***STANDARD OPERATING PROCEDURE – E002***

**Freeze drying – Cryodos machine**

1. **Objectives**

The objective of this document is to establish standard operating procedures for the freeze drying – Cryodos 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 – Cryodos 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 chemicals.
* 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 – Cryodos 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**

**WARNING:**

* Freeze dryer should not be used for evaporating samples containing **organic solvents** (except for aqueous acetonitrile < 70% is acceptable).
* Samples containing high concentration (more than 10%) of volatile acids and bases should not be used with the freeze dryer.
* The freezer dryer should not be used if there are visible signs of damage or malfunction.
1. Preparation
* Ensure that all personnel should be properly trained in its operation and safety measures prior to use of the Cryodos machine. Prepare samples in accordance with established protocols, confirming they are suitable for freeze-drying.
* Before operating the freeze dryer, inspect the machine to verify that the temperature is set between -70°C and -80°C and that the pressure is below 1 MBar.
* Pre-freeze samples using liquid nitrogen or a dry ice/ acetone mixture before placing them in the freeze dryer.
* The internal parts of the freeze dryer should be kept dry and kept away from water.
* Flammables should be kept away from the machine.
* Internal pressure of the equipment should be recorded in a logbook and records should be kept. Ensure the pressure is reduced to below 1 Mbar prior to leaving the equipment.
* Internal parts be required for changing prior to the start of the equipment run cycle; contact the equipment custodian. **DO NOT** move heavy parts alone.
1. Operation
* Place the prepared samples into containers that are appropriate for freeze-drying.
* Arrange the samples within the machine’s drying chamber to ensure adequate airflow and prevent overcrowding.
* Ensure that no other flasks or vessels are connected to the freeze dryer while attaching or detaching the drum.
* Select the appropriate parameters for the freeze-drying cycle, including freezing temperature, drying temperature, and vacuum level, based on the type of sample.
* Verify that the temperature and vacuum gauges are set to the desired values before initiating the cycle.
* Close and secure the chamber door.
* Start the freeze-drying cycle following the manufacturer’s guidelines.
1. Process Monitoring
* Continuously monitor the machine during the operation to ensure all parameters remain within the specified limits.
* Pay attention to any alarms or alerts. If an alarm is triggered, investigate the issue and follow the troubleshooting procedures outlined by the manufacturer.
1. Post-Process
* After the freeze-drying process is finished, allow the chamber to return to atmospheric pressure before opening the door.
* Carefully remove the dried samples, ensuring proper personal protective equipment (PPE) is worn.
* Open both valves by turning them a quarter turn to the right and wait approximately ten seconds or pressure release.
* Clean the machine according to the manufacturer’s instructions, ensuring all areas are free of residual materials.
* Always use a step ladder / stool if required to move objects safely from height.
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\_SMB014: Freeze drying – Cryodos Machine.* Risk Assessment. The University of Sydney.
* Coleman, N. & Atkinson, F. & Shepherd, N. & Nikolic, A. (2014). *SOP SMB014.3 (AN NC FA NS 0314): Freeze drying-Cryodos machine.* The University of Sydney.