Technical Bulletin

Arginase I (ARG1) Inhibitor Screening Kit (Colorimetric)

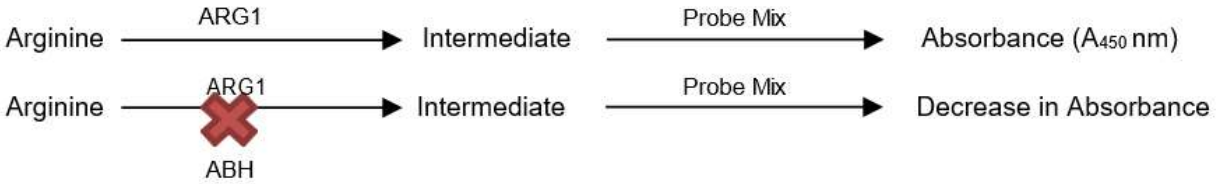
Catalog Number MAK392

Product Description

Arginase 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 have shown ARG1 expression by mature myeloid cells in a 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 the 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, induces T-cell apoptosis and supports antigenic cell proliferation.

The Arginase I (ARG1) Inhibitor Screening Kit is designed for the screening of ARG1 inhibitors. Two substituted-2-amino-6-hexanoic acids have been studied as arginase inhibitors; in this kit, Amino-2-Borono-6-Hexanoic Acid (ABH) is provided as a positive control. ARG1 activity is monitored by the increase in absorbance readings at 450 nm (A_{450}), 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.

The kit is suitable for the screening for inhibitors of human arginase I (ARG1).



Components

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

- Assay Buffer 25 mL
  Catalog Number MAK392A
- ARG1 Substrate 1 vial
  Catalog Number MAK392B
- ARG1 Probe Mix A 12 mL
  Catalog Number MAK392C
- ARG1 Probe Mix B 12 mL
  Catalog Number MAK392D
- Human ARG1 1 vial
  Catalog Number MAK392E
- ABH (in DMSO) 20 µL
  Catalog Number MAK392F

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (including multichannel pipettor)
- 96-well flat-bottom plate. It is recommended to use clear plates for colorimetric assays. Cell culture or tissue culture treated plates are **not** recommended.
- Spectrophotometric multiwell plate reader

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 ºC, protected from light. Use kit within two months of opening.

Preparation Instructions

Briefly centrifuge small vials at low speed prior to opening.

Assay Buffer: Warm to room temperature before use. Store at 2-8 ºC or -20 ºC.

ARG1 Substrate: Reconstitute vial with 250 µL of purified water. Pipette up and down to dissolve. Store at -20 ºC.

ARG1 Probe Mix A: Ready to use as supplied. Warm to room temperature prior to use. Store at 2-8 ºC or -20 ºC, protected from light.

ARG1 Probe Mix B: Ready to use as supplied. Warm to room temperature prior to use. Store at 2-8 ºC or -20 ºC, protected from light.

Human ARG1: Reconstitute with 220 µL of Assay Buffer. Pipette up and down to dissolve. Aliquot and store at -20 ºC. Avoid repeated freeze/thaw cycles. Keep on ice while in use.

ABH (in DMSO): Ready to use as supplied. Warm to room temperature prior to use

Procedure

Test Compound Preparation

1. For unknown inhibitor samples, it is recommended to test several inhibitor concentrations.
2. Dissolve candidate inhibitors at 1000× highest final test concentration into an appropriate solvent. See also Solvent Control instructions below.
3. Further dilute the sample solution from Step 2 to 5× the desired test concentration with Assay Buffer.
4. Add 10 µL of the diluted test inhibitor into designated wells as Sample (S).

Enzyme Control

Add 10 µL of Assay Buffer to a well designated as Enzyme Control (EC) (no inhibitor).

Inhibitor Control and Background Control

1. Dilute ABH (in DMSO) by adding 2 µL of the stock solution into 18 µL of Assay Buffer.
2. For the Inhibitor Control, add 10 µL of the diluted ABH inhibitor from Step 1 into one well labeled as Inhibitor Control (IC).
3. If screening test compounds that have significant absorbance at 450 nm at the 5× final concentration, prepare background controls by adding 10 µL of the diluted ABH inhibitor prepared in Step 1 and 30 µL of Assay Buffer in a well designated as Background Control (BC).
4. Discard remaining diluted ABH control. Do **not** store diluted ABH control.

