Standard Operating Procedure #505
Protein Testing of Honey Using Known Standards

Laboratory: Bioresearch
Location: RM 169
SOP prepared by: R. Sanders
Last Revision: 5/20/23

General

This assay is suitable for the simple and rapid estimation of protein concentration. This assay is based on a single Coomassie dye-based reagent known as Bradford Solution. The binding of protein to the dye results in a change of color from brown to blue. The change in color intensity is proportional to protein concentration.

Safety: Eye Protection, gloves if available
Note: Bradford reagent can stain your skin and fingernails!! (They contain proteins!)

Materials

honey samples
micro tubes
Bradford indicator dye
protein standards (Bovine Serum Albumin, BSA)
micropipettes & tips
micro tubes and rack
vortexer

Procedure

Prep of Protein Standards for Color Comparator

1. Use the following formula to create the following known concentrations (0.25, 0.5, 0.75, 1.0, 1.25, 1.5) from a given concentrated stock solution.

   $C_1V_1 = C_2V_2$

   1. $\mathbf{C_1}$ = Given stock solution concentration
   2. $\mathbf{V_1}$ = ? (how much is needed)
   3. $\mathbf{C_2}$ = New concentration needed
   4. $\mathbf{V_2}$ = Volume needed of new concentration

   Example: make 1 mL of 2 mg/mL protein solution from the given 10mg/mL stock solution:

   5. $\mathbf{C_1}$ = 10mg/mL, $\mathbf{V_1}$= ? , $\mathbf{C_2}$ = 2mg/mL, $\mathbf{V_2}$ = 1 mL
   6. $\mathbf{V_1} = \frac{(2)(1)}{10}$

      $\mathbf{V_1}$ = 0.2 mL of 10 mg/mL needs to be added to 0.98 mL of distilled water to make 1mL of 2 mg/mL protein solution. (May need to convert mL to µL if measuring small amount)
2. Once the known concentration solutions have been made, add 30µL of the known concentrations into the appropriately labeled micro tubes.
3. Add 1.5 mL of Bradford indicator dye to each micro tube.

4. Invert micro tubes to mix, then allow samples to incubate for 5 minutes at room temperature before using as a color comparator of unknown concentrations.

Prep of Honey Samples

1. To an appropriately labeled micro tube, add 30µL of honey sample.
2. Add 1.5mL of Bradford indicator dye to the honey sample and close the cap tightly.
3. Vortex or invert the tube to completely mix the honey and dye and allow to sit for 5 minutes before reading the sample against the color comparators. Record results in data table.
4. Repeat steps 1-3 with all honey samples.

Sample identification letter or number	Color	Protein in mg/mL

Honey and Biotech