PREPARATION OF PROTEIN SAMPLES

1. Grind tissue in liquid N_2 and transfer to appropriate volume of **homogenization buffer** in a 30 ml tube. Use 3 ml buffer per g tissue.

2. Filter samples through cheesecloth into microfuge tubes (two tubes per 1 g sample) and spin 10' in a microfuge.

3. For soluble proteins, add 0.6 x volumes **2x Stop Dye** to the supernate. i.e 500 µl supernate plus 300 µl dye.

4. For insoluble proteins, extract the pellet with 0.1 x volume of original homogenate with **Pellet Buffer**. i.e. for 1 g of tissue, add 300 µl of buffer to each tube, mix and add 180 µl **2 x stop dye**.

5. Boil both samples for 10' and then place on ice before loading on a gel. Store at –70°C.

Protein concentrations should be approximately 2mgml^{-1} from young leaves but do a Bradford assay if concentration is critical.

Homogenization Buffer (50 ml) (make fresh)

18% sucrose	15 ml 60%
10 mM $MgCl_2$	0.5 ml 1 M
100 mM Tris-HCl, pH 8.0	2.5 ml 2 M
40 mM β-mercaptoethanol	140 µl

Pellet Buffer (10 ml)

2% SDS	2 ml 10%
6% sucrose	1 ml 60%
40 mM β-mercaptoethanol	28 µl

2 X Stop Dye (20ml)

1% SDS	2 ml 10%
0.1% bromophenol blue	2 ml 1%
10 mM EDTA	400 µl 0.5 M
20% Ficoll	4 g