Immunogold Diffusion on thick Cryostat Sections

Day One:
1) Make buffer stocks, A and B Epon stocks, and PBS.
   Acetate Veronal Stock = 1.15 g NaAcetate Anhydrous
   J.T. Baker 1-3470)
   + 2.943 g NaBarbituate (Veronal)
   labelled Barbital -- Sigma B-0500)
   to 100 ml with ddH2O

2) Cut Frigocut sections of fixed tissue (for kidney, generally ~ 25 microns thick), and place in vial of PBS/0.1% BSA.

3) Distribute ~4 sections with some buffer into EA borosilicate tube that will be needed for the experiment and label EA tube.

[4) For strept-avidin gold testing, a quench is necessary here. Make 0.05% Avidin in PBS. Quench for 20 min. Wash in PBS for 5 min.

5) Make 0.005% Biotin. Incubate for 20 min. Wash in PBS for 5 min.]

6) Add primary antibody in 0.1M PBS/1%BSA + 0.02% Azide. Need 100 ul per tube. Cover EA tube with parafilm. Incubate overnight on rotating wheel.

Day Two:
NO AZIDE!!!
7) Wash with 0.1M PB/0.1%BSA 4x 15 min (30 min. minimum).

8) If necessary add bridge here in 1% BSA/PBS, and follow incubation with wash as described in step 7.

9) Gold conjugates are diluted in 1% BSA/PBS/0.02% Azide. Add centrifuged gold conjugates to vials and incubate overnight at RT, covered, on rotating wheel.

Day Three:
10) Observe with dissecting microscope.

11) Add more gold if necessary.

12) Wash for 40 min. in PBS/0.1% BSA through six changes.

13) Fix with 1.5% Glutaraldehyde in 0.1M NaCacodylate + 5% Sucrose, pH 7.4, at RT for one hour.

10 ml = 5 ml 0.2M Cac stock + 1.5 ml 10% glut + 0.5 g sucrose + ddH2O to10 ml
14) Wash for 15 to 20 min. in PBS/0.1% BSA, through ~3 changes.

15) Post-fix cells with Palade's 0s04 for one hour one ice, light tight, under hood.
   5 ml Palade's 1% 0s04 = 1 ml Acetate-veronal stock
   +1.25 ml 4% 0s04
   +1 ml 0.1N HCl
   +1.75 ml ddH20

16) Two quick rinses in ddH2O.

17) Kellenberger --- 1 hr. at RT.
   10 ml Kellenberger = 2 ml Acetate Veronal Stock
   +2.8 ml 0.1N HCl
   +5.1 ml ddH20
   + 0.05g Uranyl Acetate
   Check pH with pH paper before adding UA.

18) Two quick rinses in ddH2O.

19) Dehydrate cells with graded series of ethanol (70, 95, 100, 100) and then change tube to
    scintillation tube and place tissue in 100% PO --- quickly EA (~1 min).

19) Place in 50% PO/50% Epon (can be old) overnight, rotating on wheel with caps on.

Day four:
20) Remove caps from tubes and make new Epon.

21) Embed in 100% Epon. Change two to three times throughout the day --- ~EA hour. (If
    destroying tissue, don't change Epon.)

22) Put typed or pencil-written label in dummy capsules with wooden stick, at least two capsules
    per sample. Pour tissue out of tube onto mincing dish. Place tissue in flat mold with small amount
    of Epon (to avoid curling) with a wooden stick and place in 60o oven overnight.

Day Four:
23) Remount tissue onto respective dummy block.
    Put piece of filter paper on top of hot plate and place hot plate under dissecting
    microscope. With scalpel, cut piece of tissue that you would like to remount and place on
    dummy block with small drop of Epon. Let dry in 60oC oven overnight.

Day Five:
24) Start cutting!!