Uranyl Acetate Staining protocol in the IMF

Uranyl acetate is in a 2% solution.

1. Use the designated pipettes and Radiation shield stored in the locked drawer near the fumehood in IMF SE2-90G
2. Lab coat, gloves, and safety glasses are required when handling uranyl acetate
3. Prepare samples in the workbench designated for radioactive materials located in IMF SE2-90G
4. Remove TEM grid from its grid storage using the TEM specified tweezers.
5. Place TEM grid in your grid case
6. Apply 4 µl of sample to the grid and wait 5-10 min
7. Cut out a 3x3 inch piece of parafilm and place within the specified radioactive area. place 3 drops (40 µl each) of HPLC water on the top of the parafilm.
8. Remove the uranyl acetate from the locked drawer located in IMF room SE2-90G and place in the specified radioactive area.
9. Place 3 drops (40 ul each) of uranyl acetate in the lower part of the parafilm.
10. Remove TEM grid from case and wash in HPLC water (10 seconds each droplet). Then wash in uranyl acetate (10 seconds each droplet).
11. Store back in grid case and let sit for 5-10 min.
12. Dry using a kimwipe by placing the tip of the kimwipe next to the grid.
13. Let dry overnight
14. Place uranyl acetate back in drawer and make sure the drawer is locked.

Alternative: TEM protein sample staining with uranyl acetate (ver.1, 2023.06.12)

We use carbon coated formvar-copper grids (CF400-CU-50, Electron Microscopy Sciences).

1. The grids are treated with a plasma glow discharger to make the grid surface hydrophilic.
2. Sample solution (typically use 5 µL, 0.05 - 0.1 mg/mL protein) is applied to the carbon coated side of the treated grid and incubated for 30 seconds. Excess liquid was blotted with filter paper. Wait about 5 min to make the surface dry.
3. To remove salt from the grid surface, ultra-pure water (5 µL) is applied to the grid surface and water is immediately wicked away with filter paper. Wait about 5 min to make the surface dry. If necessary, repeat this process one more time.
4. Grid is then stained with 5 µL 1 – 2 % uranyl acetate and excess stain was wicked away with filter paper. Wait about 5 min to make the surface dry. The sample grid is ready for TEM observation