

Histology for Frogs

Fixation

Two different fixes can be used:

1) Romeis Fixative. This is far superior for preserving cell layers at late stages

- 25 ml saturated mercuric chloride
- 20 ml 5% trichloroacetic acid
- 15 ml 37% formaldehyde

- 1-3 hrs RT then transfer to 100% ETOH and change 2 times.

2) PFA + glutaraldehyde. This is adequate for most things.

- 2% PFA
- 1% glutaraldehyde
- in PBS

- 5 hrs room temp. or overnight 4C, wash in PBS then dehydrate in series of ETOH.

Plastic Embedding

- JB-4 embedding kit (www.polysciences.com, datasheet #123)
- Make up infiltration solution just before use. Keep this in the dark
- Remove all buffer or ETOH and replace with infiltration solution.
- Do 2 changes and leave overnight at 4C.
- Do one more change the next day.

- Make up embedding solution according to manufacturers instructions just before use.
- Put embryos into molds and remove as much infiltration solution as possible
- Add embedding solution.
- Orient embryos
- Surround molds with saran wrap with as little airspace as possible
- Surround in aluminum foil
- Put at 4C overnight.

- The next day, remove from molds and leave overnight on paper towels to drain goopy stuff.

Sectioning

- Use a glass knife and microtome to make 7-10 um sections.
- Put sections onto slides covered with an ammonium hydroxide and water solution (1 ml ammonium hydroxide in 500 ml tap water)
- Use microscope and forceps to arrange and flatten each section (if necessary)
- Let dry over night
- Stain as desired

Toluidine Blue Stain:

- Use a 1% Toluidine Blue solution
- dip slide in solution 1-2 min
- quick dip in 70% ETOH
- wash in water 3X
- dry on hotplate
- cover in permount and coverslip.