# QuantiChrom™ Arginase Inhibitor Screening Kit (IARG-100)

## Quantitative Determination of Arginase Inhibitor Activity

## DESCRIPTION

*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 $NH_3$ 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 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.

## KEY FEATURES

**Safe.** Non-radioactive assay.

**High-throughput.** Homogenous "mix-incubate-measure" type assay. Can be readily automated on HTS liquid handling system.

**Rapid and reliable**. Can be completed in less than 2 hours and no 37℃ heater is needed.

## APPLICATIONS

HTS for inhibitor screening and evaluation of arginase inhibitors.

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

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| **Arginine Buffer (pH 9.5):** | 1 mL | **Mn Solution:** | 300 µL |
| **Reagent A:** | 12 mL | **Reagent B:** | 12 mL |

**Storage conditions**. 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.

**Precautions**: 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.

## 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 $dH_2O$. 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 $dH_2O$ 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

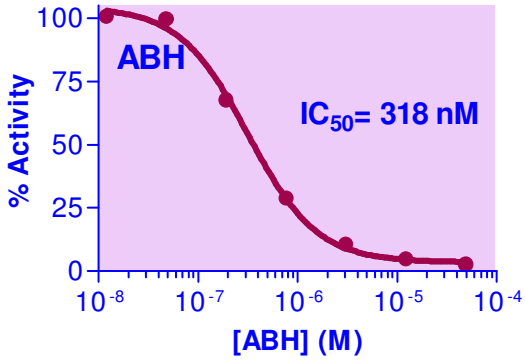
Arginase inhibition for a test compound is calculated as follows:

$$\% \text{ Inhibition} = \left(1 - \frac{\Delta OD_{\text{Test Cpd}}}{\Delta OD_{\text{No Inhibitor}}}\right) \times 100\%$$

Where $\Delta OD_{\text{Test Cpd}}$ is the $OD_{430nm}$ value of a test compound minus the $OD_{430nm}$ value of the Blank well (no substrate) at 60 min and $\Delta OD_{\text{No Inhibitor}}$ is the $OD_{430nm}$ value of the Control well (no inhibitor) minus the $OD_{430nm}$ 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). Pipeting devices and accessories (e.g. multi-channel pipettor), clear flat bottom 96-well plates (e.g. VWR cat# 82050-760), and plate reader.




**ABH titration:** Human Arginase I was incubated with various concentrations of ABH in 100 v/v% DMSO (final 10 v/v% DMSO in 50 µL reaction).

## LITERATURE

1. Segal, Robert, et al. (2012) 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.
2. Di Costanzo, Luigi, et al. (2005) Crystal structure of human arginase I at 1.29-Å resolution and exploration of inhibition in the immune response. Proceedings of the National Academy of Sciences of the United States of America 102.37: 13058-13063.
3. Pham, T. N., et al. (2016) Arginase inhibitors: from chlorogenic acid to cinnamides. Planta Medica 81.S01: P482.