Cryo EM - Negative Stain Protocol

Potential Hazards/Toxicity

Uranyl formate (UF) is a heavy metal stain, which requires radiation safety training and radiation disposal protocols to be strictly followed during use.

Procedures

A. Solution Preparation (0.75% UF)

1. Weigh out 37.5 mg of UF powder (yellow in color; from Electron Microscopy Sciences) in a small beaker (a 30–50 mL beaker should be fine).
2. Add 5 mL of boiling water to the UF powder in the beaker, then stir this mixture in the dark for 5 minutes.
3. Add 10 µL of 5M NaOH to the solution and continue stirring for 5 minutes.
4. Filter the solution with a 0.2 µm syringe filter into a 50 mL conical tube. Keep it in the dark for this step; aluminum foil works well.
5. Aliquot the mixture at your desired volume into black Eppendorf tubes. This will be good for a week or two on the bench; for long-term storage and use, flash freeze the mixture in liquid nitrogen and thaw prior to use.

B. Grid Preparation

This protocol uses the glow discharge CF-200Cu grids from Electron Microscopy Sciences.

1. Hold the grid with anti-capillary forceps with the carbon-coated (darker) side facing up.
2. Add 4.8 µL (typical concentrations range from 0.02 to 0.05 mg/mL, depending on the sample) of your protein sample to the grid and wait 15 seconds for absorption of the sample to the grid.
3. Wick the sample away with filter paper on the side of grid without touching the surface of the grid.
4. Add 4.8 µL of buffer solution to wash the grid surface, wick it away, and repeat 2 more times.
5. Add 4.8 µL of 0.75% UF solution to the grid, wick it away, and repeat 3 more times. For the fourth (final) UF addition, wait 30 seconds after adding it to the grid and, when wicking the solution away, leave a tiny film of solution on the grid.
6. Pick up the grid with forceps and air-dry it by waving it back and forth in the air to generate areas with different depths of stain.
7. Proceed to imaging the grid on a microscope.

Final Considerations

- Ensure the humidity of the room is consistent. You can obtain drastically different results depending on the conditions of the room.
- If detergent is present in your protein solution, follow the steps outlined above, but instead of using a pipette to directly add the sample to the grid, apply the carbon side of the grid to a 40 µL droplet of each of the wash buffer, protein sample, and stain following each of the above steps to ensure good staining.
- Phosphates precipitate UF, so ensure that you use a phosphate-free buffer or water to thoroughly wash the grid to improve staining if the samples contain phosphates.
- If preferred orientation is a problem, add 0.1% polylysine (from Electron Microscopy Sciences) to the grid and wick it away before adding the sample and follow the procedure as outlined above.

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