EMS Microscopy Academy

Endless Possibilities ...

Kirsch notes

Preparation of Post Staining Solutions

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Post staining of thin sections is used to increase contrast in TEM images. Typically this is accomplished in a two-step process using uranyl acetate, or a uranyl replacement, followed by a lead citrate solution.

EMS Catalog Supplies

Uranyl acetate #22400
Uranyl replacement #22405
Lead citrate #17800
Lead nitrate #17900
Sodium citrate #21140

Uranyl Acetate

Uranyl Acetate (UA) is an excellent general stain, adding high resolution contrast to membranes, nucleic acids, and proteins. It is a depleted uranium compound, less U 235 than natural uranium, but is still radioactive and will exhibit 0.51µCi·gm-1 (µCi = microcuries) of activity and is also chemically toxic, so care should be taken during handling. Besides being toxic and radioactive it is also sensitive to light, photo-labile, so must be protected from light, in storage and during use. It can degrade over time and should not be used if a cloudy precipitate forms.

UA solutions are sometimes used as an enbloc stain during processing, providing inherent contrast to the sample . Serving as a tertiary fix, after glutaraldehyde and osmium tetroxide it provides excellent preservation of membranes, DNA filaments, mitochondria and proteins and decreases some extraction during dehydration. This procedure can be done with 0.5-2.5% UA in an aqueous or 50% ETOH solution. When used in 50% ETOH it serves as the initial step in dehydration as well.

When UA is used as a post stain for thin sections it can be used on its own or as the first stain when employing lead citrate in a double staining procedure. As with the enbloc UA it is often aqueous or in a 50% ETOH or Methanol solution. Both alcoholic solutions penetrate into section more easily than the aqueous solution, reducing the staining time and potential stain precipitates. Typical staining times for these stains range from 7 – 15 min at room temp. Elevated temperatures (40°-70° C) reduce the staining times but can also increase the risk of stain contamination. Aqueous solutions of the same concentration are often employed for up to 30 minutes on epoxy sections and can also be used at elevated temperatures.

Preparing Uranyl Acetate

EXAMPLE: You need ... 25 ml of 2.5% UA How to calculate: 025 x 25 ml (dH2O or 50% ETOH) = 0.625 g

1. Measure and pour 25 ml of dH_2O ***or*** 50% ETOH into a dark stoppered bottle.
2. Add 0.625 g of uranyl acetate.
3. Drop in stir bar, seal with cap.
4. Place on stir pad, cover from light.
5. Mix for 30 minutes.
6. Remove and store in dark until use.

NOTE: When making the aqueous version of UA do not use “extra” purified water as it may cause more stain contamination.

Using Uranyl Acetate for Post Staining

Items needed: Petri dish cover, parafilm, filtered UA, dark cover, 3 rinse beakers, tweezers

1. Place appropriate size piece of parafilm on work surface.
2. Place 1 drop of filtered UA for each grid on parafilm surface.
3. Float grids section side down on top of stain drop.
4. Cover with Petri dish and dark cover.
5. After elapsed time remove grid and rinse by dipping vertically 10 times in first beaker containing same solvent as the stain, dH20 or 50% ETOH.
6. Repeat in remaining beakers containing dH2O.
7. Blot dry and store.

Lead Citrate Post Stain

Lead stains provide high contrast in most cellular components. Osmium act as a mordant for lead stains, so any structures with unsaturated lipid groups will have both Os and Pb preferential staining. Lead stains react strongly with trace amounts of carbon dioxide forming insoluble lead carbonate precipitates on stained sections. To avoid this, many procedures are taken to eliminate CO_2 from the mix: Water (2x distilled) used to make and rinse off the staining solution should be boiled for 10-15 minutes prior to use and kept air tight, the contained area for staining should be a high pH environment and protected from environmental CO_2, and the first rinse solution should be a beaker of 0.02 M NaOH.

Lead stain is very poisonous and pH is very high ~12, so care must be taken when making, working, and disposing of these solutions.

There are many recipes to make lead citrate stain with the most common being Reynolds 1963 method combining lead nitrate with sodium citrate to form lead citrate. Another more-straight forward method was developed by Venable and Coggeshall 1965 and Fahmy 1967 using just lead citrate. A more recent is a modification of Sato's lead by Takamasa Hanaich, uses all 3 of the lead compounds to form a more stable solution.

Reynolds Lead Citrate Stain

1. Add 1.33 g lead nitrate + 1.76 g sodium citrate to ~ 30 ml of boiled 2x distilled water in a 50 ml volumetric flask.
2. Shake vigorously for 1-2 minutes, and 3-4 more times over 30 minutes to ensure complete conversion of lead nitrate to lead citrate.
3. Add 8.0 ml of 1N NaOH and bring to 50 ml.
4. Invert and store in sealed bottle and is stable for at least 6 months.
5. Lead solution should be filtered or centrifuged before use.

Fahmy Lead Citrate Stain

1. Add 1 pellet (0.1-0.2g) of NaOH to 50 ml boiled 2x distilled water and shake to dissolve.
2. Add 0.25 g of lead citrate and shake to dissolve.
3. Final pH is about 12 and stable for at least 6 mo. Filter or centrifuge before use.

Staining with Lead Citrate

Needed: Parafilm, Petri dish cover, filter paper, 0.1N NaOH, 3 rinse beakers, .02 N NaOH, NaOH pellets, and boiled distilled water.

1. Soak filter paper with 0.1N NaOH, place in Petri dish.
2. Place parafilm on filter paper.
3. Peak open Petri dish cover and place 1 drop for each grid on the Parafilm and replace cover.
4. Slightly tilt cover open and float grids, section side down, on stain drop.
5. When time is up ~ 7-10 minutes remove grids and rinse in first wash beaker containing 0.02 N NaOH by dipping vertically 10 times.
6. Repeat with 2 remaining beakers containing boiled distilled water. Blot dry and store.

A Stable Lead by Modification of Sato's Method Lead Staining Solution

Calcined lead citrate is a long-term storage lead solution. Since the first introduction by Watson (1958), various lead stains have been developed in order to increase contrast and reduce contamination in sections for electron microscopy. One of the disadvantages of lead stains developed to date is that the staining solution is apt to produce precipitates of lead carbonate, resulting in difficulty for long-term storage of the stains. Now there is a new stable lead solution which is free from precipitates when kept at room temperature for over 1 year.

The stock lead solution is made up as follows:

Calcined Lead Citrate	0.20 g
Lead nitrate	0.15 g
Lead acetate	0.15 g
Sodium citrate	1.00 g
Distilled water (boiled)	41.00 ml

The calcined lead citrate is obtained by heating crystal lead citrate for several hours in a melting pot (200°C to 300°C) until the color changes to a light brownish yellow. **NOTE:** Overheated lead citrate with a dark brownish or black color can't be used.

The above reagents are placed in a 50 ml volumetric flask and mixed well to produce a yellowish milky solution. Then 9.0 ml of 1N NaOH is added to the solution and mixed well until the solution becomes clear with a light yellowish color. The solution is then transferred to an amber glass with a screw cap bottle for storage. The solution can be stored at room temperature or in the refrigerator for over 1 year.