

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

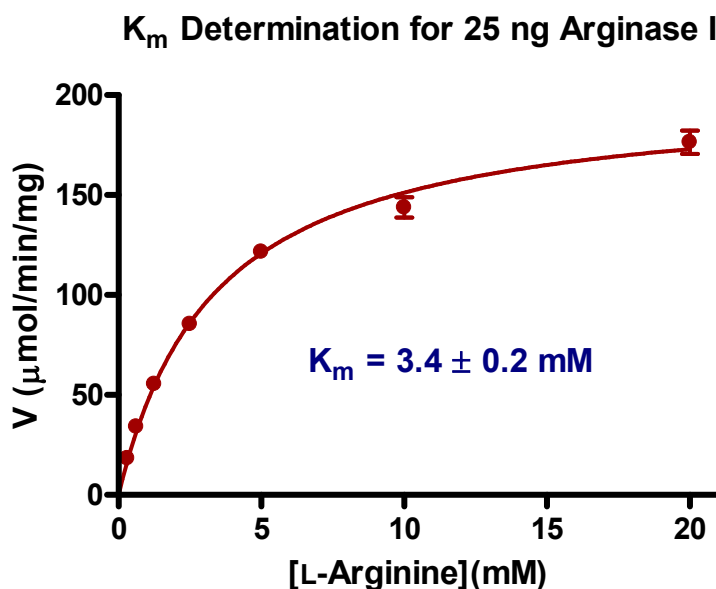
### Summary

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  $3.4 \pm 0.2$  mM. Using 3 mM L-arginine, we observed an  $IC_{50} = 0.14 \pm 0.1$   $\mu$ M for ABH. We also verified that 2 v% DMSO will not affect the assay.

### Results

#### $K_m$ of L-Arginine

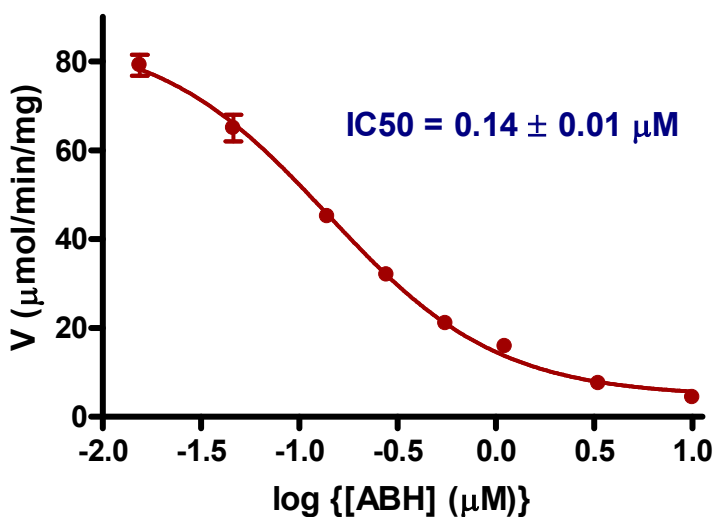
In order to determine the  $K_m$  of L-arginine for Arginase I, we titrated L-arginine from 0–20 mM with 25 ng Arginase I and computed the rates of reaction,  $V$  ( $\mu$ mol/min/mg), for each L-arginine concentration. The rates of reaction were then plotted versus L-arginine and the  $K_m$  was computed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



## 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  $\mu$ M 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 =  $0.14 \pm 0.01$   $\mu$ M.

### IC50 Determination of ABH for 3 mM L-Arginine and 25 ng Arginase I



## Effect of DMSO

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

Sample	$\Delta$ OD <sub>520</sub>	$\Delta$ OD <sub>430</sub>
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.



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## Conclusions

The results for the  $K_m$  of L-arginine for arginase I and IC<sub>50</sub> 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.