**Microtome sectioning sample preparation protocol using Technovit 7100**

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**Fixation**

Chemicals & Tools

Fixative: FAA (Formalin: Acetic acid: 50% Ethanol = 5: 5: 90)

Vacuum pump connect to desiccator

Small glass vial with cap (~ 10 mL, depends on sample size)

Protocol

1. Prepare small glass vials filled with FAA, the fixative amount must > 10X samples volume
2. Cut samples into proper size (< 1 cm2) and put into fixative immediately after collecting.
3. Put the sample glass vials in desiccator, loose the caps, and pump out the air bubbles from plant tissue. Adjust the strength of vacuum to prevent breaking tissues.
4. Slowly release the vacuum status and shake the vials to see if the samples sink to the bottom. If not, repeat step 3 until sample sink to the bottom.
5. Change to fresh FAA. If the samples are processed in the next day, put on shaker overnight in room temperature. For long term storage, store in 4 °C.

**Dehydration**

Chemicals

1% Safranin O in 50% Ethanol

50%, 70%, 80%, 90%, 95%, 100% Ethanol

Protocol

1. If the samples are not in the right size or orientation, move stored samples to petri dish with 50% ethanol. Cut the samples in 50% ethanol.
2. Transfer samples to glass sample vials with 50% ethanol. Add few drops of 1% Safranin O to stain the samples. (Samples might lose color during the dehydration, the staining help us visualize the samples.)
3. Serial dehydration (all on shaker):

50% ethanol (+ safranin O) 30min to 4 hours

70% ethanol 30min to 4 hours (can be few days)

80% ethanol 30min to 4 hours

90% ethanol 30min to 4 hours

95% ethanol 30min to 4 hours

100% ethanol 30min to 4 hours x 2 times

**Filtration**

Chemicals

“Technovit 7100”: 100mL Technovit 7100 + 1 g hardener I (1 pack)

Filtration solution prep ratio:

|  |  |  |
| --- | --- | --- |
| Prep | 100% Ethanol | “Technovit 7100” |
| I | 5 | 1 |
| II | 3 | 1 |
| III | 2 | 3 |
| IV | 1 | 5 |

\*\*Filtration solution can be reused. Keep the used solution in another bottle and filter them before use.

Protocol

Serial filtration in room temperature, wait samples sink to bottom before put on shaker. If samples do not sink, apply vacuum pump as needed.

Technovit 7100 Prep I 6-12 hours

Technovit 7100 Prep II 6-12 hours

Technovit 7100 Prep III 6-12 hours

Technovit 7100 Prep IV 6-12 hours

“Technovit 7100” 6-12 hours

“Technovit 7100” 12-24 hours (Fresh solution only, not reused)

\*\*If longer than 24 hours, put in 4 °C to prevent polymerization.

**Embedding**

Chemical & tools

“Technovit 7100”

Technovit 7100 hardener II (solid in 4 °C, return to room temperature before use)

Polyethylene glycol 400 (PEG 400)

HistoForm S

Beaker (50 or 100 mL)

Tweezers

Protocol

1. Prepare embedding medium in a beaker:

15 mL “Technovit 7100”

1 mL Technovit 7100 hardernell II

0.6 mL PEG 400 (add when the block is too hard to cut using disposable knife)

1. Fill each block of HistoForm S with the embedding medium using micropipette (P1000)
2. Transfer each sample to each block with tweezers. Be careful not to hurt the tissue. Use toothpicks or pipette tips to instead. Do not transfer too much filtration solution.
3. Wait samples sink to bottom, and change the orientation of the samples.
4. Put the HistoForm S horizontally in 4 °C, overnight (~ 1 day) for polymerization
5. Move to room temperature for 1 -2 hours.

**Sample block**

Chemical & tools

Technovit 3400

HistoBlock

Disposable paper cup

Disposable coffee stirrer or chopstick

Flat head screwdriver

Protocol

1. Move HistoForm S to fume hood
2. Put HistoBlock to fit each block of HistoForm S.
3. Quickly mix Technovit 3040 (yellow power) and Universal liquid (3:1) to disposable cup using disposable coffee stirrer or chopstick.
4. Pour the mixture into the back of each HistoBlock. Do not overfill.
5. The mixture will solidify within 5 minutes. Wait 20 minutes longer ensure it is totally solid.
6. Use flat head screwdriver to take out the sample blocks from HistoForm S.

**Trimming**

Tools

Hot plate

Razor knife

Protocol

1. Set up hot plate at 80°C
2. Let the top surface of sample block contact the hot plate to soften the sample block
3. Trim the softened sample block under microscope.
4. Dry the sample block in 60°C incubator to remove additional water from air.

**Re-embedding**

When the orientation of the samples are not ideal for sectioning, re-embedding is required for re-orientation.

1. Trim the sample block to desired orientation after softened on hot plate.
2. Clean the trimmed block by ethanol, and dry.
3. Put the trimmed sample block into HisoForm S in desired orientation to re-embed.
4. Fill the fresh embedding medium, stay in room temperature for 1-2 hours
5. Use Technovit 3400 to make new sample block as described above.
6. Trim the block before sectioning.