

Sticky Bar Staining Protocol

For Use with Uranyl Acetate Stain and Lead Citrate Stain

Make sure you have freshly boiled then cooled distilled water to use for rinsing and making up Lead stain.

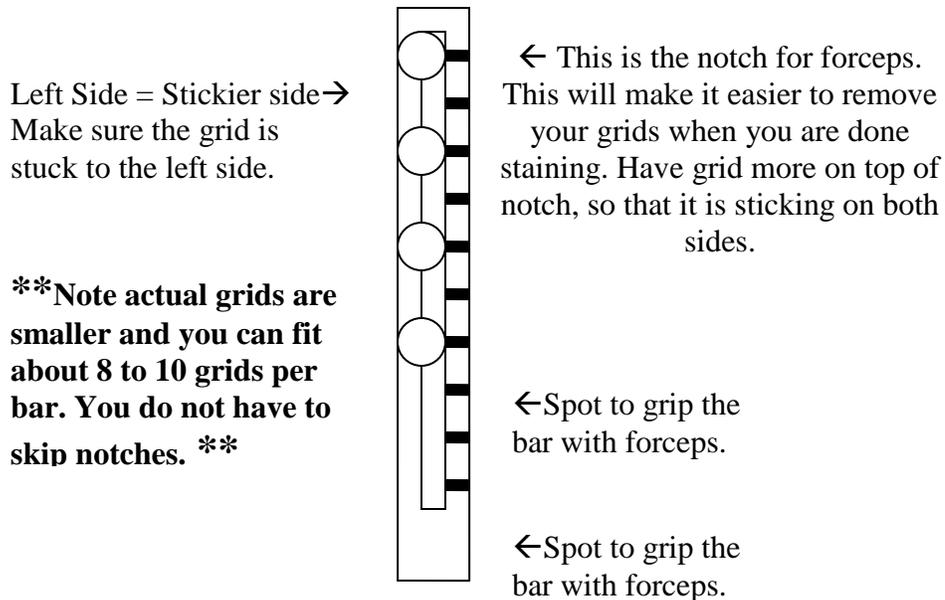
Supplies

- ~2 new 3 or 6 cc syringes
- ~ 2 new .22µm filters.
- ~15 mL centrifuge tube for Uranyl Acetate
- ~1 glass Pasteur pipet and rubber bulb
- ~15 mL centrifuge tube for Lead Citrate Stain
- ~ 4 1mL pipettes for making up Lead Citrate Stain
- ~2 100mL or 150mL beaker, filled with boiled and cooled distilled water
- ~1 pair of forceps
- ~Sticky Bar Staining Kit
 - Sticky Bar, Rubber pipette tips, a glass Sticky Bar Pipette and two glass vials for Uranyl Acetate Stain, and a glass Sticky Bar Pipette and two glass vials for Lead Citrate Stain. *Only use Uranyl Acetate supplies for Uranyl Acetate Stain and Lead Citrate Supplies for Lead Citrate Stain. **DO NOT MIX-UP!***
- ~ 25% HCl (Found in Acid Cabinet in Lab 1-225)

Wear Gloves!!!!

- 1.) Using a new glass Pasteur pipette with a rubber pipette top, remove a sufficient amount of 8% Uranyl Acetate from one of the bottles on the bench top into a 15mL centrifuge tube. **DO NOT** tip the bottle, disturb the precipitate, and **AVOID** the small area of thin film on top of the solution. The amount you use will depend on how many grids you are staining. Keep in mind: 3mLs of Uranyl Acetate stains 8-10 grids and counts as 1 kit.
- 2.) Make up the lead citrate stain using the directions for the Modified Reynold's Stain Working Solution on the cupboard door. Make up the stain in a 15mL centrifuge tube. You will use four one milliliter pipettes to measure out the different solutions: one pipette for each individual stock solution (A and B), one for 1 N NaOH, and one for freshly boiled and cooled water.
- 3.) Take the centrifuge tube with Uranyl Acetate and the centrifuge tube for Lead Citrate into the main lab (1-225). Centrifuge the vials for **3 minutes** on **level 3**. Bring the tubes back into the staining room and set them aside.
- 4.) To set up a syringe and filter, remove the syringe plunger, attach a filter to the end, and set the syringe/filter on top of a Uranyl Acetate vial. Carefully pipette Uranyl Acetate (with the glass pipette from earlier) from the centrifuge tube into the syringe. Put the plunger back into the syringe and **SLOWLY** filter the Uranyl Acetate into the vial. When it is all filtered, put the cap on the vial and set it aside.

- 5.) Repeat step four with the Lead stain. NOTE: After the stain is filtered, evaluate the cloudiness of the solution. If the solution is not clear, put the syringe/filter onto another Lead Citrate vial, and re-filter the solution into the new vial. Repeat as necessary. Finally, put the cap on the vial and set it aside.
- 6.) Tape a piece of 9cm round filter paper to the top of a plastic Petri dish half (This creates a flat, elevated surface). Use forceps to remove a sticky bar from its magnet and place it on the filter paper (sticky side up).
- 7.) Using forceps, place your grids onto the sticky bar. You want to place the grids tissue side up (dull side down) on the sticky bar. Anchor the grid to the left side (stickier side) of the sticky bar and secure it to the glue drops on the right side of the bar. Position your grids so that the middle of the grid is over the notch for forceps.

