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Technical Support Bulletin

UranyLess classic and negative staining protocols:

#29-005030, #29-005040

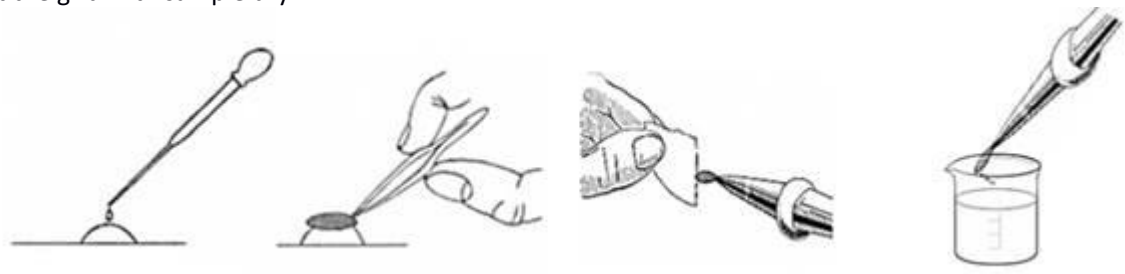
UranyLess – Protocol for classic contrast

This protocol is used for double staining with UranyLess and lead citrate on ultrathin sections.

The protocol is adapted for biological samples that have been fixed with glutaraldehyde, osmium or ruthenium and embedded in an epoxy type resin (i.e. Epon, Araldite, Spurr) or an acrylic type resin (LRWhite, HM20).

Staining protocol:

1. Place a droplet of UranyLess on parafilm or a hydrophobic slide
2. Place the TEM grid on the UranyLess droplet for 1-2 minutes
3. Blot the grid with filter paper
4. Then wash the grid in distilled water
5. Let the grid with sample dry



Place a droplet of UranyLess on parafilm | **Stain section 1-2 minutes** | **Blot with filter paper** | **Rinse in distilled water and let sample dry**

After drying, use the lead citrate protocol according to Reynolds (1963).

1. Place the grid on a 3% lead citrate droplet for 1 minute
2. Blot the grid with filter paper
3. Rinse the grid with distilled water
4. Let the grid dry

Technical tips:

- UranyLess is not air or light sensitive (unlike uranyl acetate)
- After lead citrate, rinse immediately in freshly prepared distilled water or wash with a solution containing 0.01N of NaOH
- If there is any precipitate in the solution, filter it prior to use

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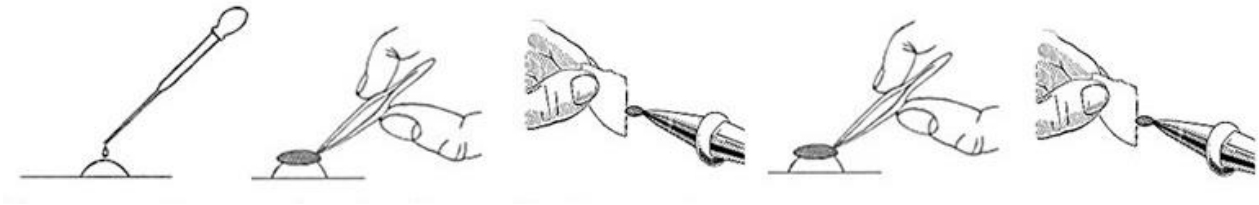
- If solution was refrigerated, allow solution to return to room temperature before use
- Do not keep lead citrate or UranyLess refrigerated

UranyLess – Protocol for negative staining

This protocol is adapted for negative staining with UranyLess. It allows characterisation of isolated particles or morphology of bacteria, viruses, protein, liposomes, exomes, nanoparticles etc.

Staining protocol:

1. Place a droplet of the sample solution (~10ul) and a droplet of UranyLess on a piece of parafilm or any other hydrophobic carrier
2. Place a formvar coated grid on the sample solution for about 1 minute; use fine tweezers.
3. Blot the grid with filter paper
4. Place the grid on the UranyLess droplet for about 1 minute
5. Blot the grid with filter paper
6. Let dry for 5 minutes and observe with a TEM



Place a sample droplet | **Place grid on sample droplet** | **Blot with filter paper** | **Place the grid on UranyLess** | **Blot with filterpaper**

Technical Tip:

- If the staining is too intense, wash with water for 1 minute.

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