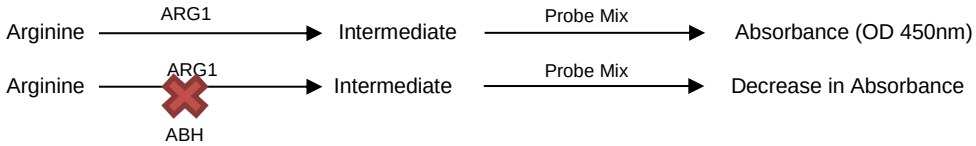
Arginase I (ARG1) Inhibitor Screening Kit (Colorimetric)

(Catalog #K567-100; 100 assays; Store at -20°C)

I. Introduction:

Arginase (EC 3.5.3.1) is a manganese-containing enzyme which catalyzes the conversion of arginine into urea and ornithine, which is the final reaction in the urea cycle. Arginase I (ARG1) is the liver isoform of arginase. Recent studies showed that ARG1 expression by mature myeloid cells in tumor environment as demonstrated in a 3LL murine lung carcinoma model causes L-Arginine depletion by tumor-associated myeloid cells (TAMC). L-arginine depletion suppresses immune-response against tumor cells due to inhibition to T-cell proliferation. In addition, the depletion of arginine increases the reactive nitrogen species (NOS) and reactive oxygen species (ROS), which, in consequence, induce T-cell apoptosis and supports antigenic cell proliferation. BioVision's Arginase I (ARG1) Inhibitor Screening Kit is designed for screening ARG1 inhibitors. Two substituted-2-amino-6-hexanoic acids have been studied as arginase inhibitor and in this kit, Amino-2-Borono-6-Hexanoic Acid (ABH) is provided as a positive control. The ARG1 activity is monitored by the increase in absorbance readings (OD 450 nm), while potential inhibitors will cause a decrease of absorbance. The assay kit is simple, quick and can be used to identify and characterize ARG1 inhibitors in a high-throughput format.



II. Applications:

- Screening for inhibitors of human arginase I (ARG1)

III. Kit Contents:

Components	K567-100	Cap Code	Part Number
Assay Buffer	25 ml	WM	K567-100-1
ARG1 Substrate	1 vial	White	K567-100-2
ARG1 Probe Mix A	12 ml	NM/Blue	K567-100-3
ARG1 Probe Mix B	12 ml	NM/Brown	K567-100-4
Human ARG1	1 vial	Green	K567-100-5
ABH (in DMSO)	20 µl	Purple	K567-100-6

IV. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)

V. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay. Use within two months of opening.

- **Assay Buffer:** Warm to room temperature before use. Store at 4ºC or -20ºC.
- **ARG1 Substrate:** Reconstitute with 250 µl dH_2O. Pipette up and down to dissolve. Store at -20ºC.
- **ARG1 Probe Mix A:** Ready to use as supplied. Warm to room temperature before use. Store at 4ºC or -20°C. Keep away from light.
- **ARG1 Probe Mix B:** Ready to use as supplied. Warm to room temperature before use. Store at 4ºC or -20°C. Keep away from light.
- **Human ARG1:** Reconstitute with 220 µl ARG1 Assay Buffer. Pipette up and down to dissolve. Aliquot and store at -20°C. Avoid repeated freeze/thaw cycles. Keep on ice while in use.
- **ABH (in DMSO):** Ready to use as supplied. Warm to room temperature before use

VI. ARG1 Inhibitor Screening Assay Protocol:

1. Test compounds, Inhibitor Control, Enzyme Control & Background Control Preparations:

Dissolve candidate inhibitors at 1000X highest final test concentration into an appropriate solvent. Dilute to 5X the desired test concentration with ARG1 Assay Buffer. Add 10 µl diluted test inhibitor or Assay buffer into designated wells as sample screen [S]. Add 10 µl of Assay Buffer to a well designated as Enzyme Control [EC] (no inhibitor) respectively. **For ABH control**: Dilute ARG1 inhibitor by adding 2 µl of the stock solution into 18 µl of ARG1 Assay Buffer. Add 10 µl of the diluted ABH inhibitor into one wells labeled as Inhibitor Control [IC]. **For Background Control [BC]**: Add 10 µl of the diluted ABH inhibitor and 30 µl of Assay Buffer in a well designated as Background Control [BC]. If you are screening test compounds that have significant absorbanced (OD 450 m) at the 5X final concentration prepare background controls as described above.

