

Array tomography protocol: tissue fixation, dehydration and embedding

Tissues are fixed, dehydrated and embedded in preparation for array tomography. Find out how to complete the process.

Overview

Tissue fixation immobilizes antigens while preserving molecular architecture. Once fixed, tissue is dehydrated using ethanol and the water is replaced with an acrylic resin suitable for ultra-thin sectioning and repeated immunostaining.

Fixation

Tissue can be processed on the bench or using a microwave. The fixative should be prepared on the same day and kept at room temperature.

1. Dissect out the brain and put into a 35 mm Petri dish containing fixative solution.
2. Isolate the region of interest.
3. Transfer the tissue into a 20 ml scintillation vial with fixative solution.

If using a microwave, use approximately 1 ml of fixative per vial, or just enough to cover the tissue. Excessive volume will cause overheating.

4. Microwave

- I. Microwave for 1 min at 100–150 W, stand for 1 min then microwave again for 1 min at 100–150 W.

After microwaving check the glass vial for overheating. If solutions are getting too warm (>37°C), decrease the amount of liquid added.

- II. Microwave three times using a cycle of 20 seconds on - 20 seconds off - 20 seconds on at 350-400 W.
- III. Incubate the tissue in fixative at room temperature for 2–3 h.

5. Bench

- I. If a microwave is not available, fix first at room temperature for 2–3 h and then leave overnight at 4°C. The volume of fixative isn't important for bench top processing, provided it covers the tissue.

Dehydration and embedding

Tissue can be processed on the bench or using a microwave.

1. Wash the tissue twice in wash buffer for 5 min each at 4°C.
2. Transfer the tissue into a 100 mm Petri dish, cover with wash buffer and dissect into smaller pieces (<1 mm in at least one dimension).

3. Return the tissue to the 20 ml scintillation vial and wash twice in wash buffer, for 15 minutes each at 4°C.

4. Microwave

- I. Replace the wash buffer with 50% ethanol at 4°C and microwave samples for 45 s at 350 W.

If using a microwave, use approximately 1 ml of liquid per vial, or just enough to cover the tissue. Excessive liquid volume will cause overheating.

- II. Replace the 50% ethanol with 70% ethanol at 4°C and microwave for 45 s at 350 W.

Stop here if processing samples with GFP or other fluorescent proteins (see below).

- III. Replace the 70% ethanol with 95% ethanol at 4°C and microwave for 45 s at 350 W.

- IV. Replace the 95% ethanol with 100% ethanol at 4°C and microwave for 45 s at 350 W.

- V. Replace the 100% ethanol with 100% ethanol:LR White resin (1:1 mixture) at 4°C . Microwave for 45 s at 350 W.

- VI. Replace the 100% ethanol:LR White resin (1:1 mixture) with 100% LR White resin at 4°C and microwave for 45 s at 350 W. Repeat twice more with fresh resin.

For preservation of GFP fluorescence omit the 95% and 100% ethanol steps. Instead, add 70% ethanol again and then place samples into a mixture of 70% ethanol:LR White resin (1:3; if it turns cloudy add one to two extra drops of LR White resin) and microwave for 45 s at 350W. Replace with 100% LR White resin and microwave for 45 s at 350 W; repeat this twice more.

5. Bench

- I. If a microwave isn't available, perform steps 4.1 to 4.6 by incubating in the indicated solutions for 5 minutes at 4°C.

6. Replace with fresh 100% LR White resin and leave overnight at 4°C.

At this point, if needed, the sample can be left for several days at 4°C in LR White resin.

7. Using a fine paintbrush, place tissue pieces at the bottom of gelatin capsules (paper labels can also be added inside the capsule, Figure 1). Fill to the rim with 100% LR White resin.

8. Close the capsules securely and place into a capsule mold.

Oxygen inhibits LR White resin polymerization, which is why gelatin capsules that completely exclude air are used. The little bubble of air that will remain at the top of the capsule will not interfere with the polymerization.

9. Put capsules into an oven at approximately 53°C for 24 h.

If the oven temperature falls below 50°C, the resin may not polymerize. It is better to set the oven temperature just above 50°C (~53°C) to ensure consistent polymerization.



Figure 1: Gel capsules with paper labels in a gel capsule mold.

Materials and reagents

- Dissection equipment
- 0.02 M PBS (2x)
- 8% Paraformaldehyde (EMS, 157-8)
- Sucrose
- Glycine
- Ethanol (200 proof/absolute)
- LR white resin (hard grade, SPI supplies, 2645)
- Petri dishes (35 mm and 100 mm)
- 20 ml glass scintillation vials
- Gelatin capsules, size 00 (EMS, 70010)
- Capsule holder (EMS, 70161)
- Oven (set at ~53°C)

Optional: PELCO 3451 laboratory microwave system with a ColdSpot set at 12°C (Ted Pella)

Preparation

Fixative: (prepare the same day, keep at room temperature): 4% paraformaldehyde, 2.5% sucrose in 0.01 M PBS.

4 ml: 2 ml 8% paraformaldehyde
2 ml 0.02 M PBS (2x)
0.1 g sucrose

Wash buffer: 3.5% sucrose and 50 mM glycine in 0.01 M PBS, use at 4°C (can be prepared in advance and stored at 4°C for up to one month; discard if appears cloudy).

50 ml: 25 mL 0.02 M PBS (2x)
25 mL H₂O
1.75 g sucrose
187.5 mg glycine

Ethanol (keep at 4°C):

50% ethanol
70% ethanol
95% ethanol