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Data sheet
ARG2 Inhibitor Screening Assay Kit
Catalog #72043-1
Size: 96 reactions

BACKGROUND: Arginase enzymes convert arginine to ornithine through hydrolysis. Two known isoforms of arginase exist, ARG1 and ARG2. These enzymes are involved in the regulation of a variety of immunological responses and are a major target in immunotherapy. ARG1/2 are 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 to 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.

DESCRIPTION: The *ARG2 Inhibitor Screening Assay Kit* is designed to measure ARG2 enzyme inhibition. The kit comes in a convenient format, with enough reaction solution and enzyme to perform a total of 96 reactions. The *ARG2 Inhibitor Screening Assay Kit* is simple to use. The test inhibitor and enzyme are added to a reaction containing thioarginine substrate. After a room temperature incubation, activity is determined by measuring the absorbance of the reaction product at λ = 410 nm.

COMPONENTS:

Catalog #	Component	Amount	Storage	
71659	ARG2	5 µg	-80 °C	Avoid multiple freeze/thaw cycles!
	10X ARG Assay Buffer	1 ml	-20 °C	
	Thioarginine	1 mg	-20 °C	
	Detection Reagent	1 mg	-20 °C	
	96-well transparent plate	1	RT	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
Spectrophotometer capable of measuring absorbance at λ = 410 nm.
Ethanol (200 proof, 100%)

APPLICATIONS: Useful for the study of ARG2 enzymology, screening inhibitors, and selectivity profiling.

CONTRAINDICATIONS: DMSO > 1 %, strong acids or bases, ionic detergents, high salt

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STABILITY: Up to 6 months from date of receipt, when stored as recommended.

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

ASSAY PROTOCOL:
All samples and controls should be tested in duplicate.

ARG Reaction Solution preparation

1. Dilute **10X ARG assay buffer** with water (1:10) to **1X ARG Assay Buffer**.
2. Solubilize 1 mg of **Thioarginine** with 367 µl **1X ARG Assay Buffer**. Keep on ice until use.
3. Solubilize 1 mg of **Detection Reagent** with 350 µl ethanol. Ensure complete solubilization by vortexing for approximately 1 minute.
4. Prepare **ARG Reaction Solution** by adding 250 µl solubilized **Thioarginine** and 56 µl solubilized **Detection Reagent** to 9.7 ml **1X ARG Assay Buffer**. Keep on ice until use.
5. *Note: Due to instability of the substrate and detection reagent it is recommended to initiate reaction within 1 hour of reaction solution preparation. These materials cannot be refrozen and thawed for later use.*

ARG2 Assay:

1. Prepare **ARG2 Reaction Solution** (see above) and aliquot 90 µl into each well of the assay plate.
2. Add 5 µl of **inhibitor solution** (including no more than 20% DMSO) to each well designated “Test Inhibitor”. For the wells labeled “Positive Control” and “Blank”, add 5 µl of the same solution without inhibitor (inhibitor buffer). Note: Keep the final DMSO concentration ≤1%.
3. Thaw **ARG2** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full contents of the tube. Aliquot **ARG2** into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. Note: **ARG2** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.
4. Dilute **ARG2** in **1X ARG Assay Buffer** to 4 ng/µl. Keep diluted protein on ice until use. Discard any unused diluted protein after use
5. Add 5 µl of **1X ARG Assay Buffer** to the well designated “Blank”.
6. Initiate reaction by adding 5 µl of diluted **ARG2** (4 ng/µl) prepared as described above to the wells labeled “Positive Control”, and “Test Inhibitor”. Incubate at room temperature for up to 30 minutes.
7. Measure absorbance at λ = 410 nm. Subtract “Blank” value from all other values. Note: If test compounds absorb at 410 nm, it is recommended to read the plate at time 0 as well as the final timepoint at 30 min. The time 0 measurement can be subtracted from the final reading to account for compound absorbance.

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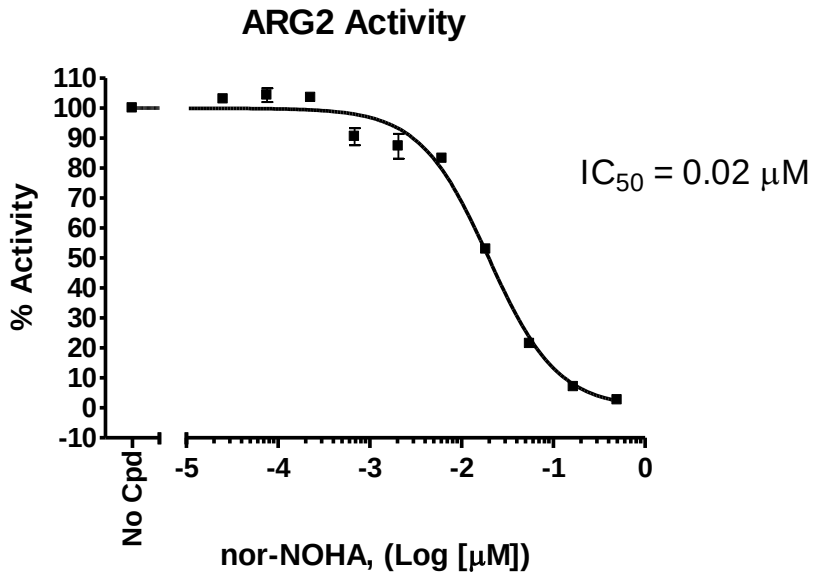
	Positive Control	Test Inhibitor	Blank
ARG Reaction Solution	90 µl	90 µl	90 µl
Test Inhibitor	-	5 µl	-
Inhibitor buffer (no inhibitor)	5 µl	-	5 µl
1X ARG Assay Buffer	-	-	5 µl
ARG2 (4 ng/µl)	5 µl	5 µl	-
Total	**100 µl**	**100 µl**	**100 µl**

Example of assay results:



ARG2 inhibition by nor-NOHA (Cayman, #10006861) measured using the *ARG2 Inhibitor Screening Assay Kit*, BPS Bioscience, #72043-1. Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.

RELATED PRODUCTS:

Product	**Catalog#**	**Size**
ARG1	71658	50 µg
ARG2	71659	50 µg
ARG1 Inhibitor Screening Assay Kit	72048-2	384 reactions
ARG2 Inhibitor Screening Assay Kit	72043-2	384 reactions