Negative staining protocol

1. Staining solution preparation (refer to the protocols below)
2. Glow-discharge EM grids coated with continuous carbon film (the grids should be used within 30 min after the glow-discharging). Note: usually 20mA* 20s will be used.
3. Apply 6 µl of protein sample solution to the EM grid and wait for 1 minute.
4. Blot the grid from the edge with a piece of filter paper (briefly touch the rim of the grid with a piece of filter paper, allow the solution to flow to the filter paper). Caution: don't let the grid to be completely dry. It is better to have a thin layer of solution.
5. Apply 3-6 µl staining solution and wait for 1-2 minutes.
6. Blot with a piece of filter paper (briefly touch the rim of the grid with a filter paper, allow the solution to go into the filter paper completely).
7. Completely dry the grid in the hood for 10 minutes.
8. Store the stained grid in a grid box and label properly.

Staining solution preparation

1. 2% Uranyl Acetate

1.1. Weight 0.2 g of uranyl acetate
1.2. Stir to dissolve the uranyl acetate particles in 10 ml ddH_2O (can take hours) in the hood.
1.3. Filter solution with a 0.22 µm filter using a syringe
1.4. Aliquot into eppendoff tubes and wrap with aluminum foil
1.5. Store at room temperature in the dark
1.6. Filter again before use after a few days

2. 2% Uranyl formate

2.1. Boil 10ml ddH_2O and let it cool to room temperature
2.2. Weight 20mg uranyl formate in Eppendorf tube.
2.3. Add 1ml water to the tube.
2.4. Vortex vigorously to dissolve the yellow powder
2.5. Add 10ul 1N NaOH to the cap of the tube.
2.6. Close the cap and vortex vigorously
2.7. Filter this solution through a 0.02 µm filter using a syringe

Here is another protocol used at LBNL to make 1% UF

2.1) Put 1 g Uranyl Formate (UF) powder into a glass bottle containing 100 ml deionized water.

2.2) Stir solution overnight at room temperature under a dark condition, in which the water bottle was wrapped with aluminum foil to prevent light.

2.3) Aluminum foil wrap a 5 ml syringe using a 0.2 μm (pore size) filter, and filter the solution gently through the syringe into an aluminum foil covered tube.

2.4) Aluminum-foil wrap a 1 ml syringe using a 0.02 μm (pore size) filter, then filter the solution followed by aliquot the filtered solution into 2 ml vials covered by aluminum foil protecting the stain from light.

2.5) Immediately after aliquot, freeze the vials by plunging the vials into liquid nitrogen container using long handled forceps.

2.6) Transfer the frozen vials into a - 80 ^{o}C freezer for storage for future usage.

2.7) Thaw a vial of 1% UF solution in a water bath set at room temperature. Make sure that the aluminum foil remains wrapped around the vial to prevent exposure to light.

2.8) Once the UF is completely thawed, using an aluminum foil wrapped 1 ml syringe mounted with a 0.02 μm filter to filter the UF solution right before usage. Save the filtered solution into a new aluminum foil wrapped vial, and then place the vial into a cap covered ice box to maintain a dark environment.

3. 2% Sodium Silicotungstate (SST)

3.1. Weight 0.2 g sodium silicotungstate and put into 10 ml ddH_2O

3.2. Stir to dissolve sodium silicotungstate particles (it can take hours to overnight)

3.3. Adjust pH using couple drops of NaOH. (Adjust PH to 7.0?)

3.4. Filter solution with a 0.22 μm filter using a syringe

3.5. Aliquot into eppendoff tubes and wrap with aluminum foil

3.6. Store at room temperature in the dark

4. **5% Ammonium molybdate (AM)**

4.1. Weight 0.05 g ammonium molybdate and place in a 1.5 ml Eppendorf tube

4.2. Add 0.8 ml distilled water and dissolve all the ammonium molybdate

4.3. Add a few drops of 10 N NaOH and adjust the solution pH to 7.0 (using pH paper)

4.4. Bring the final volume to 1.0 ml

4.5. Filter this solution through a 0.22 μm filter using a syringe.