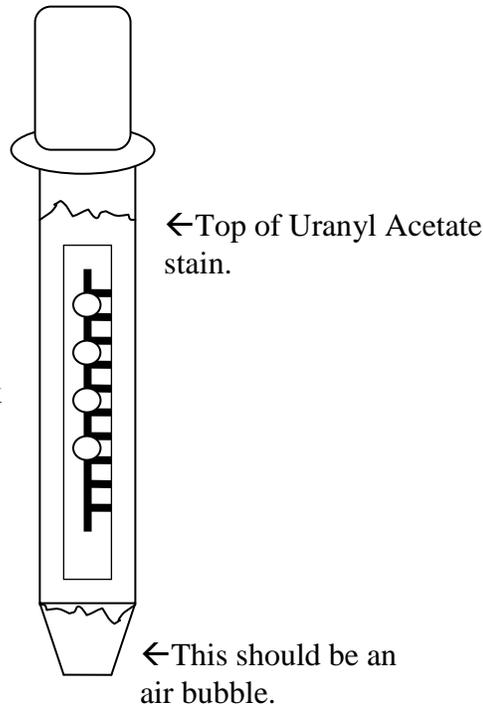


***Make sure the grids are all stuck to the bar. You do not want grids to fall off in the middle of staining. You should make a chart so you know the order of the grids and can put them back in their proper places in the grid box.

- 8.) Use forceps to place the sticky bar into the Uranyl Acetate glass pipette. Tap the pipette gently so that the bar falls to the bottom of the pipette. Attach the rubber bulb.
- 9.) Slowly pipette Uranyl Acetate up to cover the entire sticky bar. Then, let out one drop so there is an air bubble at the tip of the pipette. Do not invert the pipette. Place the pipette in an empty Uranyl Acetate vial and let it sit for 15 minutes or the desired amount of staining time. During the wait, fill a 100mL beaker with freshly boiled, then cooled distilled water.

****Please note that the pictures are not drawn to scale****

This is what the pipette should look like once you let out a drop of Uranyl Acetate. Make sure the Sticky Bar is still completely covered.



- 10.) Once the timer goes off, hold the pipette completely vertical over the Uranyl Acetate waste container and slowly expel the Uranyl Acetate from the pipette. Gently draw water from the beaker (50-100mL) filled with boiled distilled water into the pipette and slowly release it into the Uranyl Acetate waste container. Rinse the sticky bar pipette with the boiled, then cooled distilled water **5 times**. Make sure to hold the pipette straight up and down, this is so that your grids and the sticky bar are getting completely rinsed.
- 11.) After rinsing, use forceps to take the sticky bar out of the Uranyl Acetate pipette. You may need to tap the pipette to get the bar to fall to the opening. Put the sticky bar into the Lead stain pipette and attach a rubber bulb.
- 12.) Slowly pipette up Lead Stain in order to cover the entire sticky bar and create an air bubble at the tip of the pipette. Let the pipette sit in the empty Lead stain vial for 2 minutes or desired amount of staining time. Fill another 100mL beaker for rinsing during the wait time.
- 13.) When the timer goes off, release the Lead stain into the Lead Citrate waste container and rinse **5 times** with distilled water. When you are done rinsing, take the sticky bar out of the pipette and set it on the filter paper to dry for at least 20 minutes.
- 14.) When your grids look dry, carefully remove them with forceps. There is a notch on the sticky bar that will make it easier to remove your grids. Gently remove the grids and put them in your grid box. Place the sticky bar back inside its box.

Clean Up

Syringes/filter combinations need to be rinsed **3x** with distilled water into the correct waste container. Syringes can be thrown away and the filters can then be disposed of into the red Uranyl Acetate Syringe container.

Glass or plastic pipette and centrifuge tube used for Uranyl Acetate need to be rinsed **3x** with distilled water into the Uranyl Acetate waste container. If a glass pipette was used, put it in the glass waste container in the main lab (Rm 1-225). The centrifuge tube can be thrown away after rinsing.

Glass or plastic pipettes that were used to make up the Lead Citrate stain need to be rinsed **3x** with distilled water. Rinse the pipettes used for the stock solutions and the NaOH pipette into the Lead Citrate waste. If the pipettes are glass, dispose of them in the waste glass container in the main lab (Rm 1-225)

The vials and sticky bar pipettes used for Uranyl Acetate and Lead Citrate need to be rinsed **3x** with distilled water into the right waste containers and proceed to acid washing.

Acid Washing

- 1.) Take the bottle of 25% HCl from the Acid Cabinet in the main lab and bring it into the staining room.
- 2.) Rinse the vials and sticky bar pipettes with 25% HCl in the sink.
 - i. Reattach rubber bulb to sticky bar pipette, and pipette HCl up in order to rinse the inside.
 - ii. Using an acid-washed sticky bar pipette, apply HCl to around the inside of its corresponding vial until it is $\frac{1}{4}$ - $\frac{1}{2}$ full with HCl. Pipette up and down so that the sides of the vial are getting rinsed with HCl.
 - iii. Run the faucet water **lightly** in the sink when pouring the HCl down the drain.
- 3.) Repeat steps i-iii **2x** for each vial and pipette.
- 4.) Rinse each vial **3x** with distilled water after the HCl. Set the vials aside to dry.
- 5.) Repeat for the Lead Citrate items. Make sure to keep the Lead Citrate vials/pipette and Uranyl Acetate vials/pipette separate.

Dry off the sink with Paper Towels or KimWipes when you are done using it.

Put the HCl back in the main lab. Put used lab wares in the dish rack by the sink in the main lab.

Make sure everything is straightened and set up the way it was when you entered the Staining Room.

Thank You!

