

Microtome Sectioning of Paraffin-Embedded Tissue Block

1. Preparation and Setup:

- **Microtome Selection:** Ensure the microtome is clean and well-maintained.
- **Workspace:** Set up a clean, organized workspace with adequate lighting. Ensure the microtome is placed on a stable surface.
- **Personal Protective Equipment (PPE):** Wear appropriate PPE, including lab coat, gloves, and safety glasses.
- **Microtome Blade:** Check the blade for sharpness and proper alignment. Replace if necessary.
- **Water bath:** Fill a water bath with RO water and heat it to **45-48°C**.
- **Slides:** Use clean glass microscope slides. Commonly used types include plain, charged, or adhesive-coated slides.

2. Precautions and Safe Usage of Microtome:

- **Blade Handling:** Handle the microtome blade with care. Always use forceps or blade holders to avoid accidental cuts.
- **Wheel Locks:** there are two locks on the microtome wheel: The top lock secures the wheel during routine operation. The bottom lock, located below the wheel, overrides the top lock. Use the bottom lock when leaving the microtome unattended for any reason.
- **Adjustments:** Follow the protocol instructions to adjust the thickness setting (usually between 4-5 μm).
- **Clearance Angle:** Set the clearance angle to prevent blade contact with the block face.
- **Stabilize the Block:** Secure the paraffin-embedded block in the microtome block holder.
- **Safety Interlocks:** Ensure all safety interlocks are engaged before operating the microtome.

3. Trimming:

- **Cold Trimming:** Chill the block on ice to make it easier to trim. Trim the block to expose the tissue surface for sectioning.

- **Trim Thickness:** Typically, trim at a thickness of **10-15 μm** to reach the desired tissue level.

4. Sectioning:

- **Cutting Sections:** Adjust the microtome settings to the desired thickness (e.g., **5 μm**).
- **Floating Sections:** Using fine forceps, gently pick up the ribbons of sections and float them on the surface of the water in the water bath. Separate the sections carefully.
- **Transfer to Slides:** Use clean slides to pick up individual sections. Place them upright in a slide rack.

5. Picking Up Sections from the Water bath:

- **Tweezers:** Use fine-pointed tweezers to lift sections from the water bath.
- **Gently Flatten:** Place the section on a clean slide. Gently flatten it using the edge of another slide or a brush.
- **Avoid Bubbles:** Ensure there are no air bubbles trapped under the section.

6. Drying:

- **Oven Drying:** Place the slide rack with sections into an oven set at **60°C**. for 1 hr.

7. Clean Up:

- **Microtome Cleaning:** Wipe down the microtome with a damp cloth to remove any paraffin residue.
- **Dispose of Waste:** Dispose the used blades in the sharps bin and trimmings in the biohazard yellow plastic bag.
- **Slide Labeling:** Label the slides with relevant information (e.g., ID, number, stain type).
- **Storage:** Store the slides appropriately for further processing (e.g., staining).