
1. Prepare tissue: high pressure freeze, freeze substitute in acetone (plus 0.5% uranyl acetate), embed at (-) 50 C in Lowicryl HM20 (or other immunoresin).
2. Mount thin sections on nickel grids.
3. The remaining steps are via floating grids on drops of the solution, in humidity chambers.
4. Block 30 minutes in TBST (TBS with Tween 20) and 20% fetal bovine serum albumin.
5. Blot and transfer to primary antibody diluted in blocking buffer, incubate for 1-2 hours.
6. Rinse three times in blocking buffer for 30 minutes total.
7. Incubate in secondary antibody diluted in blocking buffer, for 1-2 hours.
8. Rinse once in blocking buffer, and then wash in water.
9. For additional contrast, post stain in uranyl and lead salts.