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Data Sheet

ARG1 Inhibitor Screening Assay Kit (384)

Catalog # 72048-2

DESCRIPTION: The *ARG1 Inhibitor Screening Assay Kit* is designed to measure ARG1 enzyme inhibition. The kit comes in a convenient format, with enough reaction solution and enzyme to perform a total of 400 reactions. The *ARG1 Inhibitor Screening Assay Kit* is simple to use. Inhibitor and enzyme are added to a sample containing thioarginine substrate. After a room temperature incubation, activity is determined by measuring the absorption of reaction product at $\lambda=410-415$ nm.

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.

COMPONENTS:

Catalog #	Component	Amount	Storage	
71658	ARG1 His-Tag	50 μ g	-80°C	Avoid freeze/ thaw cycles!
	10X ARG Assay Buffer	5 ml	-80°C	
	Thioarginine	3 x 1 mg	-80°C	
	Detection Reagent	3 mg	-80°C	
	UV transparent 384-well plate	1	Room Temp.	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:

Spectrophotometer capable of measuring absorbance at $\lambda = 410-415$ nm.
Ethanol (200 proof)

APPLICATIONS: Useful for the study of ARG1 enzymology, screening inhibitors, and selectivity profiling.

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

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STABILITY: Stable at least 6 months from date of receipt, when stored as directed. Kit components require different storage conditions. Be sure to store each component at the proper temperature upon arrival.

REFERENCES:

1. Sedbrook, J.C., et al., PNAS. 1999 Feb; 96(3):1140-1145.
2. Woll, P.J., et al., PNAS. 1998 Mar; 85(6):1859-1863.

ASSAY PROTOCOL:

All samples and controls should be tested in duplicate. Use slow shaking for all incubations.

Setup: ARG1 Reaction Solution

- 1) Dilute **10X ARG assay buffer** with water (1:10) to **1X ARG assay buffer**
- 2) Dissolve 3 mg of thioarginine substrate with 1.1 mL **1X ARG assay buffer**. Keep on ice until use.
- 3) Dissolve the detection reagent with 350 μ l EtOH. Ensure complete solubilization by vortexing for approximately 1 minute.
- 4) Prepare **ARG1 Reaction solution** by adding 1 ml thioarginine and 225 μ L detection reagent to 39 ml **1X ARG assay buffer**. Keep on ice until use.

Step 1:

- 1) Prepare **ARG1 Reaction Solution** (see above) and aliquot 90 μ l into each well of the assay plate. *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.*
- 2) Add 5 μ l of inhibitor solution (no more than 20% DMSO) to each well designated "Test Inhibitor". For the wells labeled "Positive Control" and "Blank", add 5 μ l of the same solution without inhibitor (inhibitor buffer). *Note: Keep the final DMSO concentration below 1 %.*
- 3) Thaw **ARG1 enzyme** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full contents of the tube. Aliquot **ARG1 enzyme** into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. *Note: ARG1 enzyme is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*
- 4) Dilute **ARG1** in **1X ARG Assay Buffer** to 18 ng/ μ l. Keep diluted protein on ice until use. Discard any unused diluted protein after use.

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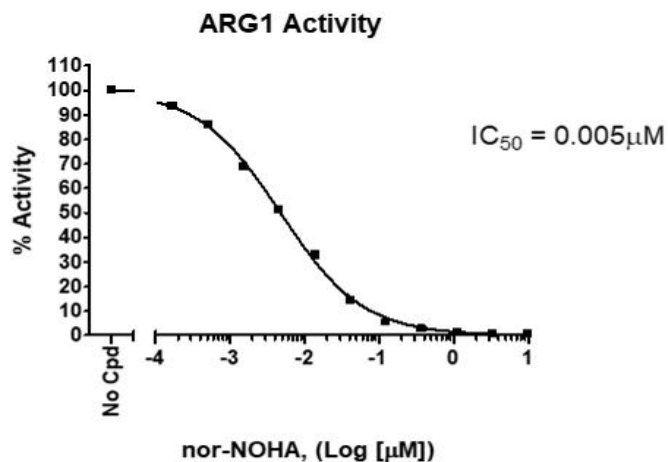
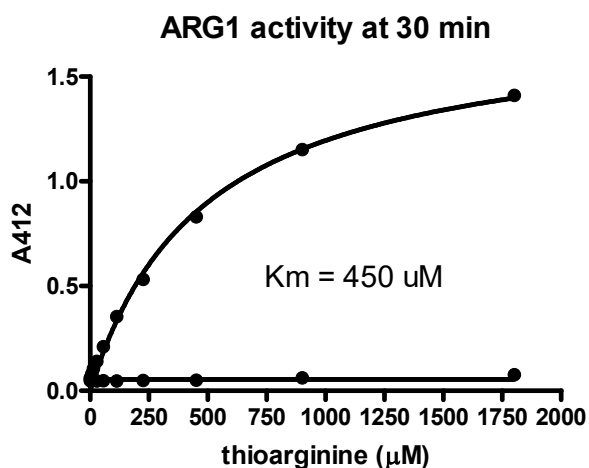
	Blank	Positive Control	Test Inhibitor
ARG1 Reaction Solution	90 μ l	90 μ l	90 μ l
Test Inhibitor	-	-	5 μ l
Inhibitor buffer (no inhibitor)	5 μ l	5 μ l	-
ARG Assay Buffer	5 μ l	-	-
ARG1 (18 ng/ μ l)	-	5 μ l	5 μ l
Total	100 μl	100 μl	100 μl

- 5) Add 5 μ l of **1X ARG Assay Buffer** to the well designated "Blank".
- 6) Initiate reaction by adding 5 μ l of diluted **ARG1** prepared as described above to the wells labeled "Positive Control", and "Test Inhibitor". Incubate at room temperature for up to 30 minutes.
- 7) Measure absorption at $\lambda=410 - 415$ nm. Subtract "Blank" value from all other values.
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 min. The time 0 measurement can be subtracted from the final reading to account for compound absorbance.

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EXAMPLE OF ASSAY RESULTS:



ARG1 activity (left) and ARG1 inhibition (right), measured using the ARG1 Inhibitor Screening Assay Kit, BPS Bioscience, Catalog #72048-2. Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.

RELATED PRODUCTS AND SERVICES:

<u>Product Name</u>	<u>Catalog#</u>	<u>Size</u>
ARG2 Inhibitor Screening Assay Kit	72043-2	384 rxns
ARG1, His-Tag	71658	50 µg
ARG2, His-Tag	71659	50 µg
ARG1 / ARG2 Screening & Profiling	Services	

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