IN VIVO LABELLING OF PLANT PROTEINS AND IMMUNOPRECIPITATION (This was placed on the electronic Arabidopsis bulletin board; July 1992.)

Janet Braam e-mail address: braam@bioc.rice.edu

* The following is a summary of replies I received regarding in vivo labelling of plant proteins and immunoprecipitation. There are several methods of labelling the proteins, one protocol for cell lysis and protein recovery, and two suggestions for carrying out the immunoprecipitation.

1. LABELLING WITH 35S * Paint the 35S on leaves in a solution of 0.05% Triton X-100, preferably on the underside because of the presence of more stomata. (Chris Sommerville)

* Paint with 35S methionine in a solution of 0.02% Tween20, allow it to be taken up for 4 h. (Sarah Gilmour)

* Cut leaf with sharp razor blade and place in 35S met/cys ("EXPRE" from NEN or TRANS-label from ICN) for 1 h. The label is diluted to 1.4 mCi/ml. This method was found to work better than painting label on leaves, getting 400,000 dpm total from one leaf. (Carrie Schneider)

* Place detached leaves in eppendorf tubes with a short vacuum infiltration (Plant Physiol. 88, 731). Note error in M&M, use 20µl, not 209µl of H2O. (Tim Caspar)

* For labeling whole plants, use 35S sulphate instead of labelled amino acids. Put seedlings just geminated in small puddles of S35 sulphate for 2, 3 or 4 days. (Russell Malmberg)

2. HOMOGENISATION AND IMMUNOPRECIPITATION (i) Freeze tissue in liquid N2. (ii) Homogenize in 2ml of (0.7M sucrose, 0.5M Tris 9.4, 50mM EDTA, 0.1M KCl, 2% mercaptoethanol, 2mM PMSF: from Hurkmans and Tanaka, Plant Physiol. (1986) 81, 802). Use a glass homogenizer. (iii) Add 2ml phenol, shake. Spin to separate and retain phenol phase. (iv) Add 5 volumes of 0.1M NH4OAc in methanol. Precipitate o/n in freezer. Spin out proteins. (v) Wash pellet with 80% acetone and dry. Resuspend in 1% SDS and boil for 1 min. (vi) Add 7 volumes of (50mM Tris 7.8, 1% Triton-X-100), spin out insolubles. (vii) Add antibody to this. Precipitate the antibody with 1% crosslinked Staphlococcus A cells. (viii) Wash the precipitate with (50mM Tris 7.5, 150mM NaCl, 5 mM EDTA). (ix) Resuspend in Laemmli buffer and load gel. (Carrie Schneider).

3. IMMUNOPRECIPITATION Any standard protocol will get you started, but I would urge you to optimise each reagent. We found that we could greatly reduce the amounts of killed Staph A cells, the amounts of rabbit-anti- mouse, and the initial amounts of the specific monoclonal we were using. When you think you have each of these reasonably optimised, do a dose response of the amount of protein in the supernatant and the amount in the pellet as a function of increasing specific antibody. This will give you a dose of your antibody that should work for routine assays. We particularly found that bridge antibodies (such as the rabbit anti-mouse) are particularly important to optimise, since bunnies and other animals usually
contain antibodies that recognize some plant epitopes.

4. IMMUNOPRECIPITATION DOSE RESPONSE CURVE  
   (i) Plant extract

   (ii) 50 mM Hepes

   (iii) Hepes-BSA 50 mM Hepes, 1% BSA

   (iv) Washed pansorbin cells (Calbiochem) Remove 55 ul pansorbin cells Spin down, resuspend in 55 ul Hepes-BSA Repeat 3 times. Let sit on ice at least 1 h.

   (v) Rabbit anti-mouse dilute 10x into Hepes-BSA (need 10 ul)

   (vi) Monoclonal dilute 100x into Hepes-BSA (need 14 ul) dilute 10x into Hepes-BSA (need 13 ul straight, undiluted (need 13 ul)

   (vii) Gently mix the following, then incubate on ice for 60 minutes A B C D E F G H 100x dil 0 1 3 10 0 0 0 0 10x dil 0 0 0 0 3 10 0 0 straight 0 0 0 0 0 3 10 oat extract 25 25 25 25 25 25 25 25 Hepes-BSA 25 24 23 21 19 17 15 13

   (viii) Add 1 ul diluted rabbit anti-mouse to each tube. Gently mix, then incubate on ice for 1 hour. (ix) Add 5 ul Pansorbin to each tube. Gently mix, then incubate on ice for 1 hr. (x) Wash by spinning down and resuspending in 100 ul Hepes-BSA. Repeat four times. (xi)