HARVARD
Campus Services
ENVIRONMENTAL HEALTH & SAFETY

Cryostat Standard Operating Procedure and Safety Guidelines

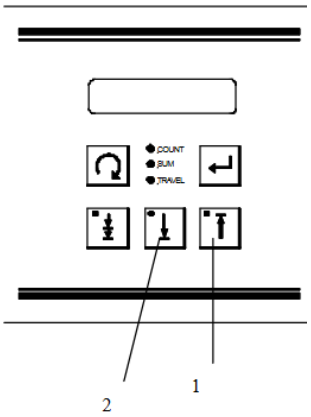
Cryostats are commonly used in laboratories for frozen tissue sectioning. This equipment poses sharps, biological (infectious/recombinant materials), ergonomic, and cryogenic hazards for the user during use, including the cleaning process. This document outlines basic operating and safety procedures for work with cryostats. Sharps safety must be emphasized. All manipulations of the blade must be done mechanically (not by hand) when possible, with the handwheel locked, and cut resistant gloves in use when engineering and work practice controls provide insufficient protection. Mechanical techniques and proper personal protective equipment (PPE) also reduce risks of frostbite and exposure to the other hazards present. For uses other than tissue sectioning, reach out to your EHS Lab Safety Advisor for a risk assessment. In the event of any injuries/exposures, immediately notify your supervisor and have them complete an incident report as soon as possible.

SET-UP

1. **Before operating the cryostat, you should be trained in this standard operating procedure by your Principal Investigator (PI) or a trained and experienced operator. Certify at the end of this document that you have been trained.**
    a. Persons are to be supervised until the trainer determines they are qualified to handle the operation on their own. **Note that safety of laboratory personnel is ultimately the responsibility of the supervising PI.**
2. Don appropriate PPE for operation of the cryostat, such as eye protection, lab coat (if BL2), and disposable gloves. Cut resistant (Kevlar or stainless-steel mesh) gloves should be available.
3. Set and cool the cryostat chamber to the desired temperature. Then cool tissue blocks, brushes, forceps, and any sectioning tools. **ENSURE THE HANDWHEEL IS LOCKED BEFORE PLACING HANDS INSIDE THE CHAMBER.**
    a. **The handwheel must be manually locked. A pin or button is usually situated on the rim of the handwheel, which needs to be pushed to lock the wheel. The blade should not be present in the chamber until immediately before sectioning. Review your manual for locking procedures, if this pin/button is not present.**
4. When ready to commence sectioning, with the handwheel locked, position the tissue block.
5. USING CAUTION, mechanically (e.g. with forceps or a magnet) place the blade in the holder and clamp to secure. Once hands are out of the chamber and the sliding window closed, the handwheel can be released and the blade advanced to the specimen block.
6. Review the various functions of the controls on your instrument with your trainer prior to sectioning.

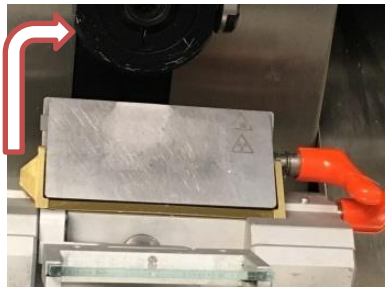
****If manual adjustments to the tissue block must be made later, the handwheel needs to be locked and the blade guarded.**

OPERATION/SECTIONING



While sectioning, the sliding window needs to remain closed. The handwheel *must be locked prior* to opening the window and the *blade immediately guarded or removed* before performing manipulations inside the chamber.

1. For sectioning, pre-select the desired section thickness using the controls.
2. Put the anti-roll plate against the blade using the knob. Turn the handwheel in a clockwise direction to carry out sectioning.
3. The section slides into the space between the blade and the anti-roll guide. Remove the anti-roll guide by means of the knob. **With the handwheel locked**, transfer section to a slide using a brush or forceps; **do not use fingers to manipulate the section.**
4. For additional adjustments to the plane of the tissue block, first attempt to use the motorized course feed system (examples, arrows 1 & 2).
    a. If manual adjustment is absolutely necessary, lock the handwheel, don cut resistant gloves, and guard the blade (flip guard upward toward blade).
    b. If blade guard is unavailable, mechanically (with forceps or a magnet) remove the blade before making adjustments to the tissue block. Replace and secure blade before releasing handwheel.



5. Be sure to take periodic breaks to prevent errors due to ergonomic fatigue.
6. **In case of an emergency during sectioning, immediately cease activity until the hazard has been resolved.**

CLEAN-UP

1. Lock the handwheel, don cut resistant gloves, and mechanically remove the blade. **Never manipulate a blade with your fingers.**
2. Dispose of unwanted blades in a biological sharps container.
3. Remove the tissue block.
4. Use a large brush to sweep tissue sections into the drop pan below the head. **NEVER USE FINGERS TO SWEEP CHAMBER SURFACES.**
5. Generally, dH_2O or pure ethanol can be used to rinse the specimen disks, brushes, and other tools. Check the manual.
    a. **Treat all cryostat cold chambers as biologically contaminated until the cryostat can be turned off for thorough disinfection with an appropriate disinfectant.** A cryostat must be marked with a biohazard sticker when used with materials requiring BL2 containment and to indicate that potential contamination exists. Review your manual for decontamination procedures and use a non-corrosive disinfectant effective against potential contaminants.
        i. **Please note that it is common for tissues that are sectioned with a cryostat to not undergo a fixation process, and these samples pose a biohazard risk to cryostat users.**
6. Ensure wastes go into the appropriate waste streams: Chemicals will be disposed as hazardous waste, used blades will go into a biological sharps container, and discarded

tissues will be processed as biohazardous waste.

7. Ensure the handwheel is locked before you leave, and the area is clean and free of sharps.

ACCESSORIES & MICROTOME USE

Many cryostats now have specialized accessories that can pose additional hazards. Some common additions can be UV crosslinkers to affix samples to slide covers and even integrated vacuums for the frozen cutting media shavings. Vacuums should have collection basins and parts that have to be cleaned/disinfected and an inline filter for the vacuum. Discuss the use of any specialized cryostat accessories with your EHS Lab Safety Advisor.

Microtomes pose many of the same physical hazards as cryostats, with the exception of the extreme temperatures; however, the manipulations and mechanism used for cutting sections is similar. Microtomes differ from cryostats as the tissue block to be sectioned normally undergoes a fixation process and is embedded in a wax block for sectioning. This may reduce the biological risk of the sample to the user. Follow the applicable guidance above when working with microtomes and ensure users are appropriately trained. Microtome users should also receive a procedure specific SOP and procedure specific training.

REFERENCES

1. COMS Policy
2. OSHA Bloodborne Pathogens Standard
3. CDC BMBL 6th edition

Cryostat Training Confirmation

The Trainees below have been educated in the proper use of the cryostats and understand the safety requirements that must be implemented.

Trainee Name (printed)	Trainee Signature	Trainer Signature	Date of Training