A Commentary On Immunolabeling

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about their systems and immunoreagents before giving up their experiments.

The choices open to us for immunocytochemistry are wide and it is often confusing to know where to start. However, I hope that I have provided some help when faced with the ubiquitous problems we have all had to face with immunocytochemistry. This is not meant to be a complete guide. For this I recommend the book by Gareth Griffiths which covers this subject in great detail, and which has been my invaluable reference book for many years (G. Griffiths, 1993).

Immunocytochemistry is a very special branch of EM that is almost impossible to provide as a service. However, this is what is being asked of EM labs all over the world and I know it this will be virtually impossible to change. For this reason, it is our responsibility to educate our colleagues and to allow them to become involved in the discovery process of how their antibodies work. I have no good solution to this, but do know that if someone is involved in their own specimen preparation and data collection, their work will progress much faster. If new EM users are taught why particular approaches should be applied instead of being given one protocol to apply exactly as written, pretty neat ideas originate from their work.

We must stop being the "black box" of science. ■

Further reading:

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Hannah, M. J., U. Weiss & W. B. Huttner. 1998. Differential extraction of proteins from paraformaldehyde-fixed cells: Lessons from synaptophysin and other membrane proteins. Methods (a companion to Meth. Enzymol.) **16**:170-181.

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LR White Flat Embedding of Cells Grown on Coverslips

Tamara Howard, Cold Spring Harbor Laboratory
howard@cshl.org

We do this two different ways, using polyethylene (PE) molds or aluminum weigh boats, depending on the sample. You can either heat- or UV-cure these molds – just change the type of "lid" on the embedding mold.

The PE molds are used when the coverslip can be made to fit in the mold. We use JB-4-type molds, available from your favorite microscopy supplier. The key to using these molds is that they have to be pre-treated…fill the inner cup of the mold with LR White, cover with an aluminum JB-4 chuck (try to have enough resin in the mold to come up around the base of the chuck), and polymerize in a 60° C oven for a day or two, until the resin is hard. The Al chuck can usually be removed by hand, but a flathead screwdriver used as a pry will help pop it off if you have trouble. Dispose of the resin block and wipe out the cup with KimWipes…the mold is ready to use for real samples. The Al chuck can be cleaned by soaking it for a day in methanol (in the hood). Any resin remaining stuck to the chuck can be scraped off with a spatula. Our guess is that there are micropores in the molds that have to be sealed; we've had problems with incomplete polymerization of blocks when fresh molds were used. The molds last until you get sick of them or until someone turns up the oven without checking to make sure it is empty and melts everything. Thermanox or other plastic coverslips can be cut to fit the molds (before the cells are plated!); glass coverslips can be broken to fit. Tissue pieces that are too large for embed-

ding capsules or that have to be oriented can also go in these molds.

The Al dishes are for samples that can't be made to fit into the PE molds. I use these for gridded coverslips when we do correlative microscopy of single cells. Gridded coverslips always seem to break "funny", leaving you with shards of glass. So, I leave them whole and embed in the Al boats. They require more resin, but I think it is worth it in terms of ease of use.

The fixation and dehydration are done in culture dishes. The coverslips are transferred to glass containers (dishes, scintillation vials, whatever) for the resin infiltration steps, since LR White eats polystyrene. Or you can infiltrate in Al boats, but the resin use increases drastically.

When you are ready to embed, put a few drops of resin in the base of the mold, then put in the coverslip, cells up. (We clip the upper right corner of Thermanox coverslips; for glass, you can usually see which side the cells are on, or check in a dissecting 'scope.) Then fill the PE mold's cup with resin. With Al boats, fill halfway with resin. If you are going to heat-cure, use Al JB-4 chucks as lids for the PE molds, or another Al boat for the Al boat molds. Make sure you have resin at least up to the lower surface of the lid, to ensure an air-free polymerization. This is tricky with the Al boats – I try to add enough resin to come close to the top of the lower (mold) boat. The resin will shrink a bit during polymerization, but there is enough depth in these molds to keep the sample in resin if you've put enough resin in to start with! Use regular lab tape to label the samples; it will stick to the Al of the chuck or boat.

To UV-cure, use Aclar or heavy Saran-type wrap to cover the top of the mold/boat. It will sit on the resin surface; try to avoid forming air pockets. Cure at 60°C or with UV for 1-2 days.

Once you've removed the resin block from the mold (you should be able to pop them out of the PE molds like ice cubes; Al molds have to be peeled), wipe away any uncured resin. Use a razorblade to remove any resin from the back of the coverslip, then peel away the coverslip (Thermanox) or use a heat/cold regimen to release glass coverslips (wear safety glasses!). The areas of interest can then be cut out with a jeweler's saw and mounted on blanks for sectioning. I also cut down the resin backs of the cell blocks to ~2 mm before mounting to avoid skyscraper blocks. ■

JEST A BIT

Three professors (a physicist, a chemist, and a statistician) are at a meeting when a fire breaks out in a wastebasket.

The physicist says, "I know what to do! We must cool down the materials until their temperature is lower than their ignition temperatures and then the fire will go out!"

The chemist says, "No! No! I know what to do! We must cut off the supply of oxygen so that the fire will go out due to the lack of one of the reactants."

As the physicist and the chemist debate what to do, the statistician actually does something. He runs around the room lighting more fires.

As the physicist and the chemist scream, "What are you doing?"

The statistician replies, "We're going to need a larger sample size."