



Purpose: To create negative stain for future room temperature microscopy.

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1. Purpose:

- 1.1. Negative stain sample preparation for future room temperature microscopy.

2. Scope:

- 2.1. There are three stains regularly made at NCCAT for negative stain workflow: Uranyl Formate (UF), Uranyl Acetate (UA) and Phosphotungstic Acid (PTA).

3. Definitions

- 3.1. For negative stain the background is stained leaving the actual specimen untouched and thus visible for room temperature microscopy.
- 3.2. To have stains made, stored properly and safely so they will be ready for use and not degrade/precipitate ahead of use.

4. Responsibilities:

- 4.1. Uranyl Formate (UF) is a light sensitive salt kept in solution at pH 4-5.
 - 4.1.1. Fill a beaker with 10mL of ddH₂O.
 - 4.1.2. Place on a hot plate until water boils.
 - 4.1.3. Grab a LD4 and fill with LN₂.
 - 4.1.4. Label a new sample box with UF 2%, the date, the pH and your initials.
 - 4.1.5. Place the sample box in the foam bucket and fill with LN₂ until the inserts of the sample box are filled halfway.
 - 4.1.6. Fill the outer slots of a tube rack with black 0.5mL centrifuge tubes.
 - 4.1.7. Attach the filter to the 10mL Luer Lock syringe and place in a beaker.
 - 4.1.8. Grab a black 15mL centrifuge tube, remove the cap, and place it in another beaker.
 - 4.1.8.1. Place them on a weight scale and tare.
 - 4.1.9. Measure out 0.2 grams of UF into the black 15mL centrifuge tube.
 - 4.1.9.1. Record UF usage in the blue radioactive binder.
 - 4.1.10. Measure out 10 mL of boiled ddH₂O using a graduated cylinder and add it to the black 15mL centrifuge tube that contains the 0.2 grams of UF.
 - 4.1.10.1. Cap the tube and vortex at medium-to-high speed for a few minutes; occasionally stopping and shaking back and forth.
 - 4.1.11. Remove the plunger of the Luer Lock syringe and pour in ~ 5mL of UF.
 - 4.1.12. Place the filter tip into the first 0.5mL centrifuge tube on the rack and slowly place plunger into the barrel of the syringe.
 - 4.1.12.1. Pressure will cause the solution to drip out of the filter tip, so be cautious.
 - 4.1.13. Move the syringe along the edge of the rack, filling each black 0.5mL centrifuge tube with about 6-7 drops.
 - 4.1.14. When the syringe is empty, cap the filled centrifuge tubes and place them in the LN₂ filled slots of your sample box.
 - 4.1.15. Remove the syringe plunger and pour in the remainder of the UF.



