

Electron Microscopy of Nucleic Acids – Aqueous Procedure

Materials

Shallow dish – approx. 10cm square x 1.5cm deep.
Teflon bars (2).
Microscope slides (one for each prep) precleaned in cleaning solution.
Microscope slides (as is from box).
Tweezers – one fine tipped, one Teflon coated.
Parlodion coated TEM grids (300 mesh) freshly prepared.
Filter paper (7 cm diam.).
Micropipettors (20 μ and 50 μ l).
Pasteur pipettes.
Grid coating apparatus (Coors porcelain funnel, hose, hose clamp, ring stand, and clamp).
Rubber or plastic gloves.
Platinum wire (8 mil diam.).

Solutions

Parlodion in amyl acetate (2.5%).
2mM EDTA (pH 6-7).
1M ammonium acetate (hyperphase).
0.25M ammonium acetate (hypophase).
0.05% cytochrome C.
DNA prep (1-2 μ g/ml).
Squeeze bottles with 50% ethanol, 90% ethanol and hypophase.
Sulfuric acid – dichromate glass cleaning solution.
0.05M uranyl acetate in 0.05M HCl stock solution (store in dark).

Note: The spreading solutions and hypophase must be prepared immediately prior to use. All glassware and utensils need to be meticulously clean.

Procedure

1. Prepare parlodion coated grids: Place grids on filter paper in the funnel beneath the water surface. Put a drop of parlodion solution on the water surface and allow to dry for 1-2 minutes. Drain water slowly from the funnel allowing the film to settle on the grids. Remove the filter paper and dry at 40°C for 45 minutes.
2. Remove glass slides from cleaning solution (use Teflon coated tweezers) and rinse thoroughly with distilled water.
3. Make 1:1 mixture of 1M ammonium acetate and 2mM EDTA (Stock A).
4. Prepare stain: 10 μ l stock solution in 10 ml 90% ethanol.
5. Prepare hypophase: Fill shallow dish with 0.2M ammonium acetate and place Teflon bars approx. 5 cm apart across top of dish (hypophase should touch bottom of bars).
6. Prepare spreading solution: 50 μ l stock A, 20 μ l H₂O, 20 μ l DNA prep (handle with gloved hands), 10 μ l cytochrome c. Mix, wait 5 minutes.

7. Place cleaned microscope slide in hypophase solution, resting one end on a Teflon bar so as to provide a ramp for the spreading solution.
8. Gently extrude 50 μ l of spreading solution onto the glass ramp approx. 1 cm above the hypophase surface.
9. Wait until all the solution has run down the slide onto the hypophase surface, then pick up specimen on a coated grid at a point about one grid diameter from the glass/hypophase interface.
10. Rinse grid with hypophase followed by 50% ethanol and 90% ethanol.
11. Stain grid on uranyl acetate for 30 seconds.
12. Rinse grid with 90% ethanol.
13. Rotary shadow specimen in the vacuum evaporator using 3 cm of 8 mil platinum wire at an 8:1 distance to height ratio.
14. Observe specimen in TEM using 40KV accelerating voltage and a 30 μ m objective aperture.