TED ROGERS CENTRE FOR HEART RESEARCH
Translational Biology and Engineering Program

Cryostat Standard Operating Procedure and Safety Guidelines

Background

- Cryostats are commonly used in laboratories for frozen tissue sectioning. You can cryo-section fresh or fixed tissues, and tissues are either frozen before placing in embedding compound on the cryostat stub, or are frozen after placing in embedding medium on the stub, which is the more common method.

- 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.

1. Before operating the cryostat, you should be trained in this standard operating procedure.
2. Don appropriate PPE for operation of the cryostat, such as eye protection, lab coat, and disposable gloves. Cut resistant (Kevlar or stainless-steel mesh) gloves should be available.
3. For this equipment we only use isopropanol to disinfect the inside surface.

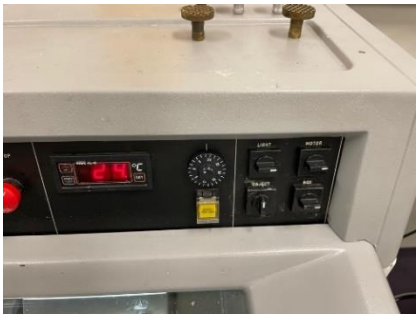
Materials/Equipment

- TissueTek or other embedding compound (viscous liquid or gel)
- fine and coarse paintbrushes to brush debris off blade and frozen tissue – these go into the cryostat before a run and stay cold
- sharp razor blades
- reasonably fine forceps – at least one pair should be in the cryostat
- sticky slides – superfrost or polylysine-coated slides
- container to collect slides with still-wet sections
- large petri dish or other to cover sections while drying – to keep off dust

Procedure

Getting Started

1. The cryostat is always on (image below); before starting, check that the temperature is correct for your tissues. At the right panel leave “object” off.



2. When you are ready to section your frozen tissue, make sure you have the complete cryostat toolkit containing forceps, razor blades, sticky slides, small paint brushes, cryostat blades, coverslips and TissueTek.

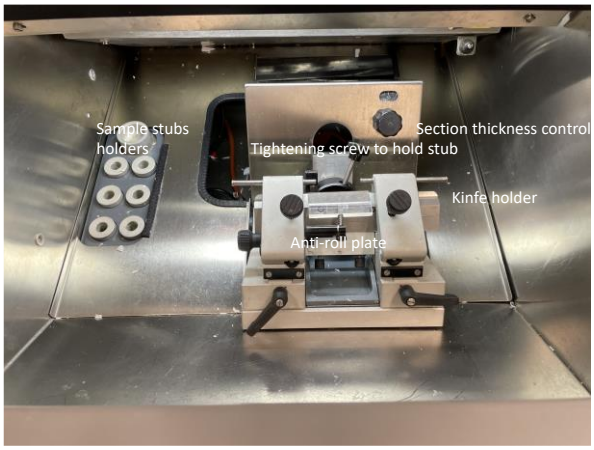
Preparing to section

3. Turn on the light in the chamber.

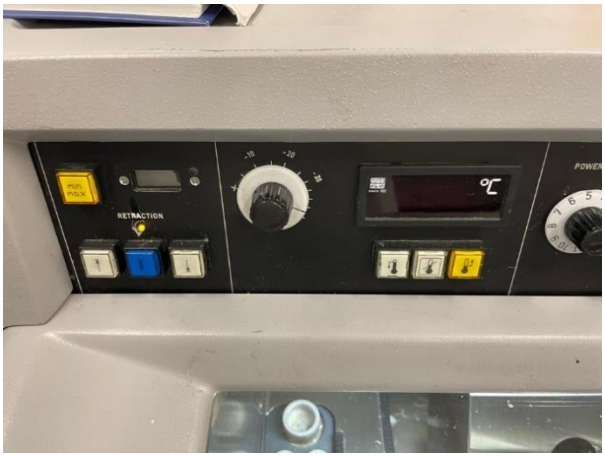
4. You now need to attach your frozen tissue to one of the cryostat stubs using TissueTek or Cryogel. This can be a messy job. Try not to drop TissueTek all over the place and note that it's easiest to clean out of the cryostat while still frozen. Put a dob of TissueTek or Cryogel on a stub, outside the cryostat chamber. The cryostat stubs used for our instrument can be seen at left below.