2. Enzyme Solution Preparation: Mix enough reagents for the number of assays to be performed. For each well, prepare 30 µl ARG1 Enzyme Solution:

Assay Buffer	28 µl
ARG1 Enzyme	2 µl

Mix and add 30 µl of the ARG1 enzyme solution into all wells except Background Control well(s). Add 30 µl of Assay Buffer into Background Control well(s). Mix well, and incubate the plate for 5 min at 37 °C.

Note:

(a) Concentration up to 10% DMSO in the sample does not affect enzymatic activity. Prepare parallel well(s) as Solvent Control [SC] to test the effect of other solvent on enzyme activity.

(b) Do not store unused diluted ABH solutions.

3. **Substrate Mix Preparation:** Mix enough reagents for the number of assays to be performed. For each well, prepare 10 µl Substrate solution:

Assay Buffer	8 µl
ARG1 Substrate	2 µl

Add 10 µl of the substrate mix into samples screen, enzyme control, solvent control, inhibitor control and background control wells. Mix well and incubate the plate for 30 min at **37°C**.

4. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare 200 µl Mix containing:

ARG1 Probe Mix A	100 µl
ARG1 Probe Mix B	100 µl

Mix and add 200 µl of the Reaction Mix to each well (sample screen, enzyme control, solvent control, inhibitor control and background wells). Mix well and incubate at **25°C** for 60 min.

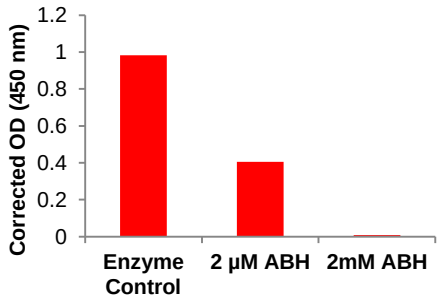
5. **Measurement:** Measure absorbance (OD: 450 nm) in a microplate reader in endpoint mode.

6. **Calculation:** Subtract the Background Control [BC] reading from all readings to obtain ΔOD for each reading (when using specific background control for a test compound subtract its signal from the signal of that particular sample only). Set the ΔOD of Enzyme Control [EC] as 100% (in case Solvent Control is significantly different from EC use that value in the formulas below). Calculate % Inhibition or % Relative Activity of the test inhibitors as follows:

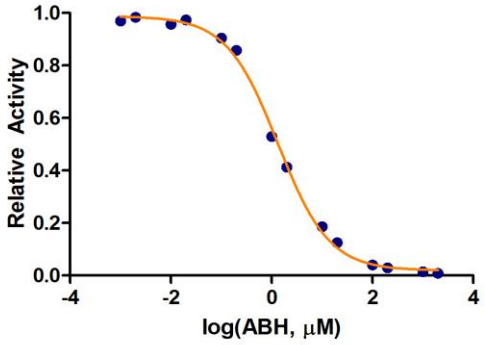
$$\% \text{ Inhibition} = \frac{\Delta OD \text{ of } EC - \Delta OD \text{ of } S}{\Delta OD \text{ of } EC} \times 100$$

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

(a)



(b)



Figures: (a) ARG1 was incubated with different concentrations of ABH for 5 minutes at 37 ºC. Then, substrate was added to the wells and mixtures were incubated for 30 minutes. Absorbance readings were taken 60 minutes after detection probe system was added. (b) Inhibition of ARG1 enzyme activity by Amino-2-Borono-6-Hexanoic Acid (ABH). IC_{50} of ABH was determined to be 1.39 ± 0.10 µM. Assay was performed following the kit protocol.

VII. RELATED PRODUCTS:

Arginase Activity Colorimetric Assay Kit (K755)
BEC (S-(2-boronoethyl)-L-cysteine) (2359)
Urea Colorimetric Assay Kit (K375)
AMI-1 (Arginine N-methyltransferase Inhibitor-1) (1943)
TREM1, Human Recombinant (7332)
Anti-ARG1 Antibody (A1080)
ARG1, Human Recombinant (P1032)
Urea Colorimetric Assay Kit II (K376)
TREM2, Human Recombinant (7357)
Meisoindigo (B1822)

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