

Negative Stain Procedure

Caution: Uranyl Acetate is made from depleted uranium, meaning that it is uranium from which the radioactive isotopes have been greatly reduced in concentration, but it is still treated as radioactive material even for diluted solution. This 2% uranyl acetate solution must be used at designated and marked work areas on benchtop with radioactive warning tape inside Imaging Suite. Do not work in other areas. Personal Protective Equipment is required when using uranyl acetate, including **Nitrile gloves, Safety glasses and Lab coat**. Dispose of contaminated gloves, filter paper, plastic pipettes, tips, absorbent paper, etc. into the waste bottle with radioactive waste sticker on the benchtop.

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 NanoClean M1070 Procedure for details.)



2. Grip the edge of the grid with a pair of anti-capillary reverse tweezers, 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 is 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.

Disposal of Waste:

- Any material that has uranyl acetate contamination (includes tips, PPE, and samples when discarded). Must be placed into a properly labeled waste container for disposal. It will be treated as radioactive waste.
- When container is full; contact the EHOS office via email tdickson@gc.cuny.edu to pick up for disposal.

Final Safety Protocols:

- Once you have completed this work and the waste is properly managed. The researcher needs to ensure they wash their hands with soap and water as the main concern with depleted uranium products is the ingestion hazard.