urea Reagent by combining equal volumes of Reagent A and Reagent B. Add 200 µL Urea Reagent to all wells. (note: Urea Reagent stops the arginase reaction). Tap the plate to mix. Incubate 60 min at room temperature and read optical density at 430 nm.

**CALCULATION**

Arginase inhibition for a test compound is calculated as follows:

\[
\text{% Inhibition} = \left(1 - \frac{\Delta \text{OD}_{\text{Test Compd}}}{\Delta \text{OD}_{\text{No Inhibitor}}} \right) \times 100\%
\]

Where \(\Delta \text{OD}_{\text{Test Compd}}\) is the OD value of a test compound minus the OD value of the Blank well (no substrate) at 60 min and \(\Delta \text{OD}_{\text{No Inhibitor}}\) is the OD value of the Control well (no inhibitor) minus the OD value of the Blank well (no substrate) at 60 min.

**MATERIALS REQUIRED, BUT NOT PROVIDED**

Purified Arginase I (e.g. Enzo Life Scientific Cat# ALX-201-081-C020) and if desired a control ABH inhibitor (e.g. ABH, Enzo Life Scientific Cat# ALX-270-420). Pipetting devices and accessories (e.g. multi-channel pipettor), clear flat bottom 96-well plates (e.g. VWR cat# 82050-760), and plate reader.

**LITERATURE**