

# FIXATION OF TISSUE FOR PARAFFIN EMBEDDING

## For general histological staining

1. Cut 2 - 3 mm leaf sections with a razor blade.
2. Fix in 3:1 ethanol:acetic acid (a few ml in a glass vial) for 30' at room temperature. Make fixative fresh each week.
3. Remove fixative and add 2 ml 70 % ethanol. Leave for 30' at room temperature.
4. Replace 70 % ethanol with fresh 70% ethanol and store indefinitely at 4°C.

## NOTES.

If fixing tissue that is tougher than leaves use **FAA** as a fixative. Overnight fixation at room temperature is usually adequate but samples can be left for longer. Transfer to 50% then 70% ethanol prior to storage at 4°C.

## FAA

50 ml ethanol  
5 ml acetic acid  
10 ml 37% formaldehyde  
35 ml H<sub>2</sub>O

## For in situ hybridization or immunolocalization

1. Make fixative fresh each time from paraformaldehyde powder.

Fixative: 4% paraformaldehyde  
0.1% Tween 20  
0.1% Triton X-100

Add solid to PBS (0.15 M NaCl, 10 mM sodium phosphate pH 7.5). Stir in fumehood at 60°C to dissolve. Will need to add NaOH pellets to get solid into solution and then re-pH with HCl. Chill on ice.

2. Cut 2 - 3 mm leaf sections with a razor blade and place in fixative. Most plant tissues have a cuticle and will thus simply float on the surface of the fixative. To allow penetration of the fixative, the tissue must be vacuum infiltrated. To do this, sometimes you have to weigh down the tissue under the surface, eg. by using a piece of wire gauze, though the use of detergents often avoids this problem. Place sample in a vacuum desiccator and apply a vacuum using an oil pump (a bench type water pump does not provide adequate suction). Care should be taken to ensure the solution does not boil under the reduced pressure. Release vacuum slowly to avoid tissue disruption. Formaldehyde vapour is volatile, so the fixative must be replaced after the vacuum treatment.
3. Fix tissue overnight at 4°C.
4. Prior to storage, transfer through 30%, 40%, 50%, 60% and 70% ethanol for an hour each at room temperature.
5. Store in 70% ethanol at 4°C