5. Jam your frozen tissue quickly into the medium and arrange in the best orientation for sectioning. Freeze the tissue onto the stub by plunging into the chamber. Add more medium around the tissue so it is well-supported for sectioning and re-freeze.

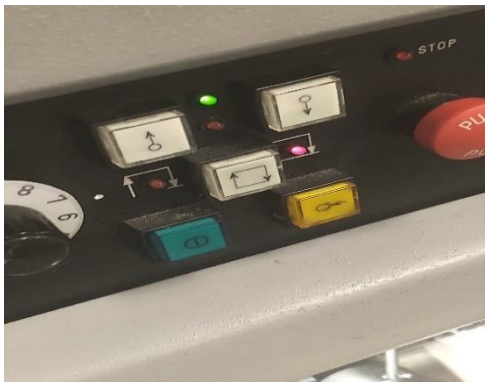
6. Now place the stub in the chuck.



If the chuck is very close to the knife holder, retract it by pressing the rapid retract button to the left, outside the chamber.

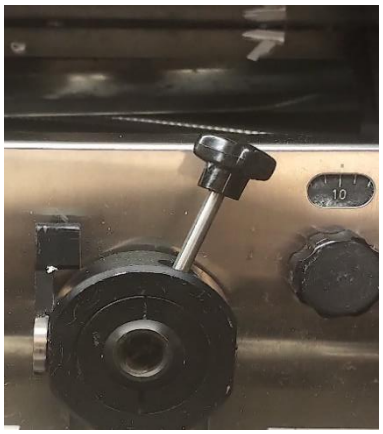


If the specimen head does not move, verify that the power to the motor control is on (green light in the picture below)



The stub is held in place by tightening the black tightening screw. Position the stub so the specimen is in the desired orientation for sectioning.

Once the stub is in the holder and oriented, fold back the anti-roll plate for trimming. Set the section thickness to at least 20-30 µm. Close the chamber lid.



7. Bring the knife close to the specimen by pressing the rapid advance buttons outside the chamber. Use the advance handle (wheel on right hand side of cryostat) to raise and lower the specimen as you are positioning the knife close to it.

   Note: Make sure the break is released before you start to turn the wheel.

Sectioning and Collecting Sections

8. Rotating the advance handle (on right hand side of machine) clockwise will move the tissue up and down past the knife. If the tissue is close enough, a section of tissue will be cut and stay on the knife edge. The chuck will also bring the tissue closer to the knife ready for the next section. Keep the anti-roll bar folded back, and sweep away trimmings until you are ready to collect sections.

9. Set the advance mechanism to the desired section thickness using dial in front of the knife. When you are ready to collect sections, you will need to either move the knife so a fresh zone will cut the tissue, or replace the knife with a new one.

10. Always close the chamber lid except when you need to reach in to make adjustments or change samples. Sectioning proceeds much more smoothly when the cryostat is not struggling to maintain temperature with the lid open. Keeping the lid closed also prevents buildup of frost inside the chamber and on your sample.

11. When you are ready to collect sections, you need to fold the anti-roll bar in place so it is resting on top of the knife edge.

12. Only cut one section at a time. To collect the sections, lower a sticky slide, sticky side down, onto the section. When the slide gets near the section it will start to melt onto the slide, at which time you can raise the slide and the section should come with it.

13. Once you have finished sectioning, remove specimens from stubs and clean stubs, and place stubs back into machine. Wipe out any residue left in the machine during sectioning. The chamber must be left as clean as possible for the next user.

Note: Please use 2-propanol to clean and disinfect the inside chamber instead of 70% ethanol. The ethanol leaves water inside to freeze, causing the turn wheel to become sticky.