





Cryostat Sectioning of Frozen Samples

General Steps



1. Reserve Cryostat in Histology Record Book in the Histology lab.
2. Precautions Before Blade Handling:
 - Lock hand wheel to immobilize the block
 - Cover exposed blade with anti-roll plate
 - Leave 'Cryostat in use' note
3. Avoid storing tissue in cryostat overnight due to defrost cycle.
4. Unlock/Lock Cryostat using the key symbol on the top control panel 
5. 'LOCKED' sign prevents parameter changes for stability. 
6. Set optimal temperatures for tissue sectioning (CT 1–3°C cooler than OT). Refer to pg52 of Leica CM3050 Cryostat instruction manual.
7. Control cryochamber light with the lightbulb symbol. 
8. Monitor temperature fluctuations when cryochamber window is open.
9. Refer to manual for troubleshooting (section 6.2) if needed.

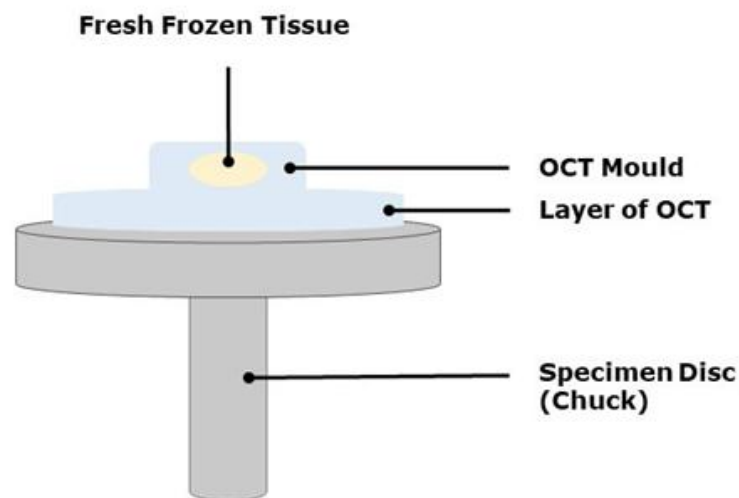
Set Up Method

1. Equipment and Materials:
 - Leica CM3050S cryostat
 - PPE: lab coat, safety glasses, gloves
 - Tork tissues, Cryomold, Frozen Section Compound, Specimen discs
 - Superfrost Plus Glass Slides, Disposable knife blades
 - Stainless-steel forceps, large and small brushes
 - Fresh frozen tissue
2. Set up 30 mins before sectioning for equilibration.


3. Unlock cryostat, turn on lights, and set parameters (thickness, CT, OT).
4. Ensure hand wheel is locked, microtome angle at 5°, and specimen holder homed using the double up arrow symbol on the left control panel 2 
5. Leave materials at room temperature.
6. Place tools inside cryostat, add fresh frozen tissue.
7. Wipe disposable blade, insert, and cover with anti-roll plate.
8. Display 'Cryostat in use' note.

Frozen Tissue Block Preparation

1. Orient and freeze tissue in cryomold with OCT.
2. Cover chuck surface with OCT.
3. Attach tissue to chuck, wait for OCT to turn white.
4. Clamp block, adjust holder using the single up and down arrow symbols on the left control panel 2   , ensure contact with the blade.



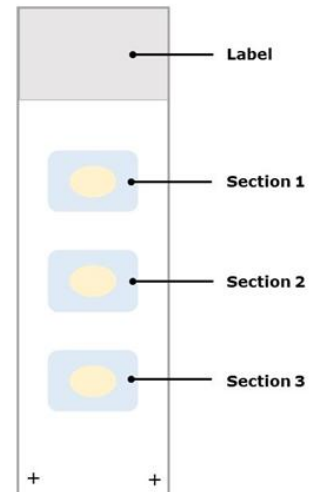
Trimming Procedure

1. Set trimming thickness to 30 µm using the plus and minus symbols on the left control panel 2.
2. Turn on 'Trim' light, unlock hand wheel, and trim block  .

3. Clear debris, switch off 'Trim,' cut and discard the first 30 μm section.
4. Lock hand wheel.

Sectioning Procedure

1. Align anti-roll plate, unlock hand wheel.
2. Section block in a slow, uniform motion.
3. Cut and mount one section at a time.
4. Uncurl sections on a labeled slide.
5. Mount 3–4 sections per slide.
6. Store slides in a chilled box.



Clean Up Method

1. Ensure hand wheel is locked, anti-roll plate covers blade.
2. Remove blocks and slides from cryochamber.
3. Partially defrost OCT-embedded tissue for removal.
4. Clean forceps and brushes with diluted trigene, rinse, and dry.
5. Discard used blade into the yellow sharps bin.
6. Dispose of debris, wipe cryochamber with ethanol, clean anti-roll plate.
7. Clean exterior surfaces of the cryostat.
8. Evaporate ethanol, set cryostat to -15°C , turn off light.
9. Lock cryostat before leaving.