



Fixing and Embedding Magnetically Levitated Cultures in Paraffin

The following outline is to be used to fix and embed magnetically levitated cultures from 35 mm dishes, 6-well, and 24-well plates. The timings and solutions for these steps are recommended and can vary with specific antigens; use your group's protocols based on the experimental parameters.

Materials Needed:

- Fixative (4% paraformaldehyde, 10% formalin)
- Saline buffer (phosphate buffered saline, tris buffered saline)
- Small glass beaker (< 50 mL)
- Magnetic concentrator
- Xylene and ethanol solutions for dehydration
- Multiwell plate/dish with magnetically levitated culture
- MagPen

Protocol

1. Place the plate of magnetically levitated cultures on the magnetic concentrator to bring 3D culture down and hold it (**This step is important throughout the protocol to hold the 3D culture while removing liquids**)
2. Remove media and add fixative solution. Fix for at least 5 h
3. After 5 h, move 3D culture from the multiwell plate to a small beaker with a MagPen
 - a. Remove plate off magnetic concentrator
 - b. Assemble MagPen and collect 3D culture on Teflon
 - c. Take out the magnet from the MagPen, leaving the 3D culture still on the Teflon
 - d. Place beaker on concentrator
 - e. Lower 3D culture into beaker, using the concentrator to pull 3D culture off the MagPen

4. Leaving the beaker on the magnetic concentrator to hold the 3D culture, add and remove the following solutions to dehydrate the tissue
 - a. 50% ethanol - 15 min
 - b. 70% ethanol - 15 min (can leave overnight on this step)
 - c. 80% ethanol - 15 min
 - d. 90% ethanol - 15 min
 - e. 100% ethanol - 15 min
 - f. 50% ethanol/50% xylene - 15 min
 - g. 100% xylene - 15 min
 - h. Paraffin - 2 h. Keep beaker warm at 60°C to allow for paraffin to permeate through the sample
5. Remove as much paraffin as possible, while keeping 3D culture held down with magnetic concentrator
6. Use MagPen to transfer the 3D culture from the beaker to a mold. If 3D culture floats, use the magnetic concentrator to hold the 3D culture down while adding paraffin
7. Add paraffin to fill mold and embedding cassette, let the block set overnight
8. Section and stain according to standard procedures