

Overview of Material and Supplies Required for LM/EM Histology

I. Animal Processing

A. Anesthesia

-Chloral hydrate (the best for perfusions) 0.4 mg/g IP or Sommitol (sodium pentobarbital) 0.01 ml/50g or whatever is required to be used for deep anesthesia by your local Animal Care Committee

B. Small operating board with restraints and an under tray for collecting fluids

C. Sterile gauze, razor blades (tall single edge [industrial type, not the ones for shaving]; some double edge blades [thin one like type used for shaving])

D. Surgical instruments including medium scissors, scissors capable of cutting bone (vertebrae and ribs), fine and very fine scissors and tweezers, hemostats, blunted perfusion needle

E. Ansell Perry X-AM brand powdered Latex gloves (medium)

F. Red wax sheets

G. Screw-top disposable glass specimen vials (large PP scintillation vials will do)

II. Perfusion – must be done in a fume hood

A. Some kind of 1 L perfusion bottle with tubing

B. 250 or 500 mL bags of sterile lactated Ringer's solution, injectable USP

C. Veni-pac or equivalent (e.g., Baxter "Continu-Flo Solution Set JC5380 10 drop)

D. Absorbent underpads with PE backing (also called absorbent bench protectors)

E. Some kind of apparatus to suspend perfusion bottle and saline bag above bench

F. Rotator for post perfusion immersion fixation step (2-4 h at 4°C)

III. Fixatives for perfusion or immersion

A. High quality LM/EM: 5% glutaraldehyde in 0.8 M sodium cacodylate buffer + 0.05% calcium chloride, pH 7.2-7.4

B. Good quality LM/EM: 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer + 0.05% calcium chloride, pH 7.2-7.4

C. Good quality LM immunohistochemistry: 4% paraformaldehyde + 0.1% glutaraldehyde in 0.08 M sodium cacodylate buffer + 0.05% calcium chloride, pH 7.2-7.4. The glutaraldehyde can be raised to as much as 0.5% for some antigens while for others it must NOT be used at all (trial and error)

D. Rodent tissues up to birth can be fixed solely by immersion preferably with enhancement by microwaving (e.g., Laboux et al. 2004, J. Histochem. Cytochem. 52:1267-1275)

IV. Washing Buffer

0.1 M sodium cacodylate buffer supplemented with 4-7% sucrose, pH 7.4 or any preferred washing buffer of choice that is near isotonic.

V. Decalcification

A. Routine, high quality LM/EM: 4.13% disodium EDTA, pH 7.2-7.4 at 4°C. Need 1 L every 2 days for 2-3 weeks (41.3 g x 3.5/week = ~145 g per week).

B. Hurry up, acceptable quality LM/EM: acidic aluminum chloride at 4°C (I sent you the components and protocol for this already).

- C. Gauze and string for making bags to suspend samples in decalcifying solution
- D. Stirring plate and bar + refrigerator or cold room to do decalcification
- E. It is important to use as large a fluid-to-tissue ratio as possible (e.g., no more than pooled jaws or bones from 5 animals per L) and to change the solution frequently (e.g., every two days)

VI. Post Processing

- A. For plastic embedding in Epon (e.g. EMBED-812 from EMSciences)
 - 1. Post-fixation with 1% osmium tetroxide in dH₂O (“oxidized osmium”) or equal parts of 3% potassium ferrocyanide + 2% osmium tetroxide in dH₂O (“reduced osmium”) WARNING: very toxic; requires special handling and disposal procedures
 - 2. For Epon embedding – acetone or alcohol + propylene oxide for dehydration + Epon embedding Kit
 - 3. “BEEM” capsules and/or molds for embedding tissues
 - 4. Rotator for specimen vials for dehydration and infiltration steps
 - 5. Vacuum apparatus for final infiltration step
 - 6. Oven for polymerizing plastic at 60°C
- B. For plastic embedding in methyl methacrylate (I have no experience with this; see Laboux et al. 2004 for details)
 - 1. Ethanol and xylene
 - 2. Methyl methacrylate kit (e.g., Technovit® 9100 from EMSciences or MMA from J-T Baker; Phillipsburg, NJ) NOTE: this plastic later can be removed from tissue sections
 - 3. UV lamp (long wave)
 - 4. Freezer or refrigerator for polymerization
- C. For paraffin embedding
 - 1. Ethanol and xylene
 - 2. Paraffin pellets and heating bath
 - 3. Heated vacuum apparatus
 - 3. Embedding molds

VII. Sectioning: specialized for each type of embedding material and goal of study. The type of microtome required for paraffin sectioning (“microtome”) is different from the type required for plastic sectioning (“ultramicrotome”). There are also frozen sections cut on a type of microtome called a cryostat (LM) or ultrafreezing microtome (EM). These machines individually are all outrageously expensive.

A. Paraffin: carbon steel or disposable blades, water bath, hotplate, oven, glass slides, slide boxes. Quick stain: 0.1% toluidine blue diluted 1:4 in dH₂O

B: Epon/Methacrylate: tempered glass for making knives, “Histoknife” (for thick sectioning at 0.25-2 μm, single and serial), ultra diamond knife (for best thin sections), hotplate, glass slides, slide boxes, EM grids, grid holders, uranyl acetate and lead citrate for staining thin sections on grids, 1% toluidine blue + 1% sodium borate for staining 1 μm thick orientation sections mounted on glass slides.

C. Coverslips (No. 1) and mounting media (e.g., Eukitt from EMSciences).