Preparation of Adherent Macrophages for Electron Microscopy

This procedure is from:
Victoria Madden, Research Analyst II
Microscopy Services Laboratory
Department of Pathology and Laboratory Medicine
CB# 7525 Brinkhous-Bullitt Bldg.
University of North Carolina at Chapel Hill
Chapel Hill, N.C. 27599-7525
Phone: (919) 966-2413
E-mail: vmadden@med.unc.edu

Supplies are from EMS: http://www.emsdiasum.com; (800) 523-5874

Culturing and Preparing Cells for Shipping:

1. Macrophages need to be grown in wells that are chemically resistant. Nunc Permanox is more resistant to epoxy resin than standard polystyrene and allows for better separation of the embedded cells from the slide. Use Sonic Seal Slide Wells (EMS cat# 70365-42) or Lab-Tek Permanox Chamber Slides (cat # 70393/2 wells or #70400/4wells). The Tabas laboratory uses the Chamber Slides. Both have detachable culture chambers, but if handled gently the wells will not leak. Sonic Seal and Lab-Tek slide chambers come with lids that can be sealed to the chambers for shipping.

2. When culturing the cells, make duplicates or triplicates of each condition if possible.

3. When fixing the cells (below), fill the chamber wells at least 3/4 full with fixative and dry the top rims of the chambers. For a waterproof seal, spread a thin layer of silicone caulk/adhesive on the inside of the lid and gently press it down on the chamber. After the adhesive sets (usually an hour or so) wrap the entire slide in Parafilm.

4. For shipping, place the sealed dishes inside two Ziplock plastic bags and put them in a Styrofoam shipping container with several cold packs. DO NOT SHIP ON DRY ICE—the cells should stay cool, not freeze. Make sure the plates are well cushioned with Styrofoam packing peanuts or bubble wrap to prevent shifting of the plates during shipping. Ship the cultures by express delivery-i.e., FedEx Overnight, or Airborne Express.

Fixation procedure:

1. Rinse cultured cells gently 2-3 times with serum-free growth medium warmed to 37° C. Be sure the cell culture medium used for rinsing is serum-free, because glutaraldehyde crosslinks the serum very quickly and will impair the fixation of the cells.

2. Remove most of the serum-free rinsing medium and fill the wells with 2.5% glutaraldehyde in 0.1M sodium cacodylate buffer, pH 7.4. The Tabas laboratory also uses 2.5% glutaraldehyde/2.5% paraformaldehyde in cacodylate buffer purchased from EMS. Victoria Madden noted in an e-mail: "The fixative solution I recommend is 2.5% glutaraldehyde in 0.1M sodium cacodylate (EMS cat # 15960) but if the sodium cacodylate buffer (arsenic) is a complication for shipping, then 2.5% glutaraldehyde in 0.1M Sorensen's phosphate buffer (EMS cat #15980) will work fine."

NB: Do not remove the dishes from the incubator until you are ready to fix them for optimal results. Use the fixative at room temperature, not cold. Let cultures rest in the fixative at room temperature for one hour before refrigeration or transporting.

NB: Keep a small amount of rinsing medium or fixative solution over the cells at all times to prevent the cells from drying. Once the cells dry even slightly, they are no longer of any value for electron microscopy. When storing fixed cells before processing, keep a large volume of fixative solution in the dish and seal the dishes to prevent evaporation.

3. Prior to shipping, we keep the cells in fixative for several days and then ship in fresh fixative.

4. **IMPORTANT:** When the EM technicians receive the samples, they must remove the chamber from the chamber slides before using propylene oxide (a dehydrating agent). Otherwise, the chamber will melt.

5. **IMPORTANT:** For embedding, the EM technicians should use “Low Viscosity Embedding Media Spurr’s Kit” (Catalog # 14300 from EMS). The use standard embedding materials may not result in adequate hardening of the resin.

For EM work, the services of UNC (Victoria Madden) or Paragon Bioservices can be used.

University of North Carolina:
http://www.med.unc.edu/microscopy/electron.htm

Contact: Victoria Madden (vmadden@med.unc.edu)

Microscopy Services Laboratory
Pathology and Laboratory Medicine
B-05 Brinkhous-Bullitt Building/ Medical Drive
University of North Carolina at Chapel Hill
Chapel Hill, NC 27599
Phone: (919) 966-2413

Paragon Bioservices:
http://www.paragonbioservices.com/Histology/electron_microscopy.htm

Contact: Xianhai An (xan@paragonbioservices.com)

Paragon Bioservices, Inc.
Johns Hopkins Bayview Research Campus Alpha Center
5210 Eastern Avenue
Baltimore, Maryland 21224
Phone: (800) 545-6569
Phone: (410) 550-2919
Fax: (410) 550-2924