

1. Policy

- 1.1. Any person working off this protocol has previously received training on this specific piece of equipment by the RESTORE Lab Manager or Technical Director with explicit instruction for independent equipment usage. Any persons operating equipment without prior training and/or permission will be removed from the facility immediately.
- 1.2. Food and beverages are prohibited in all areas of the RESTORE facility.

2. Purpose

- 2.1. The purpose of this procedure establishes standardized requirements for the safe and proper operation of Epredia HM 355S Rotary Microtome to ensure user safety, instrument integrity, and regulatory compliance.

3. Responsibilities

- 3.1. The equipment operator is responsible for the proper handling, care, and oversight of all samples, animals, and associated materials while they are under the operator's control during use of the instrument.
- 3.2. Users must follow safety procedures and log usage.
- 3.3. Report issues immediately to the lab manager.

4. Procedure

4.1. Initial Setup/ Startup

- Turn on the power strip located behind the microtome (**Figure 4.1-A**).
 - Ensure the water bath powers on simultaneously.

If the water bath does not turn on, press the power button located on top of the microtome (**Figure 4.1-B**).

- Verify that the hand brake is engaged.
 - The microtome display will show a stop sign when the hand brake is activated.
- Fill the water bath and reservoir with DI water:
 - Fill the water bath with 800 mL of DI water.
 - Fill the water reservoir with 600 mL of DI water. The reservoir may be filled through the waste grid.
- Confirm that the water bath contains sufficient water and that the reservoir is properly filled before beginning operation.

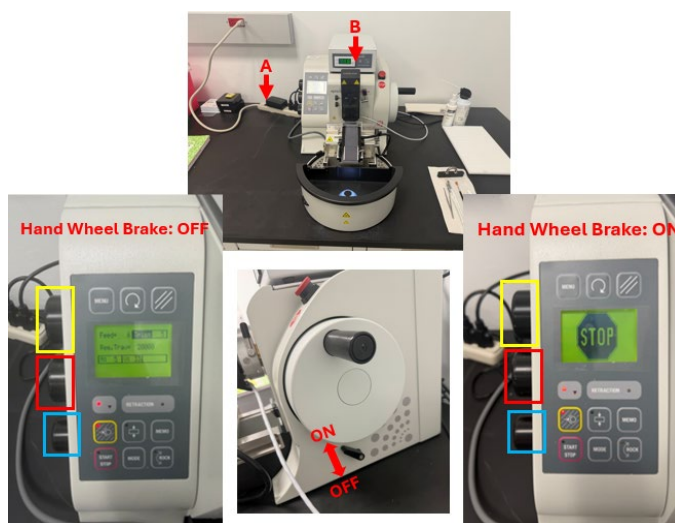


Figure 4.1: Microtome Setup with extension cord switch (A) and water bath switch (B). Screen when rotary handbrake ON (Right Image) versus OFF (Left Image)

4.2. Blade Installation/STS Water Bath Setup

Microtome blades are extremely sharp and can cause severe injuries – always use good laboratory practice when handling them. Always cover the blade with the blade/knife guard and activate then brake prior to making any adjustments to the blade/knife and specimen or changing the specimen. ****NOTE:** do not move or replace blade while water slide is on. Adjust water flow wheel located on the front left of the equipment by the water bath.

- Ensure hand brake is on.
- Open the blade carrier knife guard and insert a new blade into the blade holder
- Ensure the blade is centered and secure using the clamping lever by pushing it forward
- Start the water slide (adjustable wheel, front left side of the bath, set to ~3.5), make sure to wet the entirety of the slide.

NOTE *** If the water speed is too high water will start accumulating on the backside of the slide and in the waste container. Avoid this by making sure to slow the water and wet the entirety of the water slide.

4.3. Specimen Setup

Our microtome is set up with a cool-cut paraffin block holder that is supposed to maintain the block at a lower temp for better cutting.

- To insert the specimen into the cool-cut pull the lever towards you and insert cassette with slanted edge facing down.

4.4. Sectioning/Using STS Water Bath

Toggle between trimming and sectioning (also known as feed) by pressing the knob on the upper left of the screen (**Figure 4.1-Yellow Box**) Set thickness (μm) by turning the same knob.

- If starting with a fresh (uncut) block set the machine to trim and leave the transfer bridge open during trimming, i.e. the lever of the transfer bridge must be pushed backwards.
- Retract the waterslide, and trim block to expose tissue.
- Set to feed section thickness (**Figure 4.1-Yellow Box**).
- Extend the transfer bridge into the water bath and section using smooth, controlled motion.
- Finally pull back the transfer bridge to minimize the movement in the bath to allow collection of sections with slides

4.5. Removing Used Blades with the Magnetic Blade Tool

The magnetic blade tool allows the user to safely remove and dispose of used blades from the blade holder.

- Pull the blade clamping lever toward the front of the instrument to release the clamping plate.
- Position the magnetic blade tool over the blade.
- Carefully lift the blade out of the blade holder using the magnetic blade tool.
- Dispose of the used blade immediately in an approved sharps container or in the blade disposal compartment on the back of the blade box.

4.6. Shutdown/Draining the STS Water Bath

- Ensure the handbrake is engaged.
- Remove the specimen and dispose of it according to biohazard waste protocols.
- Drain and clean the water bath:



RESTORE

Resources for Expanding Stem
cell-derived Tissues and Organs
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STANDARD OPERATING PROCEDURE

Epredia HM 355S Rotary Microtome

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- Remove the water bath from the heating plate. This will deactivate both the heater and the section illumination.
- While emptying the water bath into the sink, discard any large paraffin sections in the trash.
- Remove the trim tray and clean out any remaining paraffin.
- Ensure the water bath and reservoir are clean, dry, and free of paraffin residue.
- Remove the waste tray located behind the water bath and clean out any accumulated paraffin and excess water.
- Turn off the power strip.
- Wipe down the work surface and ensure all brushes are clean.
- Sign the logbook to document completion of equipment use.

5. References

5.1. Please refer to the manual of the Epredia HM 355S Rotary Microtome (On the RESTORE website)

6. Troubleshooting

Issue	Possible Cause	Action
Tissue crumbles	Too cold	Wait for water bath to warm
Tissue smears	Too warm	Put block face down on paper towel in ice bucket
No cooling	Power/compressor issue	Contact lab manager
Consistent vertical Tears in sections	A nick in a blade	New area on blade/New blade