**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
* Pipettes P10, P200, P1000 and tips
* 1.5ml microfuge tubes
* DI water
* 1M NaOH (for insoluble only)
* 0.1M HCl (for insoluble only)

Protocol

**Sample Preparation to extract Insoluble Protein (OPTIONAL)**

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 solubilize 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.

**Sample Preparation for Soluble Protein from Host**

1. Thaw the 500 μL aliquot of host only supernatant.
2. Prepare plate map

**Prepare BSA Standards**

1. Label nine 1.5mL tubes with the letters A – I, and another tube “stock”
2. Beak the glass BSA stock vial and pipet it into the stock tube
3. Use the following table as a guide to prepare a set of protein standards. Diluent is DI water Type II.

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

(# standards + # unknowns) x (# replicates) x (volof WR per sample) = total vol WR required

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

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.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| # samples | # standards | # Replicates | Total vol required (μL) | Vol / 51 (rounded) (μL) | Reagent A (μL) | Reagent B (μL) |
| 10 | 9 | 2 | 7600 | 155 | 7750 | 155 |
| 20 | 9 | 2 | 11600 | 230 | 11500 | 230 |
| 40 | 9 | 2 | 19600 | 385 | 19250 | 385 |
| 80 | 9 | 2 | 39200 | 775 | 38750 | 775 |

**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. Place the plate in the platereader and run the protein absorbance protocol (562nm).
5. 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.
6. 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>