- 4.1.16. Store the stain in a -80C freezer when done.
- 4.2. Uranyl Acetate (UA) is a light sensitive salt kept in solution at pH 4
 - 4.2.1. Fill a beaker with 10mL of ddH₂O.
 - 4.2.2. Place on a hot plate until water boils.
 - 4.2.3. Grab a LD4 and fill with nitrogen.
 - 4.2.4. Label a new sample box with UA 2%, the date, the pH and your initials.
 - 4.2.5. Fill the outer slots of a tube rack with black 0.5mL centrifuge tubes.
 - 4.2.6. Attach the filter to the 10mL Luer Lock syringe and place in a beaker.
 - 4.2.7. Grab a black 15mL centrifuge tube, remove the cap, and place it in another beaker.
 - 4.2.7.1. Place them on a weight scale and tare.
 - 4.2.8. Measure out 0.2 grams of UA into the black 15mL centrifuge tube.
 - 4.2.8.1. Record UA usage in the blue radioactive binder.
 - 4.2.9. Measure out 10 mL of boiled ddH₂O using a graduated cylinder and add to the black 15mL centrifuge tube that contains the 0.2 grams of UA.
 - 4.2.9.1. Cap the tube and vortex at medium-to-high speed for a few minutes; occasionally stopping and shaking back and forth.
 - 4.2.10. Remove the plunger of the Luer Lock syringe and pour in ~ 5mL of UA.
 - 4.2.11. Place the filter tip into the first 0.5mL centrifuge tube on the rack and slowly place plunger into the barrel of the syringe,
 - 4.2.11.1. Pressure will cause the solution to drip out of the filter tip, so be cautious.
 - 4.2.12. Move the syringe along the edge of the rack, filling each black 0.5mL centrifuge tube with about 6-7 drops.
 - 4.2.13. When the syringe is empty, cap the filled centrifuge tubes and place them in your sample box.
 - 4.2.14. Remove the syringe plunger and pour in the remainder of the UA.
 - 4.2.15. You may store the stain at room temperature when done.
- 4.3. Phosphotungstic Acid (PTA) (metal) 2% solution of neutral PTA (buffered to pH 7 using sodium hydroxide if not pH 7 already)
 - 4.3.1. Fill a beaker with 10mL of ddH₂O.
 - 4.3.2. Place on a hot plate until water boils.
 - 4.3.3. Label a new sample box with PTA 2%, the date, the pH and your initials.
 - 4.3.4. Fill the outer slots of a tube rack with black 0.5mL centrifuge tubes.
 - 4.3.5. Attach the filter to the 10mL Luer Lock syringe and place in a beaker.
 - 4.3.6. Grab a black 15mL centrifuge tube, remove the cap, and place it in another beaker.
 - 4.3.6.1. Place them on a weight scale and tare.
 - 4.3.7. Measure out 0.2 grams of PTA into the black 15mL centrifuge tube.
 - 4.3.8. Measure out 10 mL of boiled ddH₂O using a graduated cylinder and add to the black 15mL centrifuge tube that contains the 0.2 grams of PTA.
 - 4.3.8.1. Cap the tube and vortex at medium-to-high speed for a few minutes; occasionally stopping and shaking back and forth.
 - 4.3.9. Remove the plunger of the Luer Lock syringe and pour in ~ 5mL of PTA.
 - 4.3.10. Place the filter tip into the first 0.5mL centrifuge tube on the rack and slowly place plunger into the barrel of the syringe.
 - 4.3.10.1. Pressure will cause the solution to drip out of the filter tip, so be cautious.



- 4.3.11. Move the syringe along the edge of the rack, filling each black 0.5mL centrifuge tube with about 6-7 drops.
- 4.3.12. When the syringe is empty, cap the filled centrifuge tubes and place them in your sample box.
- 4.3.13. Remove the syringe plunger and pour in the remainder of the PTA.
- 4.3.14. You may store the stain at room temperature when done.

5. Personal protective Equipment (PPE):

- 5.1. Laboratory coat
- 5.2. Nitrile gloves
- 5.3. Goggles

6. Chemicals:

- 6.1. Uranyl Formate
- 6.2. Uranyl Acetate
- 6.3. Phosphotungstic Acid
- 6.4. Isopropyl Alcohol 70%

7. Equipment

- 7.1. 3 beakers ~100mL
- 7.2. LD4
- 7.3. Hot plate
- 7.4. Large Foam Bucket
- 7.5. Test Tube Rack
- 7.6. Sample box (with grid insert)
- 7.7. Black 0.5mL centrifuge tubes
- 7.8. Black 15 mL centrifuge tube
- 7.9. 10 mL Luer Lock syringe
- 7.10. 0.2um PVDF Filter Media (25mm)
- 7.11. ddH₂O
- 7.12. Plastic Pipette
- 7.13. Radioactive Binder
- 7.14. Weight Scale
- 7.15. Spatula

8. Waste Disposal:

- 8.1. All residual Uranyl stains to be washed out from all syringes, filter, centrifuge tubes with ddH₂O and disposed in a designated waste disposal area such as a bottle properly labeled with chemical name.