ab283402 – Liver Arginase (ARG1) Inhibitor Screening Kit (Colorimetric)

For the screening of Liver Arginase (ARG1) inhibitors.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab283402

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light. Upon opening, use kit within 2 months.

Materials Supplied

Item	Quantity	Storage Condition
Assay Buffer	25 mL	-20°C
ARG1 Substrate	1 vial	-20°C
ARG1 Probe Mix A	12 mL	-20°C
ARG1 Probe Mix B	12 mL	-20°C
Human ARG1	1 vial	-20°C
ABH (in DMSO)	20 µl	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Temperature-controlled plate reader
- 96-well clear plate with flat bottom

Reagent Preparation

- Before using the kit, spin the tubes prior to opening.

Assay Buffer: Warm to room temperature (RT) before use. Store at 4°C or -20°C.

ARG1 Substrate: Reconstitute with 250 µl dH2O. Pipette up and down to dissolve. Store at -20°C.

ARG1 Probe Mix A: Ready to use as supplied. Warm to room temperature before use. Store at 4°C or -20°C. Keep away from light.

ARG1 Probe Mix B: Ready to use as supplied. Warm to room temperature before use. Store at 4°C 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.

Assay Protocol

Test compounds, Inhibitor Control, Enzyme Control and Background Control Preparations:

1. Dissolve candidate inhibitors at 1000X highest final test concentration into an appropriate solvent.
2. Dilute to 5X the desired test concentration with ARG1 Assay Buffer.
3. Add 10 µl diluted test inhibitor or Assay buffer into designated wells as sample screen [S].

4. Add 10 µl of Assay Buffer to a well designated as Enzyme Control [EC] (no inhibitor) respectively.

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 well labelled as Inhibitor Control [IC].

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 absorbance (OD 450 m) at the 5X final concentration prepare background controls as described above.

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

	Volume
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 minutes at 37°C.

Δ 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.*

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

	Volume
Assay Buffer	8 µl
ARG1 Enzyme	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 minutes at 37°C.

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

	Volume
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 minutes.

Measurement

Measure absorbance (OD 450 nm) in a microplate reader in endpoint mode.

Calculation:

1. 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).
2. 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:

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

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

Technical Support

For all technical or commercial enquiries please go to:

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www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)