

**CBIS Protocol for use of Leica CM1850- Cryostat**

1. Select the appropriate (cryochamber temperature) sectioning temperature for sample (see appendix 1 for table of temperature selection).
2. Assemble knife onto knife holder: insert the precooled knife in the knife holder and clamp; adjust the appropriate clearance angle on the knife holder; align the knife holder/knife with the specimen; select the section thickness setting.
3. Adjust anti-roll guide: position the anti-roll guide on the knife and align with the cutting edge. Readjust the anti roll plate if necessary.
4. Specimen cutting: cut the specimen to appropriate size; apply enough cryo M-OCT to a specimen disc at room temperature; place the specimen on the disc and orient
5. Specimen frozen: Place the specimen disc in liquid nitrogen to be frozen. Leave the disc in of the holes of the quick freeze shelf until whole batch of sample is finished follow step 2 to 3.
6. Specimen mounting: insert the specimen disc in the specimen head; lock the handle of the handwheel in the upper position; cover the edge of the knife guard (eg. Knife holder); loosen the screw on the specimen head; insert the shaft of the specimen disc in the location hole of the specimen head. The entire rear surface of the prism must have a good contact with the specimen head. Retighten screw.
7. Select section thickness with the control knob on the microtome. sectioning at 20um and reduce the section thickness gradually down to desired value
8. Trim specimen: Remove the knife guard; unlock the hand wheel; move the specimen towards the knife by using the coarse feed buttons; trim the specimen by turning the hand wheel
9. To terminate the work, lock the handwheel, take the knife out of the knife holder and place it back in the knife box; remove frozen section waste with a brush, empty the section waste tray; clean the storage shelves and brush shelves; remove all specimen from cryostat; close the sliding door; switch off the cyrochamber illumination (if you switched it on earlier)
10. Lock control panel 1, with the KEY button; NEVER turn the cryostat off by switching off the main switch. The machine will not have time to cool down.
11. To disinfect: set the cyrochamber temperature to -20°C; remove knife from holder; remove specimen and debris; once the cryochamber reach -20°C, spray disinfectant on contaminated area; after 15-20 minutes, wipe the disinfectant off with a scoots towel; dispose the towel into the biohazard bin; set the cyrochamber temperature back to its working desired temperature.

Appendix 1: Table of temperature selection for various types tissues for the cryostat (Leica.,2003)

#### 7.4 Temperaturew selection chart (in minus °C)

Tissue	-10°C – -15°C	-15°C – -25°C	-25°C – -35°C
Adrenal	*	*	
Bone marrow		*	
Brain		*	
Bladder		*	
Breast - fatty			*
Breast - little fat		*	
Cartilage	*	*	
Cervical		*	
Fatty			*
Heart and vascular		*	
Inte stinal		*	
Kidney		*	
Laryngeal		*	
Lip		*	
Liver		*	
Lung			*
Lymphoid		*	
Muscular		*	
Nose		*	
Pancreatic		*	
Prostate		*	
Ovarian		*	
Rectal		*	
Skin with fat			*
Skin without fat		*	
Spleenal or bloody tissue		*	
Testicular	*	*	
Thyroid		*	
Tongue		*	
Uterus curettage	*		

The temperature values given above are based on long-term experience, however, these are only approximate values, as any tissue may require particular adjustments.

Appendix 2: Labeled parts of cryochamber quick freeze shelf (Leica.,2003)

