section and Epon in between the slides and cure. Our procedure for doing this is:

1) Acid clean and rinse the slides before use.

2) Dry in a drying oven overnight (the type used for drying cleaned laboratory glassware), then let cool to room temperature.

3) Dip slides in the LRA.

4) Stand slides upright and allow to dry in a dust-free area.

5) Tissue was sectioned at 50 to 100 µm in a Vibratome, then processed into 100% epoxy. After the final change, rather than place in molds, place the sections on a coated slide, add some Epon, and then a second slide. The section is held flat by putting whatever weight is needed (5 g to 10 g typically; clips may also be used).

6) Place in the curing oven for the recommended time for the epoxy formulation used.

The Epon separates easily from the slides, and the residual LRA can be washed off because it is water soluble. (I understand this is a material that is used to release plastic parts from molds.) The clean slides are dipped in the liquid and allowed to dry ovenight before use. Slides can be washed in water and redipped. These embedded sections can be viewed in the light microscope after popping off one slide, and the appropriate part may be cut from the slab and remounted on a blank stub for thin sectioning.

We have used this technique for TEM identification of HRP labelled neurons.

John Chandler, Colorado State University
chandler@lamar.colostate.edu

A Brief Tip on Decolorizing DAB Staining for Immunohistochemistry

Diaminobenzidine (DAB) can be solubilized by incubating in weak (1% or less) bleach, which removes the orange-brown color. The bleach will completely remove the color if the slides reaction is allowed to run long enough. Run a test slide and check every 2 minutes at first in a microscope to get a handle on how long it will take for a given preparation. Acceptable endpoints could take less than 5 minutes or more than 2 hours depending on the particular section thickness, initial DAB intensity, etc.. It may be best to just lighten the DAB, and not decolorize it completely. Subsequent immunostaining success will depend on the tissue, antigen, and antibody, and the immunostaining may have to be modified to work with the DAB decolorization.

Subsequent immunostaining in my experience has not been affected, but in principle the bleaching process could oxidize an epitope so caveat lector.

Mike King, University of Florida College of Medicine
making@nersp.nerdc.ufl.edu