

Protocol for JB - 4 embedding plastic

- 1.) Fixation of tissue. The structure of plant and animal tissue can be observed microscopically in a variety of ways, some of which include living specimens. However, for many applications, samples are best-studied using material that has been killed. This approach requires a technique that limits the amount of damage that normally occurs in cells after they die. The process of fixation refers to any method designed to preserve the original structure of a tissue after cell death.

There are a number of different fixatives that can be used for plants and they typically have different applications that depend on the structure you are seeking to preserve. Some fixatives are alcohol-based and others are aqueous. The advantage of alcohol fixatives includes rapid penetration into a tissue sample; aqueous fixatives penetrate slowly and are thus generally most useful for tiny samples. A disadvantage of alcohol fixatives is that there tends to be a greater chance of distortion of certain features due to shrinkage. As an example, the protoplast is prone to collapse within the cell walls of plant cells due to dehydration. In some applications, this is a problem. In other cases, it is relatively unimportant.

For field-based studies, a commonly used fixative is FAA, which is formalin:glacial acetic acid:50% ethanol in a ratio of 5:5:90. You can also add 1-2 drops of Triton X detergent to improve fixative penetration in tissues that are especially hydrophobic. This fixative is practical because tissues can be stored for long periods of time in the fixative with no ill effects. Sometimes, tissue collection in the field makes it impossible to immediately process samples further. The wetting properties of ethanol can also be a benefit when the air that often occurs within samples cannot be removed by aspiration in the field (aspiration involves applying a low vacuum to the sample to remove air and thereby draw fixative completely into the material).

When indoors, work in a hood when handling FAA. Material fixed for at least 24 h in FAA with 50% ethanol should be transferred to 50% ethanol overnight. Waste FAA and the first 50% ethanol wash go into a waste jar in the hood. It is best to label the sample vials and not their caps – caps can get mixed up if you remove more than one at a time. Once you have removed the fixative with an ethanol wash, you are ready to prepare the samples for plastic embedding.

Note: the times given below are generally the shortest interval recommended. Any step can be allowed to go longer (e.g., materials fixed in FAA can stay in fixative indefinitely.)

- 2.) Dehydration. Even if an aqueous fixative has been used, samples must ultimately be dehydrated to embed them in plastic. Dehydrate tissues through a graded ethanol series (50% - 70% - 85% - 95% - 100% - 100%), 8-12h per step is optimum but can be less. An absolute alcohol step is not required, but it removes excess moisture that tends to make the final plastic too soft. Don't lose small pieces of tissue as the ethanols are decanted off! Once you are past the FAA-containing steps, the waste ethanol decanted/pipetted off in each step can go into a waste beaker. This helps to prevent accidentally losing small samples.

3.) Infiltration. Preparation of the infiltration solution (I generally make 25ml at a time):

In a small disposable plastic beaker, combine:

- 25ml of component 'A' (Large brown bottle stored in fridge, let it warm up) first)
- 0.24g of catalyst (White powder in plastic bag in fridge)

It takes a while for the catalyst to dissolve, so the solution should be set up with a magnetic stirrer for at least 20-30 minutes.

Decant off the 100% ethanol and replace with plastic infiltration solution. Samples saturated in ethanol will generally float in the plastic at first. The alcohol that remains in the tissue will gradually evaporate off and the samples will sink. Wait until they sink before putting them on the tissue rotator (waiting overnight is fine). Floating samples placed immediately on the rotator will sometimes crawl up the sides of the vial and dry out. That's not good.

Place the vials on the tissue rotator and allow infiltration to proceed for about three days. Again, you can let the tissue stay in the infiltration solution longer if scheduling requires it. Longer times are sometimes needed for recalcitrant tissues anyway.

4.) Embedding. The plastic embedding media is prepared in a small (~20ml) disposable plastic beaker agitated with a magnetic stir bar, and consists of :

- 5ml of the infiltration solution, as prepared in step #3 above
- 8 drops of solution 'B' (which is a polymerization accelerator) from a Pasteur pipette

To embed:

Place a gray plastic 35mm film canister cap into a glass petri dish and view under a dissecting scope. The petri dish keeps the microscope stage clean in the event that plastic embedding materials get spilled and it makes it easier to move the tissue in liquid plastic around. Add enough infiltration plastic from the vial containing your samples to the film cap to just cover the tissue. Then transfer as many of the tissue samples to the cap as will fit. After 5 ml of embedding plastic has been prepared and accelerator has been stirred into solution for about 1 minute, remove infiltration plastic from around samples in the film cap with a Pasteur pipette. Cover the tissue samples with embedding plastic. With the aid of a dissecting scope, periodically try to suspend tissue in the liquid plastic by lifting it with pins. When plastic begins to solidify at the bottom (material will no longer sink to the bottom of the film cap), add more embedding plastic to the cap so it is completely full. Then cover the whole film cap with a small, square piece of parafilm and put the petri dish containing the samples into the fridge. **NOW REMOVE THE STIR BAR FROM THE DISPOSABLE BEAKER BEFORE YOU EMBED THAT TOO!** The plastic should be solid in about an hour. After polymerization is complete, plastic can be stored in a low heat (30C) oven to drive off any moisture in the material.

Now the fun really begins.