



Negative staining. NCCAT specific procedures.

Safety

General Precautions:

- Use the Personal Protective Equipment (PPE) provided by NCCAT: nitrile gloves, goggles (or eyeglasses), and a lab coat the extends to the wrists.
- Inspect equipment and tools for damage before beginning work. If you suspect any damage, or encounter any problems during use, please fix immediately and ask for help from staff if needed.
- Report all accidents, no matter how minor.
- *You must have read and signed the NYSBC Standard Operating Procedure for Work with Uranyl Salts, even if using only non-uranyl stains.*

Mandatory PPE for working with negative stains



General negative stain bench use

- There is a designated set of pipettors, and tweezers/forceps at the negative stain bench, labeled for negative stain use. This is to prevent contamination of radioactivity into other areas of the lab.
- **Please use only the designated pipettors and forceps while working at the negative stain bench and do not move these tools to other areas of the lab.**



Figure 1. Set up of the negative stain bench. Leave it exactly as you found it at the end of your session.



Site specific additions to the general SOP:

3.1.1 Stains

Aliquots of 2% UA and PTA are in boxes on the negative stain bench (Figure 1). Aliquots of 2% UF are stored in the -80 freezer in the SEMC lab. Stains are aliquoted into opaque black tubes, intended as working concentration solutions for single use. Do not put partially unused tubes back in the boxes. When you are done, please dispose of all tubes as hazardous waste.

3.2.2 Glow discharge.

We typically glow discharge continuous carbon grids used for negative stain on the Solarus Plasma System, located in the SEMC lab. The “Carbon Film Standard” recipe (30 s of plasma using H₂ and O₂) should be sufficient for most grids.

3.5 Cleaning up

3.5.1./3.5.3.

All non-sharps materials (parafilm, kimwipes, blot paper, gloves) that have had contact with uranyl or other heavy metal stains should be discarded in the red-bag lined cardboard biohazard waste bin to the left of the negative stain bench (Figure 3).

Pipette tips should be disposed of as sharps in the hard sided container on the bench (Figure 3)

4.1 Waste disposal

See section 3.5

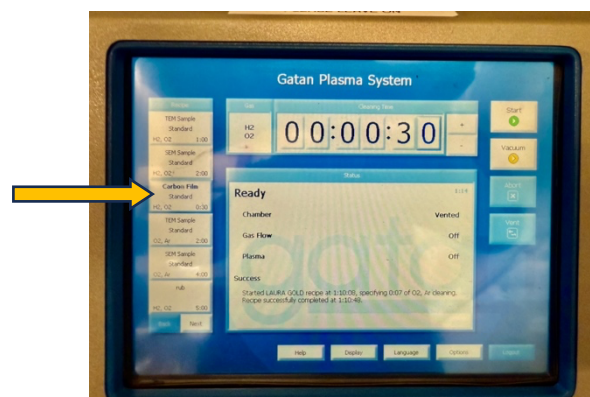


Figure 2. Use the "Carbon Film Standard" recipe for most negative stain applications.

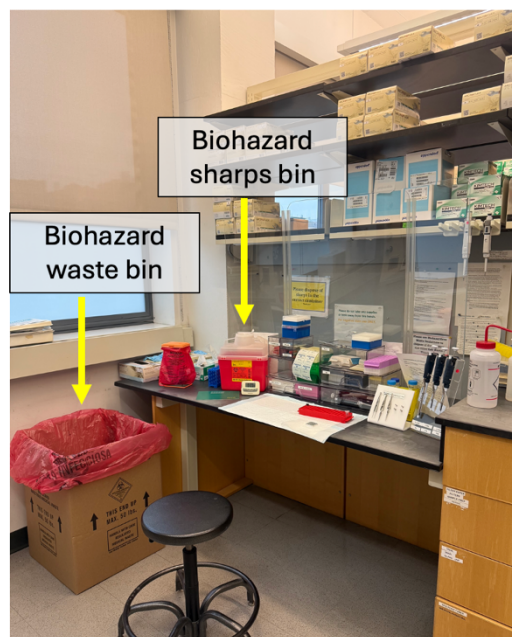


Figure 3. Dispose of all materials into waste bins at the negative stain bench.