FIXATION & EMBEDDING PROTOCOLS FOR HISTOLOGY AND IMMUNOCYTOCHEMISTRY OF ARABIDOPSIS INFLORESCENCES

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1. SOLUTIONS

(i) 12% PARAFORMALDEHYDE STOCK

* All manipulations should be performed in a fume hood.

Heat 100 ml of distilled water to 80oC then add 12 g paraformaldehyde - stirring constantly. Most should dissolve. Remove from heat and add dilute (approx 2%) sodium hydroxide dropwise until all the paraformaldehyde has dissolved. Cool on ice. Bring the pH back down to 7.0 using H_2SO_4

(ii) 0.2M PHOSPHATE BUFFER STOCK

A: Dissolve 3.12 g of sodium dihydrogen phosphate in 100 ml distilled water.
B: Dissolve 6.162 g of disodium hydrogen phosphate in 100 ml distilled water.
Mix 39 ml of A and 61 ml of B to obtain 0.2M stock solution pH 7.0.

(iii) SUBBED SLIDES

* All manipulations should be performed in a fume hood.

Wash slides in detergent, rinse thoroughly and completly dry. Dip slides for 10 sec in a 2% solution of aminopropyltriethoxysilane (TESPA) in acetone. Rinse twice in 100% acetone then rinse in distilled water. Air dry in a dust free environment.

* Slides may be stored for several months.

2. FIXATION

(i) Excise Arabidopsis inflorescences and immediately place into a freshly prepared solution containing 3% paraformaldehyde and 1.25% gluteraldehyde in 0.05M phosphate buffer, pH 7.0. Fix o/n at r.t. with gentle agitation using a 45o rotating platform at 2rpm.

* There should be at least 10 vol fixative relative to the amount of tissue. Each infloresence contains air, trapped inside the tissue and around the trichromes, causing it to float in the solution. An overnight fixation protocol was used to ensure complete penetration of fixative. In our experience, vacuum infiltration (using shorter infiltration times) gives poorer morphological preservation. Post fixation in osmium should be avoided as this can prevent polymerisation of L.R. White resin.

3. DEHYDRATION

(i) Dehydrate for 60 min in each of the following percentages of ethanol: 12, 25, 50, 75, 95, 100, 100%. Dehydrate at r.t. with gentle agitation and change the solution every 30 min.

* Ethanol dried using molecular sieves should be avoided as tiny, hard particles from the beads can enter the tissue preventing good tissue sectioning.

4. EMBEDDING IN L.R. WHITE

* When performing histochemical or immunological studies substrate retention and antigenicity may be improved by shortening dehydration times and infiltrating in L.R. White from 70% ethanol.

(i) Infiltrate tissues o/n at r.t. in a 1:1 mixture of 100% ethanol:L.R. White (medium grade) with gentle agitation.
(ii) Infiltrate tissues in 100% L.R. White for 36 h at r.t. with gentle agitation changing the solution twice daily.
* Always allow the L.R. White to equilibrate to r.t. before using to prevent the absorption of atmospheric water.

(iii) Embed inflorescences in polypropylene embedding capsules using fresh L.R. White and polymerise overnight in a 60oC oven.
* Use only polypropylene or gelatin embedding capsules and completely fill with resin as the presence of air prevents polymerisation. Polymerising at lower temperatures (55-58oC) may improve tissue permeability. L.R. White may be polymerised at r.t. using chemical accelerators though their use is not recommended for histochemical or immunological studies.

5. EMBEDDING IN WAX
(i) Infiltrate tissues o/n in a 1:1 mixture of 100% ethanol:Histoclear with gentle agitation.
(ii) Infiltrate tissues in 100% Histoclear for 24 h with gentle agitation changing the solution twice daily.
* There are alternative reagents available such as 1,1,1, trichloroethane (Inhibisol), xylene and chloroform. However, Histoclear circumvents the safety hazards associated with others.

(iii) Infiltrate tissues in a 1:1 mixture of Histoclear:wax at 56oC overnight.
(iv) Infiltrate tissues in 100% wax at 56oC for 36 h changing the solution twice daily.
(v) Embed in fresh wax.
* The high temperature and long incubation period necessary during the wax infiltration steps causes loss of much enzyme activity. In our experience, this protocol gave poor histochemical results. A variety of low melting point waxes are now available which may reduce this problem. We found the sectioning quality of Paraplast plus to be superior, ribbons of 2 um sections are possible if wax blocks are first cooled on ice.

6. EMBEDDING IN PEG 1000
* PEG 1000, polyethylene glycol 1000, is a polymer of average molecular weight 950-1050.

(i) Infiltrate tissues o/n in 1:1 mixture of dry ethanol:PEG 1000 at 42oC
(ii) Infiltrate tissues in PEG 1000 for 48 h at 42oC changing the solution twice daily.
(iii) Embed in fresh PEG 1000 in suitably sized moulds.

* We found PEG 1000 unsuitable for gross morphological studies as various organs within the inflorescence became mobile when sections were floated onto water on the glass slides. The solubility and low melting point of PEG 1000 also makes sectioning difficult. Polypropylene beakers should be avoided when using PEG 1000.

7. NOTES
* Block orientation: use pencil if labels inside block. Use syringe & filter. Knives must be sharp. Care on temp of hot plate when drying down. Care on temp of slide/section when staining with tol blue. Allow dpx to set hard before viewing slides.