Plant Protein Isolation

Rapid isolation of protein for SDS-PAGE analsyis
(Essentially the same protocol as that described for **GUS Assays**)

A. Method for ~1g or more of tissue.

1. Label all tubes. Prepare solutions and have ready at hand.
2. Remove the tissue from the –80^{o}C freezer and thaw on ice. If the tissue is fresh, keep on ice (or alternatively work in a cold room).
3. Place tissue in a mortar and pestle.
4. Add ~ 2ml of QB per ~1g tissue.
5. Grind tissue until no more chunks are visible.
6. Remove ~1ml of the liquid grindate into a microfuge tube.
7. Place on ice.
8. Rinse mortar and pestle (and any other paraphernalia that came into contact with the sample) to remove all traces of sample and proceed to the protein isolation of the next tissue sample.
9. Spin samples at top speed in the microfuge (4^{o}C for 15+ minutes).
10. Transfer the liquid supernatant into a second (new) microfuge tube.
11. 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.
12. Store samples in the –80^{o}C.

B. Alternative method for small (<1g) quantities of tissue.

1. Working in the fume hood, prepare a pestle by flaming the end of a blue pipette tip and sealing the end by gently smashing it into a microfuge tube. Prepare as many pestles as tissue samples to be isolated.
2. Using the newly created pestle, grind the tissue directly in a microfuge tube.
3. Add ~1ml of QB to the ground tissue, mix and transfer the supernatant to a second microfuge tube.
4. Follow the procedure in A above the rest of the way.

C. Solutions and stuff

A. Solutions

1. QB

Stock	For 100ml	Final []
2M KPO_4 (pH 7.8)	5ml	100mM
0.5M EDTA	200µL	1mM
Triton X-100	1ml	1%
80% Glycerol	12.5ml	10%
dH_2O	81.1ml	

Store RT

DTT (1.0M) 100µL 1mM
(Alternatively directly add 15.4mg DTT per 100ml)

Add DTT immediately before using. Store QB w/DTT at –20°C.

2. 2M KPO_4 (pH 7.8)

Stock	For 200ml	Final []
K_2HPO_4	63.2g	
KH_2PO_4	5.0g	
pH should be	~7.8	
If not, adjust pH to	7.8	
F, a/c		

3. 0.5M EDTA (pH 8.0)

Stock	For 250ml	Final []
EDTA	46.52g	0.5M
H_2O to	250ml	
pH w/ 10N NaOH to	8.0	

(Alternatively use ~5 pellets of NaOH.)
f, a/c.
Store RT.

Note: EDTA will not completely go into solution until the pH approaches 8.0 and the H_2O is almost at final volume. Essentially, the pH needs to be continuously adjusted as the EDTA dissolves.

4. 10N NaOH

Stock	For 250ml	Final []
NaOH	100g	10N
dH_2O to	250ml	

Store at RT in a PLASTIC bottle. (NaOH will react with glass.)

5. 80% Glycerol

Stock	For 100ml	Final []
100% Glycerol	80ml	80%
dH_2O	20ml	
a/c		

6. 1M DTT

Stock	For 10ml	Final []
DTT	1.545g	1M
0.01M NaOAc (pH 5.2)	to 10ml	

Filter sterilize
Aliquot into 1ml portions
Store at –80°C

0.01M NaOAc is 33µL of 3M NaOAc pH~5.2 in 9.67ml dH_2O.)

B. Stuff

1. Mortar and pestle and/or flame seal blue tips.
2. Microfuge tubes, pipette tips.
3. Test Tubes

VI. References

Bradford, M.M. (1976) A dye binding assay for protein. *Anal. Biochem.* **72**:248-254.

QB: Ni, M., Dehesh, K., Tepperman, J.M., and Quail, P.H. (1996) GT-2: In vivo transcriptional activation activity and definition of novel twin DNA binding domains with reciprocal target sequence selectivity. *Plant Cell* **8**:1041-1059.