

Negative Stain Protocol

WILLIAM ARNOLD

Table of Contents

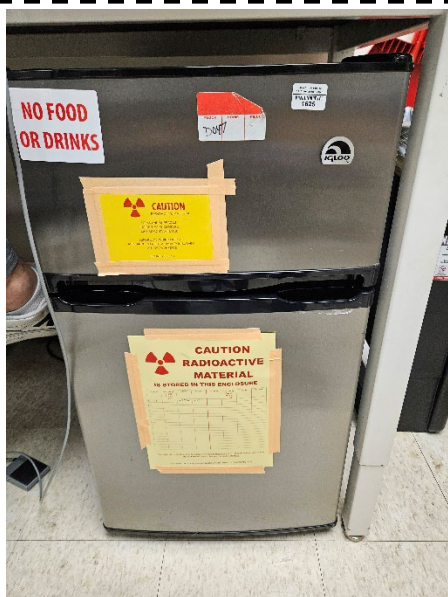
Uranium-238 Radioactivity.....	2
Materials.....	2
Protocol.....	3

Uranium-238 radioactivity

Users who want to do negative staining with U-238 need to take EHS-5250 for radiation safety training. U-238 is mildly radioactive, emitting alpha particles that are easily blocked by gloves and the outer layer of dead skin. Uranium-238 is most dangerous if ingested, and relatively safe otherwise. Users should not feel alarmed working within or around the radiation area. However, users should exercise caution and adhere to the radiation procedures by EHS. Dispose of anything that touched uranium in the radioactive waste bin. As we can only throw away solid waste, liquid waste needs to be absorbed onto a filter paper prior to disposal. DO NOT dispose of any other radioactive substance into our waste other than U-238. Users planning on working with radioactive materials (including U-238) need to take EHS-5250 Radiation Safety prior to using materials. **Always dispose radioactive waste into the radioactive waste container (dry waste only; soak up liquids with filter paper).**

Materials

1. Negative Stain (uranyl acetate 1%) is kept in the fridge in the Sample Prep Room. Aliquots with dates will be in a black 15-mL conical tube to protect from light.



2. Other materials are found on the Negative Stain Bench. Note: Only use the pipettes and tweezers that are located on the negative stain bench.



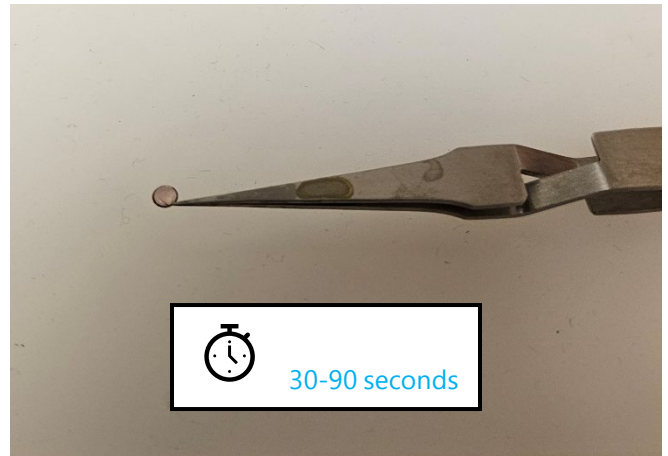
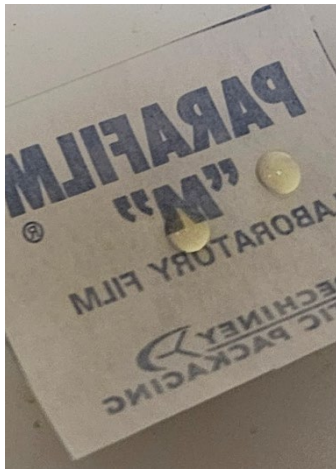
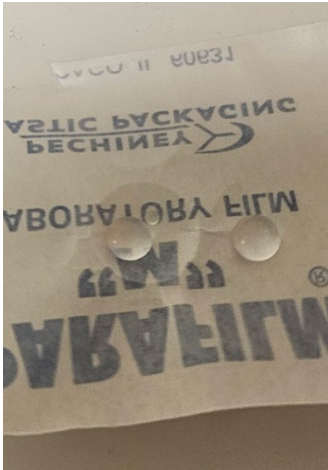
Protocol

3. Grab two pieces of filter paper two pieces of parafilm and arrange as shown in the picture above. The left side will be for non-radioactive liquid and the right for uranyl acetate (radioactive). Tip: Fold up one end of the filter paper so that 1) the liquid stays in the filter paper, 2) it is easier to pick up later, and 3) it is easier to blot dry (see below). Tip 2: You can make indents in the parafilm to better hold the liquid drops by pressing the film onto the Eppendorf tube rack as shown to the right.



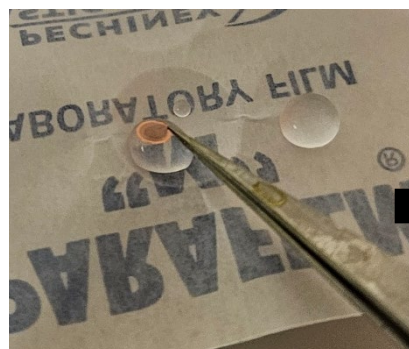
4. Apply 2 x 30 μ L drops of water per grid (can use buffer, but ultrapure water is best) onto the left parafilm. Place 2 x 30 μ L drops of uranyl acetate per grid on the right parafilm. Place the drops in the divots, if desired.

5. Use negative-pressure tweezers to grab and hold onto a grid. Apply 3 μ L onto carbon-side of grid. Apply to grid for 30-90 sec (45 sec works well). Final concentration should be ~ 0.03 mg/mL, or about 1/100th the concentration used in cryo.



6. Side-blot the grid onto the non-radioactive filter paper. Completely blot away the liquid.

7. Wash the grid by touching the carbon/sample side to the first water drop and then side-blotting the liquid away. Repeat for the second drop.



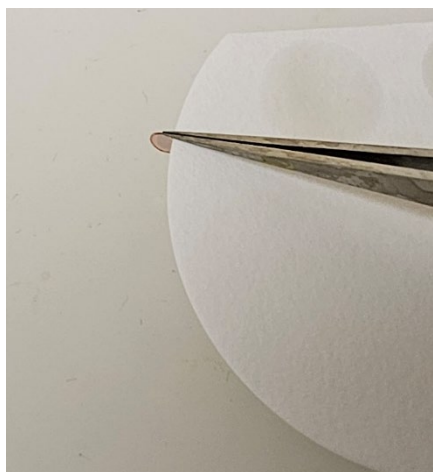
8. Wash with the first drop of uranyl acetate like the water in Step 7. Blot on the radioactive filter paper.



9. Touch the grid to carbon/sample-side down to the 2nd drop of uranyl acetate. Hold in position for 30-90 sec (45 sec works well).



10. Side-blot away the liquid like before. Blot where the tweezers touch the grid using the edge of the filter paper to remove any liquid trapped there.



11. Repeat Steps 5 to 10 with the remaining grids.

12. Non-radioactive waste can go into normal trash. Soak up remaining uranyl acetate onto filter paper and dispose in the radiation waste. Dispose of anything that touched uranium (including gloves) into the radiation waste.

