IMMUNOELECTRON MICROSCOPY OF BRAIN TISSUES EMBEDDED IN L R WHITE RESIN

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Tissue processing

[1] Fixation.

Human tissues: formalin-fixed autopsy material

Animal tissues: 4% para-formaldehyde-0.1 M phosphate buffer, intracardiac perfusion, then immersion in the same fixative overnight, 4°C. If you cannot perfuse, then immersion is OK,

Cultured cells: 4% para-formaldehyde-0.1 M phosphate buffer, I hr at room temperature (r.t.)

[2] Washing. 0.1M phosphate buffer. 10 min X 3 (tissues); 5 min x 3 (cells), r.t.

[3] Dehydration. 30%, 50%, 70%, 90% EtOH, 10 min each (tissues); 5 min each (cells), r.t.

[4] Infiltration. 90% EtOH: LR White (1:1), 20 min (tissues); 10 min (cells), r.t.
(1:2), 40 min (tissues); 20 min (cells), r.t.
pure LR White, 1 hr (tissues); 40 min (cells) r.t.
pure LR White, overnight (tissues and cells), r.t.

LR White resin from Polysciences, Inc., Warrington, PA, item # 17411. Keep in fridge (4°C) when not in use.

[5] Embed in BEEM capsules and polymerize in a vacuum oven at 50°C, 2 days. Fill resin to the top of capsule and cap it. Put some weight to hold down the cap in the vacuum oven. LR White won't polymerize in the presence of oxygen. If you don't have a vacuum oven, use gelatin capsules. Fill up to the top and cap it. These capsules are impermeable to air, so you can use a regular oven.

Post-embedding immunogold labeling

[1] Collect thin sections on film-coated nickel grids (Formvar-coated). Float each grid with sections side down on a drop of 20 ul solution. Put blocking serum, primary and secondary antibodies on Parafilm inside a moisture chamber. Use a 24-well cell culture plate for washings, one well for each grid. Fill the wells to the top. Use a wire loop to transfer grids from well to well. Make the solution in last washing well doom-up, so that it will be easy to pick up and transfer the grid by forceps to the drops on Parafilm.

[2] Block with normal serum of the same species as secondary antibody, 30 min, r.t.

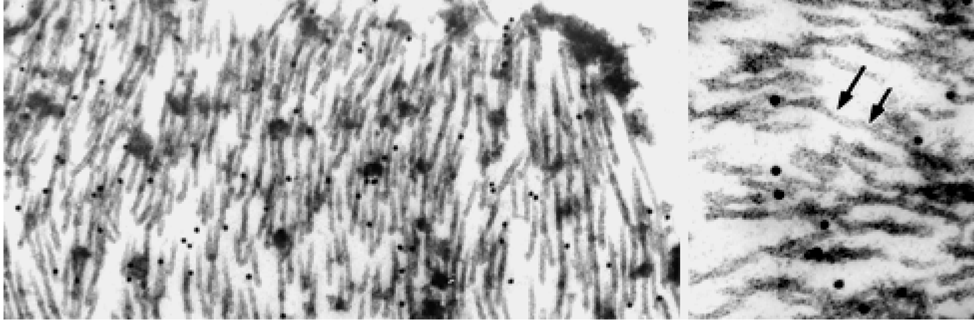
[3] Primary antibody, overnight at 4°C.

[4] Wash in 3 wells of PBS, 5 min each, 1 well of0.05 M Tris-HCl-0.1% Tween 20, 5 min.

[5] Colloidal gold-conjugated-2nd antibody, 1:20, 30 min, r.t.

[6] Wash in 2 wells of PBS, 5 min each, 1 well of Tris-Tween20, 1 well of dist. Water, 5 min each.

[7] Blot water from grids and let them dry in a Petri dish. Stain with 1% uranyl acetate in water, 0.5-1 min; wash under 20 drops of water from water bottle, then lead citrate, 0.5-1 min, wash with a jet stream from water bottle then 20 drops. Blot dry.



Left figure shows aggregated filaments in brain of Alzheimer's disease heavily labeled with PHF-1 (a monoclonal antibody against phosphorylated tau, a gift of Dr. Peter Davies, Albert Einstein College of Medicine, Bronx, NY). Right figure shows the characteristic twists (arrows) of paired helical filaments labeled with PHF-1 antibody.