**Total Protein Content Protocol**

Adapted from Putnam lab protocols by T. Lindsay

Materials

* Pierce BCA Protein Assay Kit from Thermo Scientific.
* clear 96 Well plate
* Incubator or Waterbath with range from 37°C to 50°C.
* Plate reader Spectrophotometer
* 1M NaOH
* 0.1M HCl
* Pipettes P10, P200, P1000 and tips
* 1.5ml microfuge tubes
* DI water

Protocol

**Adult Tissue Sample Preparation for Soluble and Insoluble Protein from Holobiont**

1. Thaw a 500 μL aliquot of tissue homogenate.
2. Vortex to re-suspend the symbiont cell pellet.
3. Add 10 μL of 1M NaOH (pH should be ~10) to the tube. Experiments may be needed prior to use to determine if volume is appropriate for species of choice.
4. Pipette a very small amount of sample onto pH paper to confirm the pH ~10.
5. Incubate the tube at 50°C for 4 hours flicking to mix throughout to solublize protein.
6. Add 280 μL of 0.1M HCl to the tube to neutralize the sample. Add this volume in small amounts and continue to test the pH of the sample using pH paper. pH needs to be at 7.0 to move onto the next steps.
7. It is critical to record exactly how much volume of NaOH and HCl was added

**Adult Tissue Sample Preparation for Soluble Protein from Host**

1. Thaw the 500 μL aliquot of host only supernatant.
2. Standard Table

Preparation of Diluted Albumin (BSA) Standards

These standards can be made during the 4 hour incubation period in the sample preparation section.

1. Use the following table as a guide to prepare a set of protein standards. For this project we will use the microplate procedure. Diluent is DI water Type II. Each vial will be a sterile 1.5 mL microcentrifuge tube. Label the cap of the microcentrifuge tube with the Vial ID ("A", "B", etc.).

|  |  |  |  |
| --- | --- | --- | --- |
| Vial | Volume of Dilutent (uL) | Volume of Source of BSA (uL) | Final BSA Concentration (ug/mL) |
| A | 0 | 300 of Stock | 2000 |
| B | 125 | 375 of Stock | 1500 |
| C | 325 | 325 of Stock | 1000 |
| D | 175 | 175 of vial B dilution | 750 |
| E | 325 | 325 of vial C dilution | 500 |
| F | 325 | 325 of vial E dilution | 250 |
| G | 325 | 325 of vial F dilution | 125 |
| H | 400 | 100 of vial G dilution | 25 |
| I | 400 | 0 (Blank) | 0 |

**Preparation of the BCA Working Reagent (WR)**

1. Use the following formula to determine the total volume of WR required:

(# standards + # unknowns) x (# replicates) x (volume of WR per sample) = total volume WR required

For this project, we will use 9 standards and 200 μL of WR is required for each sample in the microplate procedure.

(9 standards + # samples) x (2 replicates) x (200 μL of WR) = total volume WR required

(9 standards + 10 samples) x (2 replicates) x (200 μL of WR) = 7,600 μL WR

(9 standards + 20 samples) x (2 replicates) x (200 μL of WR) = 11,600 μL WR

(9 standards + 40 samples) x (2 replicates) x (200 μL of WR) = 19,600 μL WR

1. Prepare WR by mixing 50 parts of BCA Reagent A with 1 part of BCA Reagent B (50:1, Reagent A:B) in a clean protein-free container of the appropriate size, based on how many samples are going to be run.

**Microplate Procedure (Sample to WR ratio = 1:8) from Pierce BCA Protein Assay Kit:**

1. Pipette 25 μL of each standard or unknown sample into duplicate microplate wells.
2. Add 200 μL of the working reagent (WR) to each well and mix.
3. Cover the plate and incubate at 37°C for 30 minutes.
4. Subtract the average 562 nm absorbance measurement of the Blank standard replicates from the 562 nm measurements of all other individual standard and unknown sample replicates.
5. Calculate the standard curve by plotting the average Blank-corrected 562nm measurement for each BSA standard vs. its concentration in μg/mL. Use the standard curve equation to determine the protein concentration of each unknown sample.

References

Pierce BCA Protein Assay: <https://assets.thermofisher.com/TFS-Assets/LSG/manuals/MAN0011430_Pierce_BCA_Protein_Asy_UG.pdf>