## Section 1 - Personal Protective Equipment

1. Lab Coat or Lab Gown
2. Hair tied back if long
3. Proper enclosed footwear
4. Safety glasses or goggles
5. Nitrile or latex gloves

## Section 2 – Potential Hazards + Safety precautions

1. Do not use freeze drier for evaporating samples containing organic solvents (Aqueous acetonitrile at <70% is OK).
2. Do not use freeze drier with samples containing high concentrations (>10%) of volatile acids or bases.
3. Freeze dryer generates a high vacuum which can cause implosion of glass ampoules – this poses a threat especially to eyes – wear safety glasses at all times when using the freeze dryer.
4. Glass vessels can break if mishandled, and the sharps generated pose a risk of cuts – such cuts are especially dangerous if hazardous biologicals or chemicals are present. Handle glassware with care at all times, and know the risks and properties of the biological and chemicals you are working on.
5. Freeze dryer generates low temperatures. Glass vessels and other parts of the machine may cause cold burns if touched to exposed skin. Wear gloves when handling these cold parts, or avoid contact.
6. Freeze dryer is an electrical device and thus poses electrical shock hazard if misused or if faulty. Inspect machine for any problems before use (e.g. problems with power supply, sparks, burning smell etc). If any problems present, do not use machine, notify service centre and/or manufacturer. Do not allow water to enter the internal parts of the machine. Keep flammables away from the machine.

## Section 3 – Procedure (Specific for Cryodos machine)

1. Consult equipment custodian BEFORE using the freeze drier. (Nick Shepherd or Angela Nikolic). You need to undergo a formal induction and be added to a list of approved users before starting work.
2. There is now a weekly booking slot for the Drum use which HNU will be using for their samples, as follows: Drum on: 11am each Tuesday. Drum off: 11am each Wednesday. If other users wish to freeze dry using the chamber outlets during the time that the drum is on, they can do so as follows: Attach your samples after midday, Tuesday, in the usual manner. Have them removed by 10.30 am on Wednesday, the next day.
3. Do not turn the freeze dryer on or off. This is done fortnightly by the custodian only.
4. Before using the freeze drier, inspect the machine and make sure that the temperature is between -70 and -80 °C and the pressure is below 1 MBar. If this is the case, proceed. If not, see custodian and report.
5. Before using the freeze drier, make sure you have pre-frozen your samples in liquid nitrogen or dry ice/acetone (see Cryogenics SOP if using liquid N₂)
6. Book your exact start time for freeze drying into the log book. Please include your name, Lab you are working in and a contact number. Ensure that at least 15 minutes have passed since the last user to give the freeze drier time to stabilise.
7. Note the pressure in the log book. Attach your vessel or flask containing your pre-frozen sample/s to an outlet of the freeze dryer chamber. You may need to remove the blue and/or metal attachments depending on the diameter of your flask/vessel opening. Turn the outlet dial 180 degrees in either direction.
8. You will notice the pressure of the machine rise. You must wait until the pressure goes back
down below 1 Mbar and put a tick in the log book before you leave the machine.

9. To remove your samples, turn the outlet dial 180 degrees in either direction. The pressure will be released from the flask/vessel. Take off your vessel and replace the blue/metal attachment if you had previously removed them.

10. If you need to use the large drum chamber, consult with custodian before each run – the drum chamber is not routinely connected.

Procedure specific for large drum use

11. Fiona Atkinson (HNU) will be in charge of the large metal drum use. Please undergo training with Fiona before attempting to use the drum unit.

12. No other flasks/vessels should be attached to the freeze dryer at the times that the drum is being attached and removed. If you need to use the machine when the drum is in use, ensure your samples are attached AFTER the drum is set up, and taken off BEFORE the drum is taken off.

13. Open two of the outlet valves of the freeze dryer chamber to release the pressure. Do this by applying a quarter turn to the right to each valve. You will hear the pressure being released. Press ‘OFF’ button on the machine.

14. Remove the small perspex lid (with knob) from the freeze dryer chamber. Replace the lid with the metal doughnut shaped disc. Ensure that the black rubber seal fits tightly around the disc.

15. **TWO PEOPLE ARE REQUIRED FOR THIS STEP (HEAVY!):** Lift the large metal drum onto the metal disc and line up the small base with the central hole on the metal disc. Position the large metal plate so it sits inside the drum and the long screw faces up into the main body of the drum. Load samples into the metal drum. Use the step stool for required height to see into the drum. Reinstall the rubber seal. Make sure that the top surface of the seal is greased. If seal needs extra grease, the jar of grease is located in the bottom drawer on the beige drawer unit, underneath the bench to the left of the freeze dryer. Slide on the large Perspex lid. Check that there is 0.5 cm buffer around the top to ensure a good seal.

16. Make sure that all valves are closed. Check this by making sure that the long wing of the valves on the valve outlets faces up. Press the “ON” button. After a short period the temperature will begin to decrease to -40 °C. You will hear periodic clicking and sucking sounds coming from the machine – this is normal. Once the temperature reaches -40 °C the vacuum pump will start. Check that the pressure drops below 1Mbar and that the temperature drops to -80 °C. It should take less than 30 minutes for the pressure to drop below the required level. Allow freeze drying to proceed for 16-24 hours.

17. When freeze-drying is complete, open 2 valves, by applying a quarter turn to the right to both valves. Wait about 10 seconds for the pressure to be released. Turn the machine “OFF”. Flick the valves up so the wings are facing up again. The vacuum will then be released.

18. **TWO PEOPLE ARE REQUIRED FOR THIS STEP (HEAVY!):** Use the step ladder for the required height. Slide the large Perspex lid off. Put it down on a work surface safely. Remove your samples out of the drum. Pull the metal base out by grabbing the large metal spike. Lift the drum vertically up and off the unit. Remove the metal doughnut disc. The large metal disc with spike, the drum chamber and the smaller metal doughnut-shaped disc can all be stored on the lab bench to the left of the freeze dryer until next required. Replace the disc with the small Perspex lid with the black round knob. Turn the machine back “ON”.

19. Wait until the pressure goes back down below 1Mbar before leaving the machine.

**Section 4 – Disposal / Spills / Incidents**

1. Spills of samples that are biohazards must be done according to the biohazard spills SOP – see this document.

2. Incidents must be reported immediately to your supervisor and with 24 hours on the online incident reporting system

**Section 5 – Repairs / Certification / Validation**

1. N/A

**Section 6 – Relevant safety data sheets**

1. All users should be familiar with the risks of the biological or chemical samples used in the machine – consult SDS if these are available

**Section 7 - References**

1. See also Biohazard Spill SOP (SMB004) and Cryogenics SOP (SMB010)
SOP Consultation, Training and Approval
Print names and enter signatures and dates to certify that the persons named in this section have been consulted/trained in relation to the development and implementation of this Standard Operating Procedure. WHS Representative (WHS Committee) certifies that consultation has taken place.

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**Name Authorising (Printed):** DIANNE FISHER ............................................................... 

**Signature:** ............................................................... **Date:** 3/6/14 ............................... 

**WHS Committee Representative Name (Printed):** MARKUS HOFER ............................... 

**Signature:** ............................................................... **Date:** 18/7/14 ............................... 

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