Dimethoxypropane Dehydration of Plant Tissue

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Abstract

Dimethoxypropane (DMP) is an organic compound that reacts with water under acidified conditions to form methanol and acetone. The compound can also be used in place of an ethanol series to dehydrate formalin-fixed plant tissues for serial sectioning.

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Before start

Prepare and fix the tissue according to your normal lab protocol. I have been using fixation in FAA under a vacuum followed by a wash with 50% ethanol, but fixation in ethanol:acetic acid also works. I have done all of these steps in 25mL glass scintiallation vials. Additionally, to keep track of samples through all the solution changes, we place a small paper label (written in pencil) in the vial with the samples.

Materials

- Acetone by Contributed by users
- Histoclear HS2001GLL by Contributed by users
- Paraplast 39501006 by Contributed by users
- 37-% Hydrochloric acid 1.00317.1000 by Merck Millipore
- 2,2-dimethoxypropane AC115630010 by Fisher Scientific

Protocol

Prepare DMP

Step 1.

Prepare an acidified solution of DMP. You will need roughly 6 times the volume of your tissue to be fixed.

Component	Volume

DMP	100mL
conc. HCl	1 drop

This solution can scale linearly.

Dehydrate

Step 2.

Pour off the fixative or ethanol, and for every volume of tissue to be fixed, add 3 volumes of acidified DMP solution. Incubate this at room temperature for 15-30 minutes with occasional gently shaking.

DURATION

00:30:00 : Incubation

Dehydrate

Step 3.

Pour off the reacted DMP solution and replace with the same volume of fresh DMP solution for another dehydration step. Incubate again at room temperature for 15-30 minutes with occasional gently shaking.

DURATION

00:30:00 : Incubation

Wash

Step 4.

Pour off the reacted DMP solution, and replace with an equal volume of extra-dry acetone. This helps to wash away any methanol that was produced in the dehydration reaction. Over the course of 15 minutes pour off and replace the extra-dry acetone 1-2 additional times.

Transfer to paraffin

Step 5.

Pour off the acetone and replace with a 1:1 solution of acetone and Histoclear. Incubate at room temp for several hours.

Citrisolv also subsitutes fine for Histoclear, but I have not tried this with xylene. The 1:1 ratio is very approximate and can be made as you pour them into the vial with the tissue.

Transfer to paraffin

Step 6.

Pour off the Histoclear:acetone mixture and replace with enough pure Histoclear to just barely cover the tissue. Fill the rest of your scintillation vial with paraffin chips and place in a 60º over overnight.

Transfer to paraffin

Step 7.

The next morning, replace the paraffin:Histoclear mixture with melted paraffin. Over the next two days chaange the paraffin 2-3 times per day until you are ready to make molds/blocks for sectioning.

Warnings

DMP is a lung and skin irritant and should be used in a fume hood. Concentrated HCl is very caustic and proper skin and eye protection shuld be used. The dehydration reaction is slightly endothermic.