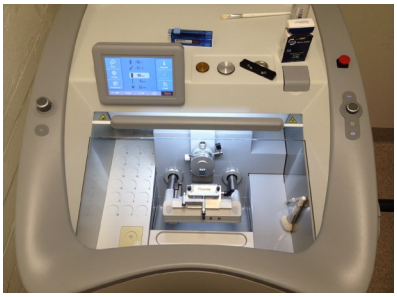
School of Biomedical Sciences
Standard Operating Procedures

Title	Operating the Thermo Cryostar NX70	
Date	11/04/2022	
Location	**Bld: 65**	**Room: 215**
Equipment Custodian	**Contact: Darryl Whitehead**	**Expert User: Darryl Whitehead**

Task	Working with cryostats, whether preparing or cutting a sample, is a true art. You need specific hands-on training from the laboratory supervisor before using a cryostat. Because of the associated hazards, safety must be incorporated into every step of the process to keep fingers, hands and other items protected. Follow the safety tips provided below to keep your fingers, hands, and your artistic touch in perfect condition and to prevent exposure to solvents and biological tissues.
Pre start checks	• Complete SBMS OH&S Induction and local Histology Facility induction • Obtain equipment specific training provided by the Senior Histologist • Read and understand all relevant SOP and Risk Assessments (Riskware ID: #1535 cryo use,#2916 disposal of waste, #2917 dissection, #2301 ethanol usage) • Book appropriate device using the SBMS Online Booking System
Safety considerations	**Personal Protective Equipment (PPE):** 1. Lab coat or gown 2. Fully closed shoes 3. Gloves 4. Safety glasses as required **General precautions**: • Handle blades very carefully when installing or removing. Follow the manufacturer's guidelines explicitly. • Store blades in a closed container. Use a container that restricts access to the blade edge. • Never leave blades on countertops or within the cryostat chamber. Lacerations can occur when reaching across the countertop and inadvertently contacting an unprotected blade. • When setting up the cryostat, ALWAYS position the sample first then put in the blade. • When applying the brake, ensure that it is locked. Most accidents occur when the brake slips and the operator's hand is drawn to the blade. • When leaving the cryostat, even for a short time, ensure that the blade guard is in place. Place the Identification sign, including name and contact details, on the unit guarding the blade and sample. If away for a

	long period of time please remove the blade. • To avoid compression or knife marks, ensure that your blade is clean. Follow the histologist's guidelines for cleaning the work area, items #16,18-21 as below. Always use a brush for cleaning the blade, thus removing your hands from potential contact. • Dispose of blades when appropriate or upon completion of work into an approved sharps container. ▪ **Emergency Procedures:** In the case of emergency, All incidents should be reported to the Facility Staff and Manager, Ext 51929, Safety Coordinator, Ext 53221, and/or Security 53333. All injuries must be reported to SBMS HSW Management, Ext 53221 or 51269, Building Management, Ext 53105. All incidents and injuries must be recorded in the online UQ Incident and Injury and Illness Database.
Procedure	1. Press unlock/lock wheel button in order to activate the machine. There are 3 ways of locking/unlocking the cutting wheel: the blue mechanical lock on the wheel, the wheel lock button on the touch screen, and the wheel lock button on the right arm of the cryostat. **If in doubt, press the red emergency button on the right arm of the cryostat to stop all wheel motion.**  2. Validate that NO knife is present in the knife carrier.  3. Place the brushes and other tools you will be using in the cold tray within the chamber. 4. Using the touch screen, set the specimen and knife temperatures. The knife temperature should always be set 1-2°C warmer than the specimen. 5. Use OCT to freeze mount specimen/sample block onto the mounting stage. The Cryostar NX70 uses stages with flat back surfaces only. Leave to freeze on the cryobar (square gold panel on the bottom left of the chamber). Press 'cryobar' on the touch screen. When it is highlighted blue, it is 

actively freezing to a set temp of -55°C.

6. Use the joystick on the left arm to retract the knife carrier to a safe distance from the sample clamp.

7. Place mounted sample into sample clamp and validate locking.

8. Unlock angle clamp and adjust angle of sample surface to be parallel with the knife carrier. Once even with the knife, re-clamp. **If you need to adjust the angle surface while cutting, always ensure the knife guard is in place and the cutting wheel is locked prior to adjusting the angle.**

9. Lift the anti-roll plate to the left, remove the knife guard and place on right shelf within the chamber. Unlock knife clamp and insert knife from the right side. Use forceps or end of a pencil to push the knife into position. **DO NOT USE YOUR FINGERS.**

10. Validate knife edges to be level on the knife ridge and lock the knife clamp. Do not overstress the lock clamp. It should be approximately in line with the knife.

11. Bring knife to specimen by using the joystick (to bring knife forward move away from user). When knife is approaching the sample, unlock the cutting wheel to lower the specimen in line with the knife to validate cutting angle.

12. Using the touch screen, set the trim and fine cutting thickness. To set/adjust thickness, hold down the trim/fine button for 3 seconds and release. This will bring up a new screen for you to adjust trim/fine thickness. You can adjust the thickness by either turning the joystick knob left to right, or simply pressing the arrows on the screen.

13. Use trim feed to minimise distance to the sample face or to trim excess OCT.

14. Validate cutting mode is in the 'single' cut mode on the touch screen, and begin sampling tissue on your desired 'fine' thickness setting. **Always rotate the cutting wheel in a clock-wise direction to cut.**

15. Pick up slices by slide mounting or free-floating sections.

16. After each cut or when necessary, clean stage and blade mount by brushing with a paint brush AWAY from the blade edge.

17. If walking away from the cryostat for a short-intermediate period, always ensure the anti-roll plate is set over the blade for protection, that the cutting wheel is locked, and the chamber window is down.

18. At the end of each work session, lock the cutting wheel and remove the knife blade. Dispose of the knife into a yellow sharps bin, and clean the cryostat by

	brushing all waste samples and tissues into the waste collection tray below the stage. 19. Remove the tray and empty all debris into the yellow clinical waste bin. 20. To remove the frozen sample from the sample stage, place the stage in the benchtop clamp located behind the cryostat. Once clamped tightly, use a single edge blade holder and cut against the base of the frozen block. ALWAYS hold the blade holder with both hands. If keeping sample, re-seal surface with OCT and place back in -20°C or -80°C freezer. If disposing of sample, discard in yellow clinical waste bin. 21. Wipe the chamber, sample stages, knife mount and other areas with 70% ethanol. If there is a lot of OCT dried on the sample stages, wash with distilled water and then 70% ethanol. 22. 'Home' the knife stage using the joystick (pull back towards user until the stage is fully retracted). 23. Close the chamber window and allow machine to go to standby. Lifting the Samples 1. Use glass guard when possible or brushes to capture the specimen cuts 2. Flatten samples with fine brushes 3. Rapidly contact slide with the specimen for clean mounting. 4. Remove slide from the chamber to maintain temperature differences for easier slide mounting.
Legislative requirements	▪ AS 2243.6 Safety in laboratories, mechanical aspects

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