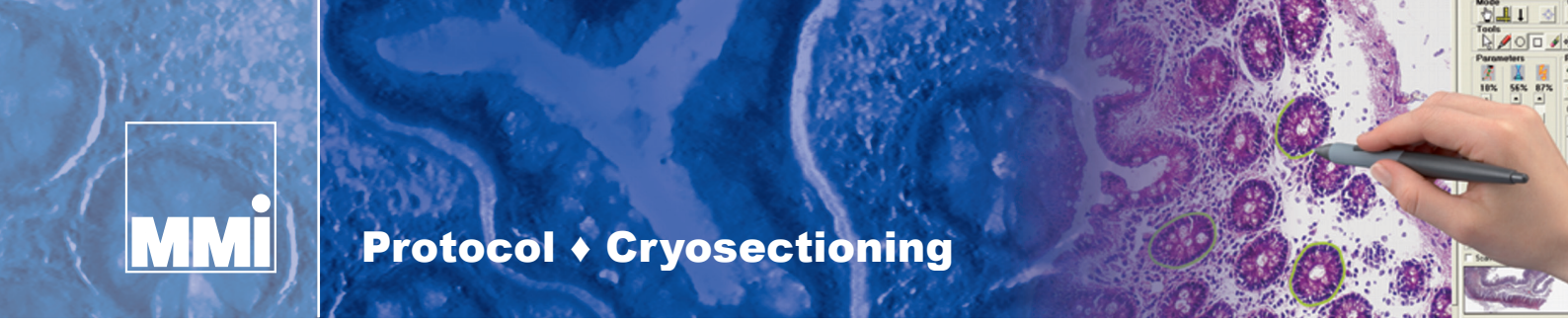


Protocol ♦ Cryosectioning

Once your samples are collected, they need to sectioned and mounted onto an MMI Membrane Slide prior to laser microdissection. The procedure for cryosectioning is simple and can be performed quickly. Please note the cryostat manufacturer's and/or institutional safety guidelines.

Materials:

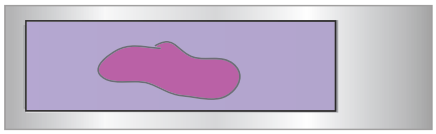
- Frozen embedding media
- Empty slide box
- Dry ice
- Cryostat and disposable blades
- Specimen mount
- MMI Membrane Slides (PN: 50102, 50103)
- Optional:
  - Poly-L-Lysine
  - Gelatine
  - Agarose

Note: Frozen sections must be either fixed immediately, kept in the cryostat until all sectioning is completed, or stored at - 80 °C (or dry ice) immediately to prevent thawing. The frozen section slide must be on dry ice at all times to avoid degradation of molecules of interest in downstream analysis (RNA, DNA, Protein)

Method:

Cryostat Preparation:

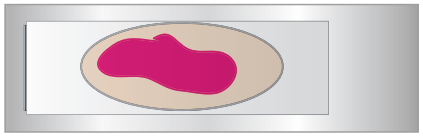
1. Clean the cryostat with proper cleaning agents
2. Insert new sterile blade
3. For fresh frozen and frozen embedded block specimens, use a drop of frozen embedding media to adhere the sample to the specimen mount - make sure you are adhering the specimen in the correct cutting position (cutting surface should be parallel to the blade)
4. Allow frozen block with specimen to equilibrate to the cryostat temperature for about 20 minutes
5. Place empty slide box in cryostat to equilibrate to cryostat temperature (- 20 °C)



Note: Section must be placed on the flat side of the MMI Membrane Slide

Frozen Section Preparation:

1. Cut ~ 5 - 10 µm thick sections onto the flat side of the MMI Membrane Slide.
2. Use brushes or toothpicks to move the cryosection samples after cutting.
3. Remove wrinkles and folds of cut sections
4. Use a room temperature MMI Membrane Slide to allow cut section to adhere to flat side of slide - gently rub the underside of the tissue section with your gloved finger to transfer heat to the sample and help with adhesion.
5. UV treatment of slide to sterilize and increase adherence (optional): To help with adhesion of the sample to the slide one can incubate the slide under UV light for 15 - 30 minutes. A UV sterilization hood works best for this. The UV light will breakdown the membrane slightly and make it tacky, helping adhesion. Additionally this will sterilize the slide. Do not incubate for longer than 30 minutes or risk damaging the membrane.



Use of MMI Support Slides:

To prepare the slide, thaw the section by placing the MMI Support Slide at room temperature in the indentation of the membrane slide. Fix the section on the flat side of the slide.

Coating of Slide to increase adherence (optional):

Additional coatings (poly-L-Lysine, Agarose, or Gelatine) are recommended for tissues that are fatty, hard, fibrous, or contain carilage/bone. The most common method is coating the MMI Membrane Slide with 0.1 % poly-L-Lysine solution. Incubate the slides for 1 hour at room temperature or 30 minutes at 37 °C. Gelatine or agarose can be used as well by preparing a 0.01 % solution and incubating the slides using the above guidelines.