



## Beefing up an enzyme lab: Acetylcholinesterase activity in extracts from bean beetles standardized for protein content

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### Why use this system?

- Bean beetles are common pests
- Bean beetles are easy to maintain in the laboratory
- Organophosphate insecticides are commonly used to control insect pests and insects are developing resistance
- Ties in several course topics and lab skills: Introduction to enzymes, colorimetric assays, serial dilution and standard curves, the role of inhibitors in enzyme activity, cell-cell signaling, and potential ecological consequences of insecticide use

### Materials

- Bean beetles, *Callosobruchus maculatus*
- Incubator with light and temperature control
- Insecticide malaoxon
- ATCI and DTNB
- Bradford reagent
- Spectrophotometer, vortexer, centrifuge
- Water bath, autoclave, micropipettes
- Other standard laboratory equipment

### What should students know?

- An introduction to the life cycle of the insects
- Basic structure of proteins
- Basics about enzymes
- The basics of enzyme inhibition
- Basics of synaptic signaling
- Function of acetylcholinesterase

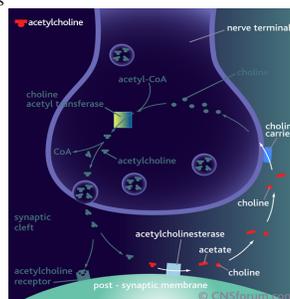


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### Sample Student Results

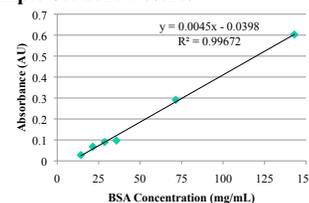


Figure 1: Standard curve for the Bradford assay.

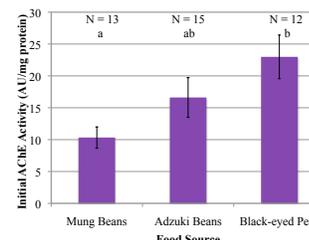


Figure 2: Initial AChE activity measured in crude extracts of bean beetles. The values are means±SEM. Different letters indicate significant differences.

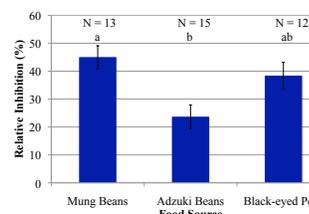
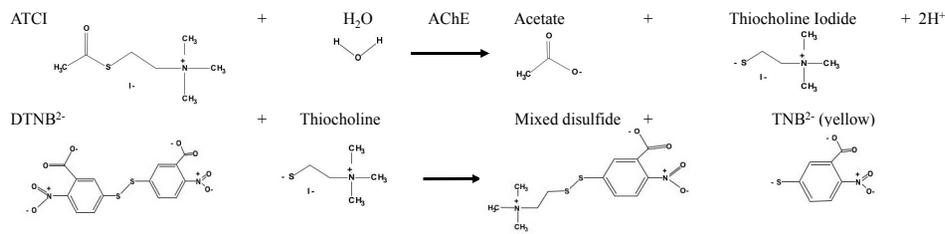


Figure 3: Relative inhibition of AChE due to malaoxon in crude extracts of bean beetles. The values are means±SEM. Different letters indicate significant differences. Relative inhibition is calculated as % reduction in AChE activity.

### Acetylcholinesterase (AChE) Enzyme Assay

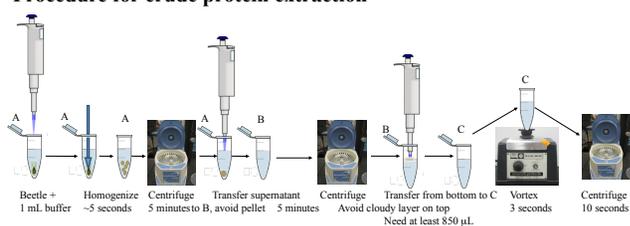
Based on the work done by Ellman et al. (1961), Ffrench-Constant and Bonning (1989), Spencer et al. (1998), and Gbaye et al. (2012). TNB<sup>2-</sup> is yellow and can be detected spectrophotometrically.



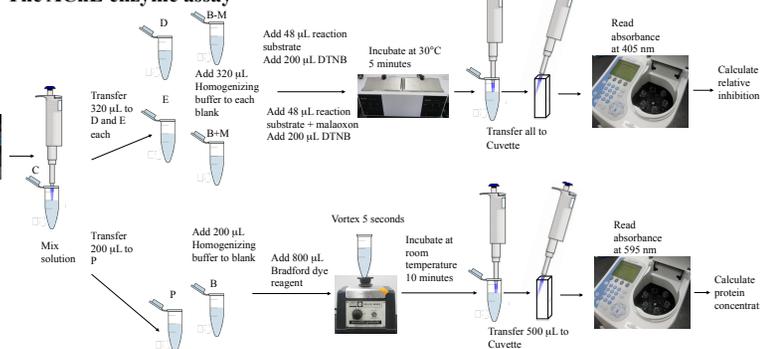
### Bradford Assay

Based on Bradford (1976) to measure protein concentration in crude extracts.

### Procedure for crude protein extraction



### The AChE enzyme assay



### The Bradford assay for protein concentration

### Comments

- There is variability in AChE activity and relative inhibition of AChE due to malaoxon
- Food source affects:
  - Initial AChE activity
  - Sensitivity of AChE to malaoxon
- Can be done in regular 3-hour laboratories
- Can be supplemented with readings from the primary literature for more advanced students:
  - Gbaye et al. (2012): variability in AChE activity and sensitivity to malaoxon in different geographic strains reared on different foods
  - Magaña et al. (2008): resistance due to point mutation in AChE, correlation between AChE activity and sensitivity to malaoxon

### Current Research

- To develop longer-term student projects to determine whether food source affects detoxification enzymes such as general esterases
- To use an artificial bean system based on Guo et al. (2012) that can be supplemented with plant defensive compounds to determine their effects on detoxification enzymes and AChE
- To isolate malaoxon-resistant bean beetle cultures and study their method of resistance to the insecticide

### Acknowledgements

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