



Sample Preparation

Negative Staining Protocol:

Work Bench Location (Room G238, Sample Preparation room)

Following protocol are the instruction guidelines for all user, please go through regular training

Optimization parameter: *Protein concentration and blotting time.*

Precaution: *While working with Uranyl salts please use all protections such as Eye glasses, Gloves, and dispose the contaminated things in designated trash bag for radioactive/uranyl waste.*

All tweezers and utilities in the workbench should not be moved to other areas to prevent cross contamination.

Staining Solution preparation. (0.75 % Uranyl Formate)

1. Heat to Boil 5 ml of ddH₂O on magnetic stirrer.
2. Add 0.0375 mg of Uranyl formate to 5ml of heated ddH₂O to make 0.75% solution.
3. Switch of the heating and add 4 ul of 10 N NaOH.
4. Filter through 0.22um filter in the test tube and label with your name, date and concentration of uranyl salt and let it cool to room temperature.
5. The stain preparation may be stable for 2-3 days or more, one can filter and reuse it.

It is advised to make a new stain each time.

Negative staining protocol.

1. Glow-discharge EM grids coated with continuous carbon film (the grids should be used within 30 min after the glow-discharging). Please follow the instructions during training how to use Pelco Glow Discharger.
2. Apply 3-4 ul of Protein sample to the EM grid and wait for 1 minute.
3. Cut a parafilm strip and make 2 drops of water or buffer (20ul each) and 2 drops of staining solution (20 ul) each.



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4. Blot the grid from edge to blotting paper followed by washing the grid surface with water /buffer drop and blot the grid from edge on the filter paper.
5. Repeat the steps with each drop, *imp*: while washing with staining solution, one can wait (seconds to minute) before you blot on filter paper to get proper/optimized staining.
6. Let the Grid air dry for 5 minutes.
7. Discard the parafilm and filter paper in uranyl waste basket.
8. Collect your grid in grid box and take grid to the microscope. ***Don't take tweezers to the microscope.***
9. Clean the workbench.