Solvent Control

A concentration of up to 10% DMSO in the sample does not affect enzymatic activity. If the test compound is dissolved in DMSO ≥ 10% or in other organic solvent(s), prepare a Solvent Control (SC) to test the effect of the solvent on the enzyme activity. In parallel well(s), add 10 µL of 5× (5× final well solvent concentration) solvent that the test compound was prepared in.

Enzyme Solution Preparation

1. Mix enough reagents for the number of assays to be performed. For each well except Background Control well(s), prepare 30 µL of ARG1 Enzyme Solution according to Table 1. Mix well.

Table 1.

Preparation of ARG1 Enzyme Solution

Reagent	**Volume**
Assay Buffer	28 µL
Human ARG1	2 µL

2. 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.
3. Cover plate and incubate for 5 minutes at **37 ºC** (**not** 25 ºC).

Substrate Mix Preparation

1. Mix enough reagents for the number of assays to be performed. For each well, prepare 10 µL of Substrate Mix according to Table 2. Mix well.

Table 2.

Preparation of Substrate Mix

Reagent	**Volume**
Assay Buffer	8 µL
ARG1 Substrate	2 µL

2. Add 10 µL of the Substrate Mix into each well. Mix well.
3. Cover plate and incubate for 30 minutes at **37 ºC** (**not** 25 ºC).

Reaction Mix

1. Mix enough reagents for the number of assays to be performed. For each well, prepare 200 µL of Reaction Mix according to Table 3. Mix well.

Table 3.

Preparation of Reaction Mix

Reagent	**Volume**
ARG1 Probe Mix A	100 µL
ARG1 Probe Mix B	100 µL

2. Add 200 µL of the Reaction Mix into each well. Mix well.
3. Cover plate and incubate for 60 minutes at **25 ºC** (**not** 37 ºC).

Measurement

Measure absorbance at 450 nm (A_{450}) in a microplate reader in endpoint mode.

Results

1. Subtract the Background Control (BC) reading from all readings to obtain ΔA_{450} for each reading. When using a specific Background Control for a test compound subtract its signal from the signal of that Sample (S) only.
2. Set the ΔA_{450} of Enzyme Control (EC) as 100%. Note: In case Solvent Control (SC) is significantly different from EC, use the ΔA_{450} value for SC in the formulas below. Calculate % Inhibition or % Relative Activity of the test inhibitors as follows:

% Inhibition =

$$\frac{\Delta A_{450}\ EC - \Delta A_{450}\ S}{\Delta A_{450}\ EC} \times 100\%$$

% Relative Activity =

$$\frac{\Delta A_{450}\ S}{\Delta A_{450}\ EC} \times 100\%$$

Figure 1.
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 Reaction Mix was added.

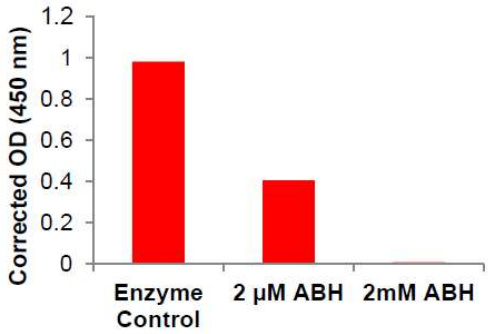
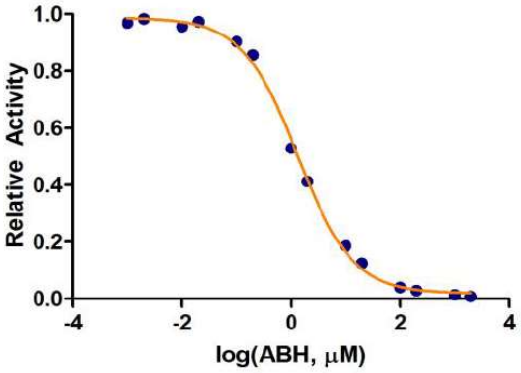


Figure 2.
Inhibition of ARG1 enzyme activity by Amino-2-Borono-6-Hexanoic Acid (ABH). IC_{50} of ABH was determined to be 1.39 ± 0.10 mM. Assay was performed following the kit protocol.



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