Plant Tissue Fixation

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Abstract

This protocol is to fix freshly collected plant tissue for serial sectioning. Although this protocol is similar to ones used for *in situ* mRNA hybridization, it should not be used for that purpose as it is not nearly careful/gently enough. This protocol has been tested with tomato, tobacco, and date fruits with good results.

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Guidelines

Tissue collected for this procedure should be small in order to inflitrate well. Samples approaching 1cm^3 may need to be cut apart or scored in order to let the fixative penatrate well. Plant organs with a continuous and/or waterproof membrane, like small fruits, should also be scored.

Before start

Label tubes using an ultrapermanent marker with the sample information (e.g. “3 DPA, Plant #1”), or alternatively, write a label with pencil and paper (not pen) and place it in the container with the sample.

Materials

Acetic acid, glacial 537020 by Sigma Aldrich

Ethanol by Contributed by users

Formaldehyde 37% Solution C5300-1.SIZE.1L by Bio Basic Inc.

Protocol

Prepare Fixative

Step 1.

Many different fixative recipes are possible. This is the most common. It can be made with either 100% ethanol or (more cheaply) 95% ethanol. This recipe can be scaled up or down proportionally. Prepared fixative can be store for many months, and can even be reused.

	(mL) with 100% EtOH	(mL) with 95% EtOH
Ethyl alcohol (100%)	50	53
Glacial acetic acid	5	5
Formaldehyde (37-40%)	10	10
Distilled H2O	35	32

Fix Tissue

Step 2.

Fill each sample tube with about 2 volumes of FAA. For example, if the tissue is $1cm^3$, add 2 mL of FAA. If the tissue is not being fixed for *in situs*, previously used FAA can be used.

Fix Tissue

Step 3.

Open the tubes and place them in a vacuum chamber in the fume hood. Seal the chamber and pull a vacuum to about -0.08 mPa. During this step, watch the samples in the fixative. They may start to create small bubbles as the air escapes them; this is normal. If you pull a vacuum too fast, the fixative will bubble rapidly, like it's boiling (it is). This is bad and means that you need to slow down the vacuum or potentially release some of the vacuum to stop the boiling.

Fix Tissue

Step 4.

Seal the chamber by turning the bleeder valve straight, and turn off the pump.

Fix Tissue

Step 5.

Allow the samples to infiltrate under the vacuum for at least 1 hour. Tissue larger than $1cm^3$ may need longer under vacuum.

DURATION

01:00:00 : infiltrate under vacuum

Fix Tissue

Step 6.

At the end of the hour, open the bleeder valve to allow the vacuum to dissipate slowly. This process should take about 1-2 minutes. Do not go too fast or you will damage the tissues. The color may have been lost from the tissues, this is also normal.

Fix Tissue

Step 7.

Once the chamber is at equilibrium, open it up and close all of the tubes.

Fix Tissue

Step 8.

Arrange the sealed tubes in a rack in the fume hood and allow them to soak in the fixative overnight.

Fix Tissue

Step 9.

In the morning, decant the used fixative into a hazardous waste container, and replace it with a solution of 50% ethanol.

Fix Tissue

Step 10.

The samples can be stored in this solution until needed later.

Warnings

The fixative is both an irritant and produces hazardous fumes. It should be handled in a fume hood and with gloves.