CRYOPRESERVATION WITH OCT

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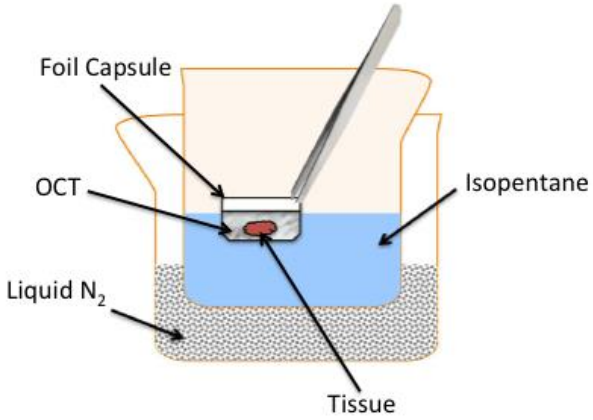
BACKGROUND

This technique is used to cryopreserve tissues for fluorescence-based immunohistochemistry.

MATERIALS

- Liquid nitrogen
- Dry ice
- Isopentane (2-methylbutane)
- OCT compound
- Pyrex beaker
- Long forceps
- Thermal safety gloves and safety glasses
- Marker/pen
- Heavy duty aluminum foil
- Small vial to shape foil capsules



PROTOCOL

1. To create a foil capsule which will hold the OCT and tissue:
    a. Use a small vial with a flat top/bottom, wrap a 3”x3” piece of tinfoil around the flat surface of the vial.
    b. Remove the foil from the vial and ensure that the shape is maintained and that there are no holes in the bottom of the foil capsule. Prepare 1 foil capsule per sample.
2. Pour ¼” OCT compound into the bottom of each foil capsule.
3. Orient the 1mm thick piece of tissue in OCT keeping in mind that the bottom of the foil capsule will become the cutting surface on the cryostat.
4. Pour isopentane into a Pyrex beaker (~20X volume of tissue) and cool in liquid nitrogen until isopentane becomes viscous (white covers bottom of beaker).
5. Place foil capsule containing OCT and tissue into isopentane without allowing isopentane to overflow into foil capsule but deep enough to surround OCT
6. When frozen, quickly remove capsule and fold extra tin foil to cover frozen OCT and tissue, then immerse closed capsule into liquid nitrogen for ~3-5 seconds. Then place on dry ice for storage while completing the other tissues.
7. Frozen tissues can be stored at -70°C until future use.