BioAssay Systems
3191 CORPORATE PLACE
HAYWARD, CA 94545. U. S. A

TEL: 510. 782. 9988
FAX: 510. 782. 1588
www.bioassaysys.com
info@bioassaysys.com

Title: Acetylcholinesterase Inhibitor Screening Assay Development: K_m for AChE and IC50 Determination for Donepezil and Physostigmine

Summary

In order to develop an Acetylcholinesterase inhibitor screening assay kit, we performed some initial experiments to establish appropriate assay conditions. We assessed conditions for Acetylcholinesterase from electric eel. First we titrated the AChE using 1 mM Acetylthiocholine iodide as the substrate and DTNB as the chromagen. From this titration we decided to use 0.0035 Units/reaction AChE for assay development and IC50 determinations. The K_m of Acetylthiocholine iodide for AChE was then determined. We next titrated DMSO to determine the highest concentration that would be tolerated by AChE. Finally, the IC50's for two known Acetylcholinesterase inhibitors, Donepezil and Physostigmine, were measured. We determined the K_m to be 0.425 mM and the maximum DMSO concentration to be 100 v%. Using 0.425 mM acetylthiocholine iodide, we observed an IC50 = 48.3 nM for Donepezil and an IC50 = 46.7 nM for Physostigmine when each inhibitor was dissolved in 20v% DMSO.

Results

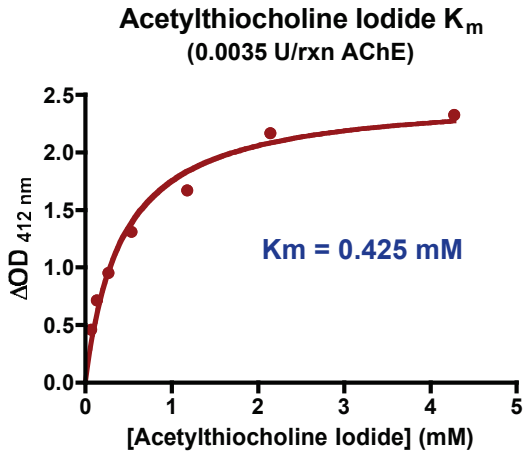
AChE Titration

In order to determine an appropriate amount of AChE to use for the inhibitor screening assay and for IC50 determinations, we titrated AChE. The reaction was performed using 1 mM Acetylthiocholine Iodide and 0.4 mM DTNB dissolved in Assay Buffer pH 7.6. From these results we opted to use 0.0035 U/rxn AChE. At this concentration the reaction would remain linear for at least 10 minutes and the ΔOD should be significant (>0.2) even at the Acetylthiocholine Iodide K_m.

K_m of Acetylthiocholine Iodide

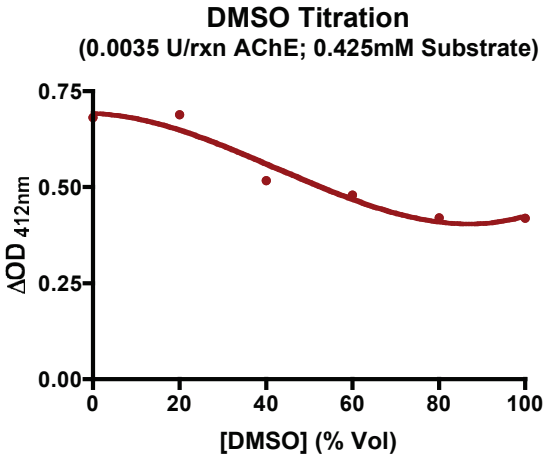
In order to determine the K_m of Acetylthiocholine iodide for Acetylcholinesterase (electric eel), we titrated acetylthiocholine iodide from 0–4.3 mM with 0.0035 U/rxn AChE and computed ΔOD (OD_{10min} – OD_{0min}), which is proportional to the rate of reaction, for acetylthiocholine iodide. The ΔOD's were then plotted versus acetylthiocholine iodide concentration and the K_m was computed using Prism 4 (GraphPad Software Inc.). We found the K_m = 0.425 mM for Acetylthiocholine Iodide.

BioAssay Systems
3191 CORPORATE PLACE
HAYWARD, CA 94545. U. S. A
TEL: 510. 782. 9988
FAX: 510. 782. 1588
www.bioassaysys.com
info@bioassaysys.com



DMSO Titration

In order to determine tolerance for DMSO of Acetylcholinesterase, we ran a DMSO titration (from 0–100 v% for the 5 µL of variable compound in a 200 µL reaction). For this titration we used 0.0035 U/rxn AChE. The reaction was initiated by adding Acetylthiocholine Iodide, DTNB, and Assay buffer pH 7.6 (Final concentrations 0.425 mM Acetylthiocholine Iodide and 0.3 mM DTNB) and was allowed to proceed for 10 min. We then computed the ΔOD ($OD_{10min} - OD_{0min}$), which is proportional to the rate of reaction, for each DMSO concentration. The ΔOD's were then plotted versus DMSO concentration. From these results we discovered that that we can use up to 100 v% DMSO if needed, but decided to move forward with 20 v% DMSO for IC50 determinations.

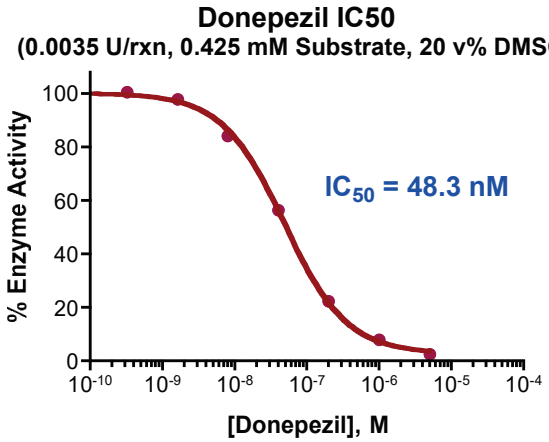


Donepezil IC50 Determination

The IC50 determination required two steps: 1) 15 min pre-incubation of 45 µL AChE (78 U/L) with 5 µL Donepezil (in 20 v% DMSO), and 2) acetylcholinesterase reaction with 150 µL Working reagent

BioAssay Systems
3191 CORPORATE PLACE
HAYWARD, CA 94545. U. S. A
TEL: 510. 782. 9988
FAX: 510. 782. 1588
www.bioassaysys.com
info@bioassaysys.com

containing acetylthiocholine iodide, DTNB, and assay buffer for 10 min (0.425 mM [Acetylthiocholine Iodide] and 0.31 mM [DTNB] final). We titrated Donepezil from 0-25 µM and computed the ΔOD for each Donepezil concentration. The ΔOD's were then plotted versus the Log [Donepezil] and the IC50 was computed using Prism 4 (GraphPad Software Inc.). We observed an IC50 = 48.3 nM.



Physostigmine IC50 Determination

The IC50 determination required two steps: 1) 15 min pre-incubation of 45 µL AChE (78 U/L) with 5 µL Physostigmine (in 20 v% DMSO), and 2) acetylcholinesterase reaction with 150 µL Working reagent containing acetylthiocholine iodide, DTNB, and assay buffer for 10 min (0.425 mM [Acetylthiocholine Iodide] and 0.31 mM [DTNB] final). We titrated Physostigmine from 0- 25 µM and computed the ΔOD for each Physostigmine concentration. The ΔOD's were then plotted versus the Log [Physostigmine] and the IC50 was computed using Prism 4 (GraphPad Software Inc.). We observed an IC50 = 46.7 nM.