SECM4 Negative Stain Procedure

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1. **Purpose**

   1.1 Rapidly visualize biological samples under an electron microscope by applying a heavy-metal stain, allowing quick assessment of particle quality, distribution, and integrity before more detailed cryo-EM analysis

2. Supplies & Equipment:

- ☐ PPE
  - ☐ Laboratory Coat
  - ☐ Nitrile Gloves
- Chemicals
  - Uranyl Acetate, 2% solution - prod#
- 1.5 mL Microcentrifuge Tubes (or similar)
- Centrifuge
- Micropipettes and Tips (for volumes ranging from 3 to 10 µL)
- Cryo-EM Grids (Carbon Film Cu 400 mesh - prod#CF400-Cu-50)
- Aluminum Foil
- PELCO TEM Grid Holder Blocks - prod#16820-25
- PELCO easiGlow™ Glow Discharge Cleaning System - prod#91000S
- Anti-capillary Tweezers - prod#510-4NM
- Chien Staining Pad - prod#10523-2
- 90mm Whatman Circular Filter Paper - cat#1001 090

3. Procedure

3.1 Preparing the Grids

3.1.1. Retrieve your Carbon Film Cu grids from the desiccator. Handle grids carefully.

3.1.2. Place the grids carefully on a **clean** PELCO TEM Grid Holder Block and insert them into a PELCO easiGlow™ Glow Discharge Cleaning System set to 15 mA of current with a 0.26 mBar vacuum for 25 seconds. (In the meantime, prepare the sample and solutions described in Sections B and C.)

3.1.3. Once complete, remove the grids and pick each one up with anti-capillary tweezers to suspend it above the counter. Ensure the film side is facing upward.

3.2 Preparing the Sample

3.2.1. Ensure the sample concentration is within the range of 0.04~0.08 mg/mL for optimal viewing with Negative Staining. For larger particles and complexes (>400kDa), use a higher concentration (by mass) to attain the same number of particles. For smaller particles (<150kDa), use a lower concentration (by mass) to attain the same number of particles.

3.2.2. Dilute or concentrate the sample with its corresponding buffer as necessary and keep it near its optimal temperature (on ice, etc.).

3.3 Preparing the Solution

3.3.1. Use a micropipette to create 20 µL droplets of ddH_2O (3 for each grid) on a clean Chien Staining Pad. Try to do this close to when you will be blotting and washing to prevent evaporation.

3.3.2. Use a micropipette to transfer ~100 µL of uranyl acetate solution into a 1.5 mL tube, wrap the tube with aluminum foil to protect it from light, and store it for future use.

3.4 Staining the Grids

3.4.1. Apply **3 µL of the sample** onto each grid using a micropipette. If preparing multiple grids, allow about 1 minute between applications to accommodate blotting later and optimize the workflow.

3.4.2. Let the grids sit with the mixture on top for **2 minutes**. During this time, prepare a filter paper for blotting by folding it in half and turning it upside down to rest on the counter.

3.4.3. Once a grid has incubated long enough, blot the film side on the filter paper. Then wash the film side in a water droplet and blot on the filter paper. Repeat the washing once more for the film side, then one last time on the bar side.

3.4.4. Use a micropipette to apply **10 µL of the 2% Uranyl Acetate** onto the grid.

3.4.5. Let the grids sit with the mixture on top for **3 minutes**.

3.4.6. Once a grid has incubated long enough, blot the film side on the filter paper until it is **completely dry**.

3.4.7. Allow the grid to dry while still on the tweezers for at least 5 minutes before transferring to a desiccator for at least 45 minutes before viewing.

4. Chemicals

4.1 Uranyl Acetate, 2% solution - prod#

5. Waste Disposal

5.1 Practice radiation safety techniques and dispose of all RAM waste properly. Contact FSU EHS for questions regarding radiation safety.