

## **Freezing and Cryopreservation of Human Tissue**

Note: This protocol is intended as a way to “flash-freeze” tissue in a manner that allows the specimen to be used in multiple analyses without compromising structural integrity of the tissue, or the quality of the sample for biochemical and/or genetic investigations.

- 1) Make the OCT rafts ahead of time by placing a modest layer of OCT on the cork raft and placing at  $-80^{\circ}\text{C}$  or in the vapor phase of liquid nitrogen – store at  $-80^{\circ}\text{C}$ .
- 2) Prepare a good number of squares of heavy-duty aluminum foil roughly 3” x 3”.
- 3) Have the sample labels ready and pre-made (if applicable).
- 4) Before collection, be sure to have a dewar (2L) of liquid nitrogen ready, as well as a Styrofoam container of dry ice.
- 5) Place the appropriate number of OCT rafts on the dry ice and proceed to collection.
- 6) Collect tissue/biopsy specimens per approved protocol.
- 7) Using forceps, carefully remove an OCT raft from the dry ice, and quickly place a single specimen directly onto the frozen OCT.
- 8) Quickly cover the specimen with additional OCT. Use just enough OCT to completely cover the sample.
- 9) Quickly place the OCT-embedded specimen in the basket found in the dewar, and suspend the sample in the vapors of the liquid nitrogen until frozen.
- 10) Carefully remove the frozen specimen from the basket and wrap in one of the foil squares.
- 11) Quickly apply the completed label to the wrapped specimen and place in the liquid nitrogen, or on dry ice.
- 12) Repeat as necessary.
- 13) When all of the specimens have been frozen, they should be stored at  $-80^{\circ}\text{C}$ .