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Negative Stain Procedure

1. Preparation of the grid surface for sample application by rendering it hydrophilic: place the grid with support film side (dark color for carbon support film) facing up on a TEM Grid Holder Block (left image below) and load to Fischione M1070 NanoClean plasma system (right image below). Using 70% power and treat for 40s for Electron Microscopy Science brand standard thickness grid (CF400-CU) or 1 min for Ted Pella brand standard thickness grid (#01824). (Refer to the Fischione NanoCleam M1070 Procedure for details.)

2. Grip the edge of the grid with a pair of anti-capillary reverse tweezer, and apply 3-5 µL of sample solution to the support film surface.

3. Allow the sample to adsorb to the grid surface for 1 min. A good concentration for protein sample is 0.05 mg/ml. Optimizing the concentration for individual samples based on the imaging results.
4. Touch the edge of the grid with a sheet of filter paper to remove the solution as much as possible.
5. (Optional) Wash the grid: Apply 3-5 µL of DI water to the grid surface right after the sample solution blotted by filter paper. Touch the edge of the grid with filter paper to remove water. (This step is repeated 0-3 times depending on the salt concentration in the sample solution)
6. Apply 3-5 µL of 2% Uranyl Acetate staining reagent to the grid surface right after the DI water blotted by filter paper. Allow the stain to adsorb to the grid surface for 30s (protein sample) or 10s -15s (DNA samples). (UA is the most popular stain solution, UF and PTA are frequently used in case UA does not work for your samples)
7. Touch the edge of the grid with filter paper to remove the stain solution until a thin layer of stain solution left on the surface. Do not let the filter paper absorb and remove the stain solution completely.
8. It is recommended to repeat staining steps (step 6-7) to achieve better stain results. (DNA samples should be stained only once.)
9. Allow the grid to air dry and put into a grid box.

Tips:

The procedure described here is standard negative staining. Generally, you need four drops of solutions to be applied to the grids in a sequential order: 1. Sample solution; 2. DI water; 3. Stain solution; 4. Stain solution.

Between each drops, try your best to remove previous drop completely and IMMEDIATELY apply next drop before the grid surface becomes dried. Do not remove the last drop of stain solution completely (always leave a thin layer of stain solution on the grid after blotting the grid to remove the last drop of stain solution).

In case you have sample solution with high salt concentration (>150 mM), you need to repeat the washing step by DI water to make total washing 2-3 times.