BioAssay Systems
3191 Coprorate Place
Hayward, CA 94545. U. S. A
Tel: 510. 782. 9988
Fax: 510. 782. 1588
www.bioassaysys.com
info@bioassaysys.com

Title: Arginase Activity Assay Control Tests: K_m for Arginase I and IC50 Determination for ABH

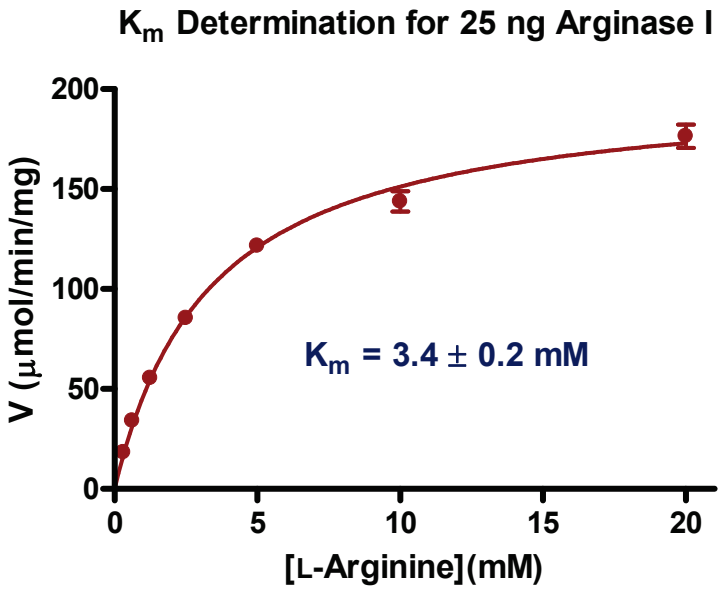
Summary

In order to test the suitability of BioAssay Systems Arginase Assay for screening for arginase inhibitors, we performed some initial experiments to determine the K_m of L-arginine for a purified human arginase and the IC50 for a known arginase inhibitor, ABH. We measured a K_m of 3.4 ± 0.2 mM. Using 3 mM L-arginine, we observed an IC50 = 0.14 ± 0.1 µM for ABH. We also verified that 2 v% DMSO will not affect the assay.

Results

K_m of L-Arginine

In order to determine the K_m of L-arginine for Arginase I, we titrated L-arginine from 0–20 mM with 25 ng Arginase I and computed the rates of reaction, V (µmol/min/mg), for each L-arginine concentration. The rates of reaction were then plotted versus L-arginine and the K_m was computed using Prism 4 (GraphPad Software Inc.). We found the K_m = 3.4 ± 0.2 mM.



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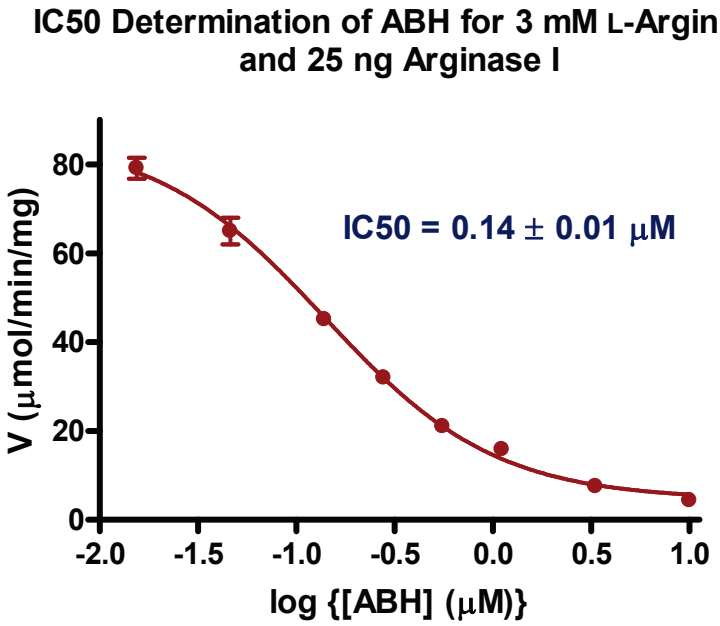
ABH IC50 Determination

The IC50 determination required three steps: 1) 30 min pre-incubation of 25 ng arginase I with ABH, 2) arginase reaction with 3 mM L-arginine for 25 min and 3) urea concentration measurement. We titrated ABH from 0-10 µM and computed the rate of reaction, V, for each ABH concentration. The rates of reaction were then plotted versus the Log [ABH] and the IC50 was computed using Prism 4 (GraphPad Software Inc.). We observed an IC50 = 0.14 ± 0.01 µM.



Effect of DMSO

In order to determine if DMSO would interfere with this arginase assay, we set up four reactions: 25 ng arginase I ± 2 v% DMSO and 0 ng arginase ± 2 v% DMSO. Following a 20 min reaction and addition of the Urea Reagent, we measured the OD_{520} and OD_{430} and computed the ΔOD ($OD_{arginase} - OD_{no\ arginase}$) for the 2 v% DMSO and 0 v% DMSO reactions at each wavelength. The following table displays the results:

Sample	ΔOD_{520}	ΔOD_{430}
2 v% DMSO	0.3995	1.4476
0 v% DMSO	0.3978	1.4181

Clearly 2 v% DMSO has no adverse effects on the assay and will not be an issue for the compound screen.

Conclusions

The results for the K_m of L-arginine for arginase I and IC50 of ABH observed in this study agree well with numbers found in literature (within 3 fold). This study also verified that 2 v% DMSO will not interfere with the assay. Thus, this arginase assay will be suitable for screening for arginase inhibitors.