MICROSCOPY 101

Post-Embedding/On-Section Immunocytochemistry

For post-embedding/on-section immunocytochemistry, most investigators work with very small volumes of their valuable primary antibodies and expensive gold labeled secondary antibodies. In an attempt to ensure that the sections are exposed to as much "fresh" antibody as possible, I have adopted the following trick. I employ nickel grids to pick up my sections. For antibody staining and for all pretreatments and washing steps, I place the grids on 6-10 µl droplets of the various solutions on a piece of parafilm on a magnetic stirring plate. The stirring plate is turned on to an appropriate speed to allow the nickel grids to rotate around on the droplets with some, but not too much, vigor. I cover the parafilm and specimens with a large (14.5 cm) plastic petri dish under which I have placed a small (60 mm) dish filled with water to maintain some humidity in the chamber. This whole setup can be placed in the refrigerator or cold room, if an extended incubation is required. Unless you're incredibly quick, it's much easier to transfer grids from droplet to droplet if you turn the stir plate off first.

When the immuno-staining protocol has been completed, I often run the sections through a "hard" fixation in 1.0% GA for 10 min., wash in buffer, and then through 0.5-1.0% OsO_4 and wash (all in the hood, of course) before a post-stain in Uranyl Acetate. The resulting images more closely resemble "routine" EM fixed material than typical lightly fixed IMC prepared material

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Cleaning Spec Holders

This is a rather important processs that should be done very carefully. I devoted several pages to discussing methods for cleaning parts for vacuum systems in my book *Vacuum Methods in Electron Microscopy*, P. 69-74.

If you are using the standard top-entry type of holder, cleaning should be straightforward - scrub it thoroughly with Tilex Soap Scum Remover, rinse with hot running water, sonicate in a strong detergent solution, rinse with hot tap water, rince with reagent grade isopropyl alcohol, dry with a gas blaster.

If you are using a side entry stage you can use essentially the same procedure, but you must then be careful to avoid getting the solutions inside the holder if it is one that has provisions for manipulating the specimen. Often, enough cleaning can be done to get rid of most contamination problems by sonicating just the end of the holder in isopropyl alcohol, then drying with a blaster. The latest method for these holders is Plasma Discharge Cleaning, and South Bay Technology markets a device that is specially designed for this purpose.

W.C. Bigelow

Readers -

As we would like to extend this feature, and include more hints and approaches to address challenges in all aspects of microscopy, we would greatly appreciate your contributions. They can be short, as the above, or longer and could include illustrations, etc.

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