Negative Stain Procedures  
Electron Microscopy Core, University of Utah

Negative staining encases objects of interest in a casting of heavy metal salt. Negative staining is a simple technique that usually provides bountiful information and beautiful images. Many variations of the procedures exist. You may need to try multiple methods or variations to find optimal staining for your sample. Below are general guidelines and three specific procedures. Search the internet for other possibilities and suggestions.

General Techniques and Variations


2. Hold glow-discharged, carbon-coated grid with a pair of tweezers (forceps).

3. Apply sample to carbon side of grid.

4. Blotting (with filter paper) should be done at the edge of the grid only.

5. Until the last step, do not let the grid dry out completely.

6. Positive staining becomes more likely the longer stain solution is incubated with sample.

7. Buffers with detergents or similar organic-dissolving agents may not be good for washing step. Water may be best in some cases.

Potentially helpful variations
a. vary time or type of glow-discharging, including not glow-discharging at all
b. time sample incubates on grid
c. time negative stain solution incubates on grid
d. volume of sample placed on grid
e. multiple applications of sample to grid
f. eliminate buffer or water wash
g. blot by putting the grid perpendicular to the filter paper (edge of grid in contact with paper) or blot by touching edge of grid to torn edge of filter paper
h. dry (final step) in vacuum
**Procedure 1 (4-Drop or 5-Drop Method)**

1. Put two 20 µl drops of buffer (preferred) or de-ionized H₂O on a piece of parafilm.
2. Place two (4-drop method) or three (5-drop method) 20 µl drops of negative stain solution (e.g. 1% ammonium molybdate, uranyl acetate, or uranyl formate) on the parafilm near the two drops from step 1.
3. Place 3.5 µl of sample on grid, wait one minute.
4. Blot with filter paper
5. Quickly (do not let grid dry out), place grid in a drop of buffer or H₂O for about one second, then blot with filter paper.
6. Repeat, but in the second drop of buffer or H₂O.
7. Repeat, but in one of the drops of stain solution. Optional (5-drop method), repeat again in stain solution
8. Place grid in next drop of stain solution for 15–20 seconds.
9. Blot with filter paper and allow to air dry.

**Procedure 2 (One Wash, One Stain)**

1. Put a relatively large drop of buffer or H₂O on a piece of parafilm.
2. Place 3.5 µl of sample on EM grid, wait one minute.
3. Blot with filter paper
4. Quickly put face down on the large drop of H₂O or buffer for 2–3 seconds
5. Blot with filter paper
6. Quickly replace H₂O with 3.5 µl of negative stain solution, wait 15–20 seconds
7. Blot with filter paper, allow to air dry.

**Procedure 3 (No Wash)**

1. Place 5–20 µl of sample on EM grid, wait one minute, then blot with filter paper.
2. Place same volume of negative stain solution on grid, wait one minute, then blot with filter paper and allow to air dry.

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by David M. Belnap, 25 Jan 2022

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¹ This method is similar to one described here, David S. Booth, Agustin Avila-Sakar, Yifan Cheng, "Visualizing Proteins and Macromolecular Complexes by Negative Stain EM: from Grid Preparation to Image Acquisition" JoVE Journal, DOI: 10.3791/3227. See, https://www.jove.com/v/3227/visualizing-proteins-macromolecular-complexes-negative-stain-em-from.