***STANDARD OPERATING PROCEDURE – E003***

**Freeze drying – Martin Christ machine**

1. **Objectives**

The objective of this document is to establish standard operating procedures when using the freeze-drying machine, ensuring the safety of laboratory personnel by mitigating potential risks associated with hazardous materials, and injuries. Additionally, this SOP aims to enhance the efficiency of experimental workflows.

1. **Personal Protective Equipment**

To ensure safety of the freeze-drying machine, appropriate personal protective equipment (PPE) must be worn. This includes:

* Long pants and closed-toe shoes to protect against spills and splashes.
* A long-sleeved, buttoned lab coat or gown to minimize skin exposure.
* Safety glasses or goggles to protect against splashes or flying debris.
* Disposable nitrile or latex gloves to prevent direct contact with hazardous materials.
* Insulated gloves for handling extremely cold samples or equipment.
* If the user has long hair, it should be tied back.

1. **Potential Hazards and Safety Precautions**

The freeze-drying machine poses various hazards that must be managed to maintain a safe working environment. These include:

* **Extreme Cold Exposure:** Handling glass vessels or cold parts of the machine can result in frostbite or cold burns. Always wear insulated gloves when handling cold items.
* **High Vacuum Exposure:** Sudden vacuum release can cause materials to expand rapidly, leading to splashes or flying debris. Safety glasses must be worn at all times.
* **Biological Hazards:** Samples containing hazardous microbiological substances may pose a risk if ampoules break. Handle with care and follow biohazard protocols.
* **Chemical Hazards:** Samples containing hazardous substances pose a risk of exposure during processing. Always review the Safety Data Sheet (SDS) for each chemical used.
* **Electrical Hazards:** Improper use or maintenance of the machine can lead to electrical shock. Do not use the machine if it appears faulty; notify the supplier or manufacturer immediately.
* **Sharps Hazards:** Glass vessels may break during mishandling. Handle glassware with care, especially if it has been in contact with hazardous biologicals or chemicals.

1. **Procedures**
2. Preparation

* Ensure that all personnel operating the freeze dryer have received adequate training on its operation and safety protocols.
* Prepare samples in accordance with established guidelines, confirming they are appropriate for freeze-drying and compatible with the machine.
* **NOTE:** Identify whether the samples are of a small or large size. For larger samples, a longer time may be required to initiate the Freeze/Dry phase, hence liquid nitrogen or dry ice/ethanol bath could be considered. Different hazards and risks exist when working with liquid nitrogen, dry ice, or ethanol. Users should acknowledge and understand the risks involved with working with these liquid or vapours. Perform a comprehensive inspection of the machine to verify that all components are functioning properly.
* Check that the temperature is set within the appropriate range, generally between -50°C and -80 °C, and that the vacuum level is below 1 MBar.
* Pre-freeze samples using liquid nitrogen or a dry ice/acetone mixture prior to placing them in the machine.
  + **NOTE**: For small volumes of samples (0.2 mL or less), pre-freezing may not be necessary.

1. Operation

* Activate the vacuum pump and allow it to warm up for 15 minutes before commencing any procedures, keeping the lever that opens the pump to the chamber in the closed position during this time.
* Move the lever on the vacuum pump to the vertical position to create a vacuum in the chamber.
* Place the prepared samples into containers that are appropriate for freeze-drying.
* Organize the samples within the drying chamber to ensure proper airflow and avoid overcrowding.
* Choose the correct parameters for the freeze-drying cycle, including freezing temperature, drying temperature, and vacuum level, based on the sample type.
* Confirm that the temperature and vacuum gauges are set to the desired levels prior to starting the cycle.
* Securely close the chamber door.
* Initiate the freeze-drying cycle in accordance with the manufacturer's instructions.

1. Process Monitoring

* Regularly monitor the machine throughout the operation to ensure all parameters stay within the designated limits.
* Be alert to any alarms. If an alarm sounds, investigate the cause and follow the manufacturer’s troubleshooting guidelines.

1. Post-treatment

* Once the freeze-drying process is complete, allow the chamber to return to atmospheric pressure and turn off the pump lever before opening the door.
* Carefully extract the dried samples while wearing the appropriate personal protective equipment (PPE).
* Open the drain valve located on the lower side of the machine to vent the vacuum. A tube should be connected to allow for any defrosted liquid to drain into a beaker. Clean the machine according to the manufacturer's instructions, ensuring that all areas are free of any residual materials.

1. **Spills or Incident Reporting**

* Notify individuals nearby about the spill, evacuate the area, and limit access to the affected zone.
* All biohazard spills must be cleaned up following Standard Operating Procedure 004 - Cleanup of Biohazard Spills.
* Report any incidents that result in injuries to the Principal Investigator (PI) or the departmental safety officer (DSO) immediately.
* For serious incidents, contact the Security Unit immediately by calling the 24-hour hotline on 2358 8999.

1. **References**

* Nikolic, A. (2016). *SOP\_SMB015: Freeze drying (Martin Christ machine).* Risk Assessment. The University of Sydney.
* Coleman, N. & Stevenson, G. & Campbell, L. (2014). *SOP SMB015.3 (GS NC LC 0414): Freeze drying (Martin Christ machine).* The University of Sydney.