

## Protein Extraction

Each group should select a source of protein for their study. The class might like to compare measured protein content in some plant-based foods to the amount reported on packaging. Protein content in fruits or seeds at various stages of development could also be assessed. Or, the class may wish to join in an investigation with the Roberts Lab. In the Roberts lab, researchers sometimes isolate proteins from plant samples to study when and where certain enzymes are expressed or to see if an attempt to knock out the production of a particular protein has been successful. If your students are collaborating with the Roberts lab, extra care must be taken with the extraction steps to ensure that the protein is not degraded during isolation. Following quantification of isolated proteins, SDS-Polyacrylimide Gel Electrophoresis (SDS-PAGE) and a Western Blot can be performed to check for expression of particular proteins.

### Buffer Preparation

- 100 mM Tris-Cl (pH 6.8) (Dilute the following Stacking Gel Buffer: 0.5 M Tris-HCl; BioRad #161-0799)
- 4% (w/v) SDS (sodium dodecyl sulfate; Biorad #161-0301)
- 200 mM DTT (dithiothreitol; BioRad # 161-0610)
- Store the SDS gel-loading buffer without DTT at room temperature. Add DTT from a 1 M stock just before the buffer is used.

### Extraction

1. Obtain ice, and use it to chill the extraction buffer and the mortar and pestle. Clearly label two 1.5 ml microcentrifuge tubes for each plant tissue from which you want to obtain protein with the name of the plant and your groups initials.
2. From each plant cut up 1 g of fresh plant tissue as fine as you can with a razor blade. Place this into a mortar with approximately 1 ml of cold QB buffer containing protease inhibitor and a pinch of clean sand. Grind until smooth with a pestle. It is very important to grind the tissue well. You may need to add more buffer but the final product should be about the consistency of slightly watery toothpaste. Keep the mortar and pestle and plant tissue as cold as possible during this process.
- 3 Transfer 1 ml of slurry into a 1.5 ml microfuge tube using a clean spatula. Place the tube on ice and perform step 2 with each plant tissue from which you plan to isolate protein.
4. Spin your samples at top speed in a microfuge at 4 degrees C for 15 minutes. (If you don't have a refrigerated centrifuge, you may be able to set up your microcentrifuge in a refrigerator. Be sure you give it plenty of time for the temperature to equilibrate prior to use.) Remember to make sure that all centrifuges and microfuges are balanced and do not walk away from any centrifuge or microfuge until it successfully reaches full speed.
5. Transfer the liquid supernatant into a second (new) microfuge tube.
6. Sometimes excess tissue is transferred over into the second microfuge tube. If this is the case, spin a second time for about 10 minutes and transfer this supernatant into a third microfuge tube.
9. Store the samples in ice only until you have finished the Bradford Protein Assay, then freeze the samples. (If these are intended for further analysis such as Western Blotting, freeze in liquid nitrogen and put them in the -80 degree freezer).