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Procedure for Freezing in OCT for Cryostat

Snap Freezing

Principle: Snap-freezing in isopentane (2-Methylbutane) is a preferred method of freezing tissues for immunohistochemistry staining due to the superb preservation of tissue elements and the lack of ice crystal artifact.
Note: Tissues should be kept moist and cool until snap freezing procedure is started

Reagents: Liquid nitrogen
2' Methybutane (Isopentane)
OCT embedding compound
Plastic embedding mold
Forceps
Metal cup
Container for liquid nitrogen

Procedure:
1. Gently blot excess liquid off of tissue.
2. Fill an appropriate container with some liquid nitrogen (black bucket).
3. Immerse metal cup filled 3/4 full with isopentane into the liquid nitrogen. The levels of these two solutions should be the same for even freezing of your specimen.
4. The isopentane will look opaque (milky) white and will have a rim of frozen isopentane when it is chilled enough to snap freeze a specimen -150 degrees C.
*This takes approximately five minutes.
*Keep adding liquid nit to your container to keep the level of the two liquids equal while you are waiting for the isopentane to chill.
5. Label the plastic embedding mold (using cryomarker) and then fill the plastic embedding mold with OCT embedding media and *orient your specimen inside the mold.
6. While holding the plastic embedding mold between a pair of long handled forceps, carefully immerse it down into the metal cup containing the chilled isopentane. Do not let the go of the embedding mold into the isopentane. Let it freeze for approx. 20-50 seconds (depending on size/thickness of tissue).
7. After freezing in the isopentane quickly place your frozen tissue on dry ice and wrap or box for -80 freezer storage or let it sit in the cryostat for 15 minutes before trying to cut it.