

CRYOSTAT USER GUIDELINES & SAFETY PRECAUTIONS

Mouse Histology & Phenotyping Laboratory

Checklist (to be provided by the user)

- ☐ Lab coat
- ☐ Disposable Gloves
- ☐ Cut-resistant gloves (for changing blades)
- ☐ Cryostat Blades
- ☐ Glass Slides
- ☐ Brushes
- ☐ Forceps
- ☐ **Histology-grade indelible** marker or pencil (for labeling slides) *[Note: Do not use SHARPIE or non-histology pens]*
- ☐ Biohazard waste bag *(to be brought back to your lab and not left at MHPL after use)*

Permitted Specimens

Strictly, only pathogen-free specimens are allowed to be sectioned with the MHPL cryostat. Please contact Tiffany Johnson at the BSL3 (Biosafety Level 3 Laboratory) to section pathogen-infected specimens:

<https://www.feinberg.northwestern.edu/research/cores/units/bsl-3.html>

SETUP

1. Minimum PPE must be worn: lab coat, disposable gloves and cut-resistant gloves (for changing blades).
2. Cool the cryostat chamber to the desired temperature. Cool tissue blocks, brushes, forceps, and other sectioning tools within the chamber.
NOTE: ENSURE THE HANDWHEEL IS LOCKED AND THE BLADE GUARD IS IN PLACE PRIOR TO PLACING TOOLS IN THE CHAMBER.
3. Follow these precautions prior to tissue block manipulation on the specimen head:
 - a. **Lock the handwheel**
 - b. **Guard and retract the blade away from the specimen head using the appropriate button.**
 - c. **Ensure there is a clear distance between the hands and specimen head.**
4. Dispose of unwanted blades in a designated sharps container. Use a magnetic tool or forceps to remove unwanted blades. **NEVER TOUCH A BLADE WITH YOUR FINGERS.**

CRYOSTAT LOGBOOK

1. All trainees and users must enter their names in the cryostat logbook.
2. Please specify the instrument status and any operation issues in the logbook.
3. **Alert staff immediately if you detect problems in the instrument.**

CRYOSTAT OPERATIONS & SECTIONING

A detailed manual for the LEICA CM1850UV is available through this link:

https://www.leicabiosystems.com/sites/default/files/media_product-download/2022-05/Leica_CM1850_UV_IFU_1v5C_en.pdf

Review the various functions of the buttons and handwheels on the instrument.

1. Ensure the tissue block is positioned as needed.
2. Move the blade towards the specimen head using the appropriate button. The blade must remain guarded until ready for sectioning. **NEVER USE FINGERS TO MANIPULATE THE BLADE.**
3. **Turn the handwheel CLOCKWISE** to begin sectioning.
4. To make adjustments to the plane of the tissue block, lock the handwheel, and guard and retract the blade using the appropriate button. Ensure proper clearance space between the hands and the blade.
5. When a section of interest is sliced, use a brush or forceps to re-position the section as necessary. Retrieve the tissue sections using brushes or any applicable tools and transfer them to a slide. **NEVER USE FINGERS TO MANIPULATE THE SECTION.**
6. When brakes are applied, ensure the brake is actually tight before proceeding.
7. **In case of emergency, stop sectioning immediately and alert MHPL staff.**

CLEAN-UP

1. Lock the handwheel. Engage the blade guard and use the appropriate button to retract the blade.
2. Ensure that there is proper clearance space between hands and blade. Remove the tissue block.
3. Use a large brush to sweep discarded tissue sections that collected in the drop pan below the head. **NEVER USE FINGERS TO SWEEP CHAMBER SURFACES.**
4. Distilled or deionized water should be used to rinse the specimen disks, brushes and other tools. **DO NOT SPRAY WATER INSIDE THE CRYOSTAT CHAMBER!**
5. Dispose of used/unwanted blades in an appropriate sharps container. **DO NOT LEAVE BLADE IN THE CRYOSTAT AFTER USE.**
6. Discarded tissues should be collected in a biohazard bag. Bring your biohazard wastes to your respective lab. **MHPL is not responsible for disposal of your biohazard wastes.**
7. Make sure the blade guard is in place and that the cryostat handwheel is locked before you leave.
8. **Close the cryostat chamber before leaving. LEAVE THE CRYOSTAT "ON" AFTER FINISHING.**
9. **TURN THE UV LAMP (UVC button) ON TO STERILIZE THE INNER CHAMBER.**
10. The surrounding area must be clean and free of sharp objects.
11. Alert MHPL staff of any problems/issues with the instrument after finishing.
12. Make sure you signed in/out in the cryostat logbook.

NOTE:

1. **MHPL will inspect the cryostat after each booking has been completed.**
2. **MHPL reserves the right to revoke permission to use the cryostat if standard procedures and safety precautions are not followed by a user.**

7.4 Temperature selection chart (in minus °C)

Tissue	-10°C – -15°C	-15°C – -25°C	-25°C – -35°C
Adrenal	*	*	
Bone marrow		*	
Brain		*	
Bladder		*	
Breast - fatty			*
Breast - little fat		*	
Cartilage	*	*	
Cervical		*	
Fatty			*
Heart and vascular		*	
Intestinal		*	
Kidney		*	
Laryngeal		*	
Lip		*	
Liver		*	
Lung			*
Lymphoid		*	
Muscular		*	
Nose		*	
Pancreatic		*	
Prostate		*	
Ovarian		*	
Rectal		*	
Skin with fat			*
Skin without fat		*	
Spleenal or bloody tissue		*	
Testicular	*	*	
Thyroid		*	
Tongue		*	
Uterus curettage	*		

The temperature values given above are based on long-term experience, however, these are only approximate values, as any tissue may require particular adjustments.

BOOKING THE CRYOSTAT AFTER COMPLETION OF TRAINING

1. The cryostat is available for independent use by authorized personnel, M-F, 9:30 am-4:30 pm.
2. Authorized users who received training from MHPL will be granted permission to book the cryostat for independent use in NUCORE:
<https://nucore.northwestern.edu/facilities/mhpl>
3. Users are still required to e-mail MHPL of their bookings to avoid double bookings or overlap with MHPL core usage:
mhpl@northwestern.edu