

TEM Grid Staining Protocol

CAUTIONS:

1. Uranyl acetate is light sensitive so it is kept in an amber bottle with aluminum foil wrapped around it.
2. Lead citrate is carbon dioxide sensitive, take two precautions:
 - a. Mark the bottle every time it is opened so air exposure can be monitored. Remake the solution as needed.
 - b. When staining, do so in a closed container with sodium hydroxide pellets in order to absorb atmospheric CO₂

MATERIALS:

1. Parafilm
2. Filter paper
3. Clean glass Pasteur pipettes
4. Double-distilled (or Millipore) water (available from Grimwade lab)
5. 2% aqueous uranyl acetate (1g/50ml)
6. 0.5% aqueous lead citrate (0.25g/50ml)
7. Sodium hydroxide pellets
8. Large plastic Petri dishes

PROTOCOL:

1. Set up stations: uranyl acetate, drying, lead citrate, and two waste containers (one for the uranyl, one for the lead).
2. Moisten the filter paper with distilled water, and apply a square of parafilm onto the filter paper as a bed for the grids.
3. Using a clean glass Pasteur pipet, place one drop of uranyl acetate on the parafilm for each grid that you intend to stain.
4. Invert each grid onto a separate drop of UA. Grids should sit on top of the drops.
5. Cover to seal out light, and wait for 1-10 minutes (we have been using 1 min with excellent results).
6. Gently dunk each grid into filtered water 5-8 times (do this in a small container).
7. Place grid right-side up (section side up) on filter paper for drying. Make sure filter paper is labeled if you have different grids. Allow grids to dry for 5 min.
8. Moisten filter paper with distilled water, and apply a square of parafilm onto the filter paper as a bed for the grids.
9. Using a clean glass Pasteur pipet, place one drop of lead citrate on the parafilm for each grid that you intend to stain. Do this inside a large Petri dish and add several pellets

of sodium hydroxide to absorb carbon dioxide. Also, set up another distilled water rinse station.

10. Using a new, clean glass Pasteur pipet, and place one drop of lead citrate on the parafilm for each grid that you intend to stain. Take lead citrate only from the center of the bottle so that precipitates do not get pipetted out.
11. Put each grid onto a separate drop of lead citrate drops, cover Petri dish with its lid, and wait three minutes.
12. Rinse each grid with distilled water quickly (5-8 dips), and then place the grids onto filter paper to dry for 5 minutes.
13. For increased contrast, re-stain grids in lead citrate and rinse again.
14. Store grids in protective TEM grid case.
15. Pipette lead citrate and uranyl acetate into separate labeled waste containers.