

Processing tissue for frozen sectioning

- Fix tissue with 10% formalin or 4% PFA at least overnight, or to the desired time listed on the protocol you are using.
- Rinse tissue in PBS (about 3 changes for about 10-20 minutes each)
- Place tissue in 30% sucrose and leave at 4 degrees until tissue sinks
- Rinse with PBS (about 3 changes for about 10 minutes each).
- Remove tissue from PBS, blot on filter paper to remove excess PBS and then drag thru OCT. Place tissue into 3 different changes of OCT to try to remove any trace of water (PBS) to prevent ice crystals in the tissue.
- Place in a mold filled with OCT and hold the mold over liquid Nitrogen. **DO NOT SUBMERGE THE ENTIRE MOLD INTO THE LN2.** This will cause cracks in the tissue and cause the tissue to become brittle and hard to section.
- Alternatively, you can place a receptacle of 2-methylbutane into the LN2. The 2-methylbutane becomes very cold like the LN2 but will not crack the tissue. It is okay to submerge the mold of tissue into this solution as it is less harsh on the tissue. **(THIS IS THE DESIRED METHOD AND THE METHOD WE USE HERE IN THE CORE).**
- Store frozen tissue at -80 degrees.

*****If you are not fixing the tissue, just start with the step that says to blot tissue on filter paper (after you quickly rinse off the blood in some PBS) and then drag it thru OCT. We do not recommend dropping unfixed tissue directly into liquid nitrogen.

We recommend only using **Tissue Tek OCT**, available from Cardinal health, catalog number # M7148-4, \$8.60 each or \$103 per case of 12 (please note: these are BIDMC quoted prices, price may vary depending on the institution you are ordering from).

We purchase the **2-Methylbutane** from Fisher Scientific, catalog number #O-3551-4. A 4 liter bottle is \$44.37 (please note this is the BIDMC quoted price, price may vary depending on the institution you are ordering from).

*** We also recommend leaving the OCT bottle inverted (up side down) to keep air bubble from accumulating at the tip of the bottle. This will cut down on the air bubbles in the actual mold and tissue block. The less air bubbles, the easier to section. The easier to section requires less time so less money!!!

Please note: If you are fixing the tissue you can always bring the tissue to our facility and we will freeze the tissue for you!

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