
1. Transport embedded samples on dry ice to the cryomicrotome
2. Place sample in the cryomicrotome box and equilibrate the box temperature to –24°C
   - Higher box temperatures (e.g., –20°C) are acceptable but can cause distortion of the acini at the periphery of the Matrigel during sectioning
   - Slide rack must be inside the cryomicrotome box to store slides after sectioning
   - Keep the slides at room temperature
3. Replace microtome blade (if disposable)
4. Spray microtome blade and anti-roll bar with a Kimwipe moistened with both ethanol and RNAse Away
   - RNAse Away by itself will freeze to the blade
   - Be careful not to cut yourself when cleaning the blade
5. Pop the embedded sample out of the cryomold, mount on the sectioning platform with Neg-50, and freeze until the sample has solidified on the sectioning platform
6. Mount the sectioning platform on the cryomicrotome and cut trim sections until ~3 mm of Matrigel is visible on the cutting surface
   - Matrigel will appear as light pink against the white Neg-50; test slides can be prepared and checked under an inverted microscope to confirm that acini are on the slides
7. Cut 8 µm sections, adjusting the anti-roll bar to prevent the section from accordioning
   - Do not graze sections (subsequent section will be 16 µm instead of 8 µm)
   - Alternatively, one can omit the anti-roll bar and use the brush technique
   - 8 µm sections are roughly one-cell thick to allow for the maximum recovery of biological material after microdissection
8. Wick sections onto Superfrost plus slides (VWR #48311-703) and move the slide immediately to the slide rack inside the cryomicrotome box
   - Do not dry the slide at room temperature (this will ruin the RNA integrity in the tissue)
   - To wick a second section per slide, warm the back of the slide with your finger for a few seconds before wicking (do not warm the part of the slide that contains the existing section)
9. Continue cutting and wicking sections until good cuts are no longer possible
   - One embedded sample typically yields 30-60 slides with two sections per slide
   - Do not return a half-sectioned sample to –80°C (the exposed sample will dehydrate and become damaged)
10. Move the slide box containing the frozen sections to dry ice and store at –80°C for up to one month