Protein Extraction from Plant Material for Lowry Assay

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11:23 AM

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Materials:
2 ml microfuge tube
Ball bearings or silicon carbide particles
25 mM Imidizole buffer pH 7.5
Protease inhibitor cocktail (Sigma P-9599)
15 ml Falcon tube
1.5 ml microfuge tubes

Procedure:

1. Label and weigh 2 ml microfuge tubes, it is helpful to weigh them with the ball bearing or silicon carbide particle already in the tube so you don't have to add it later
2. Harvest leaf material and, **push to bottom of tube**, and immediately freeze in LN2
   **** Make sure there is not too much material, ≈ 500 mg FW maximum, or it will not grind well ****
3. After freezing, quickly weigh again, to determine fresh weight
4. Store in -80º C until ready to continue with assay
5. Place Retch mill microfuge tube holder in Styrofoam box and cool with LN_2
6. Get plant samples from step 4 and place a stainless ball in each sample tube
   **** Be sure to push plant material to bottom so that ball can move freely ****
7. Place samples in cooled microfuge tube holder and place and secure holder containing vials into Retsch Mill, you will need cryo gloves
8. Tighten the outer knob first, then move the ratching set pin into the locked position and tighten further (1 -3 clicks)
   Make sure each holder has a microfuge tube holder, however the second microfuge tube holder does not have to be filled with tubes
9. Grind samples for 30 sec at a frequency of 30 (maximum speed). If the sample isn't pulverised enough, carefully open tube while cold and partially break up leaf material with a pair of chilled forceps. Grind again in Retsch mill for 30 seconds.
10. Get a bucket of ice and carry out the following procedures on ice
11. Put 15 ml of Imidizole pH 7.5 in a 15 ml falcon tube, to this add 150 µl of protease inhibitor cocktatil (1% v/v).
12. Add 750 µl of ice-cold extraction buffer to each gradient part
13. Vortex sample to mix well, you may want to sonicate samples with a constant duty cycle, output = 1 for about 5 seconds
14. Put samples back on ice for 60 seconds, and repeat sonication until the material is completely dissolved (may take 2 – 4 times)
15. Centrifuge at 4°C max speed for 5 minutes
16. Place supernatant into fresh microfuge tubes
17. Test a representative sample according to the Lowry Assay protocol, you may need to dilute your samples further

    Lowry assay is incompatible with > 1% SDS, >1 mM Hepes, > 25 mM Imidazole, Any DTT, > 1 mM EDTA, > 10% Glycerol, and many other things check product literature if unsure

Reagents
Imidazole Buffer pH 7.5

In 200 ml dH_2O
+ 25 ml Glycerol, 10 %
+ 0.34g Imidazole, 25 mM (fw 68.1)
+ 0.254 g $MgCl_2$, 5 mM (fw 203.3; Sigma M-2670)
+ 0.104 g EDTA, 1 mM (fw 416.2; Sigma ED4SS)
pH to 7.5 using HCl