Moved to Description

The ARG2 Inhibitor Screening Assay Kit is designed to measure ARG2 (arginase 2) activity for screening and profiling applications. The assay kit comes in a convenient 384-well format, with enough purified recombinant ARG2 (amino acids 22-355), thioarginine substrate, assay buffer and detection reagent for 400 enzyme reactions.

Background

Arginase enzymes convert arginine to ornithine through hydrolysis. Two known isoforms of Arginase exist, ARG1 and ARG2. This enzyme is involved in the regulation of a variety of immunological responses and is a major target in immunotherapy. ARG1/2 is overexpressed in myeloid-derived suppressor cells (MDSCs) and tumor-associated macrophages (TAMs). Overexpression of ARG1/2 results in depleted levels of arginine both intracellularly and extracellularly. As arginine levels are depleted in the microenvironment, immune cells are starved of this amino acid and the function of key immunological activators become impaired; T cell proliferation is inhibited, regulatory T cells become activated and inhibit $CD4^+$ T cells, and immunosuppressants have increased longevity. Depleted arginine also results in the release of reactive nitrogen species and reactive oxygen species from TAMs and MDSCs. These reactive species cause T cell apoptosis and the activation and growth of antigen presenting cells.

Applications

Study enzyme kinetics and screen small molecule inhibitors for drug discovery in high throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
71659-KC10	ARG2, His-Tag*	10 µg	-80°C
82982-KC5	10x ARG Assay Buffer	5 ml	-80°C
83525-KC1	Thioarginine	3 x 1 mg	-80°C
83526-KC3	Detection Reagent	3 mg	-80°C
79962	UV Transparent 384-well plate	1	Room Temp.

** The concentration of protein is lot-specific and will be indicated on the tube containing the protein.*

Materials Required but Not Supplied

- Spectrophotometer capable of measuring absorbance at λ= 410–415 nm.
- Ethanol (200 proof)

Stability

This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Contraindications

- The final concentration of DMSO in the assay should not exceed 0.5%.
- The assay should not be performed in the presence of strong acids or bases, ionic detergents, and high salt.
- Compounds that are fluorescent may interfere with the results, depending on their spectral excitation and emission properties.
- It is recommended that the compound alone be tested to determine any potential interference of the compound with the assay results.

Assay Protocol

- All samples should be run in duplicate while controls should be performed in quadruplicate.
- The assay should include "Blank", "Positive Control" and "Test inhibitor".
- We recommend using inhibitor nor-NOHA as an internal control for the assay. If not running a dose response curve for the control inhibitor, run at 0.1X, 1X and 10X the IC_{50} value shown in the validation data below.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).

1. Dilute **10X ARG Assay Buffer** 10-fold with distilled water. This makes 1x ARG Assay Buffer.

2. Dissolve each vial of Thioarginine reagent (1 mg) with 367 µl of 1x ARG Assay Buffer (total 3 mg in 1.1 ml of 1x ARG Assay Buffer).

   Note: Keep on ice until use.

3. Dissolve **Detection Reagent** (3 mg) with 350 µl of ethanol (200 proof) and vortex for 1 minute.

4. Prepare **ARG2 Reaction Solution** as follows: 1 ml of dissolved thioarginine + 225 µl of Detection Reagent + 39 ml of 1x ARG Assay Buffer.

   Note: Keep on ice until use. Use within 1 hour of preparation!

5. Add 90 µl of ARG2 Reaction Solution to each well.

6. Prepare the **Test Inhibitor** (5 µl/well): for a titration prepare serial dilutions at concentrations 20-fold higher than the desired final concentrations. The final volume of the reaction is 100 µl.

   6.1 If the Test Inhibitor is water-soluble, prepare serial dilutions 20-fold more concentrated than the desired final concentrations using 1x ARG Assay Buffer.

   For the positive and negative controls, use 1x ARG Assay Buffer as Diluent Solution.

   OR

6.2 If the Test inhibitor is soluble in DMSO, prepare the inhibitor in 100% DMSO at a concentration 200-fold higher than the highest desired concentration, then dilute the inhibitor 10-fold in 1x ARG Assay Buffer to prepare the highest concentration of the 20-fold intermediate dilutions. The concentration of DMSO is now 10%.

Use 10% DMSO in 1x ARG Assay Buffer (vol/vol) for the serial dilution to keep the concentration of DMSO constant.

For positive and negative controls, prepare 10% DMSO in 1x ARG Assay Buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

Note: The final concentration of DMSO should not exceed 0.5%.

7. Add 5 µl of the inhibitor serial dilution to the "Test Inhibitor" wells.

8. Add 5 µl of the Diluent Solution to the "Blank" and "Positive Control" wells.

9. Thaw **ARG2** on ice. Briefly spin the tube containing the enzyme to recover its full content.

10. Dilute ARG2 with 1x ARG Assay Buffer to 3.6 ng/µl (5 µl/well).

11. Initiate the reaction by adding 5 µl of diluted ARG2 to the "Positive Control" and "Test Inhibitor" wells.

12. Add 5 µl of 1x ARG Assay Buffer to the "Blank" wells.

13. Incubate at Room Temperature (RT) for 30 minutes.

Component	Blank	Positive Control	Test Inhibitor
ARG2 Reaction Solution	90 µl	90 µl	90 µl
Test Inhibitor	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
1x ARG Assay Buffer	5 µl	-	-
Diluted ARG2 (3.6 ng/µl)	-	5 µl	5 µl
Total	**100 µl**	**100 µl**	**100 µl**

14. Measure absorbance in a e plate reader capable of measuring absorbance (λ = 410-415 nm).

Note: If compounds absorb at 410-415 nm it is recommended to read the plate at time 0 as well as the final timepoint at 30 minutes. The time 0 measurement can be subtracted from the final reading to account for compound absorbance.

15. Subtract the "Blank" value from all other values.

Example of Assay Results

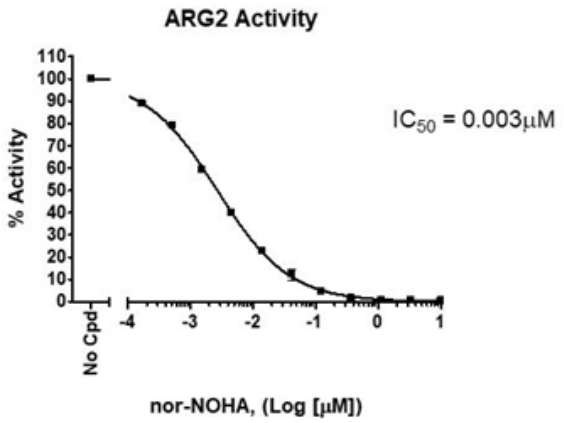

Figure 1: ARG2 activity and inhibition by nor-NOHA.
ARG2 activity was also measured in the presence of increasing concentrations of nor-NOHA.

Data shown is representative.

Troubleshooting Guide
Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit https://bpsbioscience.com/contact.

References
Sedbrook J.C., *et al.*, 1999 *PNAS* 96(3):1140-1145.
Woll P.J., *et al.*, 1998 *PNAS* 85(6):1859-1863.

Related Products

Products	*Catalog #*	*Size*
ARG1, His-Tag Recombinant	71658	50 µg
ARG1 Inhibitor Screening Assay Kit	72048	96 reactions/384 reactions

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