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THE UNIVERSITY OF ARIZONA
RESEARCH, INNOVATION & IMPACT
Core Facilities

Imaging Cores – Electron

Staining of grids SOP

This SOP outlines the staining of grids with uranyl acetate and lead citrate for TEM, and the handling and use of staining compounds.

Reagents used in this procedure are very toxic by inhalation and if swallowed. The uranyl acetate is radioactive.

Use of PPE is required (Nitrile gloves and wear lab coat) for skin and body protection. Hygiene measures include the hands wash immediately with warm water and soap after handling uranyl acetate and lead citrate compounds.

All work with the staining solutions must be performed in a fume hood, and all bottles containing uranyl acetate and lead citrate solutions must be labeled and dispose the waste according to the RLSS.

Staining Solutions Preparation

2% (w/v) of aqueous uranyl acetate

1. Weigh 2 g of uranyl acetate (UA) under the fume hood wearing protection and add it to the 100 ml volumetric flask. Pipette 98 ml of near-boiling CO2 -free double-distilled water into the flask with the UA.

2. Place on stirrer until UA dihydrate crystals are dissolved. After dissolving let the solution cool down to room temperature.

3. Filter the UA through the Whatman #1 filter into the 200ml amber glass bottle (light protection) and cap tightly and label. This solution can be stored for up to 2 months at 4°C.

4. Rinse contaminated glassware into a waste bottle for UA solution and store separately from laboratory glassware.

0.25% Lead Citrate

Weigh 0.05 g of lead citrate on a balance.

Add to 20 ml scintillation vial with white cap.

Add 2 ml of NaOH, mix until dissolved and add boiled double-distilled water to make 20 ml.

Cap tightly, label the bottle and store refrigerated. Can be used for up to 2 months. Do not leave the bottle open as the lead will precipitated with CO_2.

1N NaOH (Prepare fresh each time)

Weigh 0.81 g (~ 8 pellets) in 20 ml scintillation vial with white cap.

Add 20 ml of boiled warm ddH20. Mix well and label the bottle. Use fresh.

Note: Handle all these reagents with care and wear protection according to the LCHP in the core.

Before Staining

1. Staining is performed on 3 grids/sample. Make sure that the sections on the grids are on top (bright side) and placed on the side of the coated grids stick that has the glue.

2. Check the solutions date and replace with freshly prepared every 4 to 6 weeks as needed and record the dates.

3. All material needed, including but not limited to glass pipettes, flow-limiting bulbs, tweezers, filters, filter paper etc. must be very clean prior each use to avoid contamination and staining artifacts.

4. Make sure that the coated grids sticks are prepared with the appropriated amount of glue, to avoid a weak or strong attachment of the grids to the stick that will damage them. Coat the grids stick in advance to have them ready to use.

Procedure

- ☐ Under the magnifier lamp place 3 grids/sample with the sections on top on the coated grid stick, following the order on the grid box for sample location accuracy.

- ☐ Transfer the coated grids stick to the glass staining pipette with the flow-limiting bulb used only for the uranyl acetate staining.

- ☐ Transfer the staining pipette with the grids to a tube containing 2 % uranyl acetate solution, filtered with a 0.22 µm filter (use a syringe) and covered with aluminum foil to avoid photosensitivity.

- ☐ Staining in 2 % uranyl acetate for 30 min at RT.

- ☐ Rinse the staining pipette with the grids in double-distilled or molecular grade water for 3 min (~100 mL), thoroughly and gentle, using the flow-limiting bulb to avoid detachment of the grids from the stick.

- ☐ Transfer the coated grid stick with a tweezer to the staining pipette used only for lead citrate staining. In a clean tube filter the 0.25 % lead citrate solution with a 0.22 µm filter (use a syringe) as before, and immediately transfer the staining pipette with the grids to the tube.

- ☐ Staining in 0.25 % lead citrate solution for 1 min at RT. If the solution has been in the fridge for 3 or more weeks, then extend the time of staining to 2 min.

- ☐ Rinse in double-distilled or molecular grade water for 3 min (~100 mL), thoroughly and gentle, using the flow-limiting bulb to avoid detachment of the grids from the stick as before.

- ☐ Let the grids dry under the heat lamp for ~ 1 - 2 min. Handle the grids with care to detach them from the coated stick and avoid damage or bent, and place them in the grid box in the order of the sample, accordingly.