***STANDARD OPERATING PROCEDURE – B010***

**Protein Purification**

1. **Objectives**

The objective of this document is to establish standard operating procedures for the process of protein purification, 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 during protein purification, 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 to minimize skin exposure.
* Safety glasses or goggles to protect against splashes or flying debris.
* Disposable nitrile gloves to prevent direct contact with hazardous materials.
* Heat-resistant gloves for handling hot material or equipment.
* If the user has long hair, it should be tied back.

1. **Potential Hazards**

Protein purification presents various hazards that must be managed to maintain a safe working environment. This includes:

* **Chemical Hazards:** Flammable solvents (e.g., methanol, ethanol, acetonitrile) used in protein precipitation or chromatography pose fire and toxicity risks. All solvent handling must be conducted in a fume hood. Toxic reagents (e.g., PMSF, β-mercaptoethanol, acrylamide) require strict PPE compliance and fume hood use. Corrosives (e.g., strong acids/bases for column cleaning) can cause severe burns. Safety glasses and face shields must be worn. Carcinogens/mutagens (e.g., ethidium bromide, DEPC-treated water) should be substituted with safer alternatives where possible.
* **Physical Hazards:** High-pressure systems (HPLC, FPLC, French Press) has risk of leaks or bursts. Inspect tubing and fittings before use. Sharps injuries from broken glass, needles, or chromatography columns. Use puncture-resistant containers for disposal. Extreme temperatures (e.g. from using autoclaves, or handling liquid nitrogen). Wear insulated gloves and face protection.
* **Electrical hazards:** Faulty equipment (e.g., centrifuges, electrophoresis units) pose electrical shock risk. Ensure proper grounding and maintenance.
* **Biological Hazards:** Recombinant proteins, cell lysates, or human/animal tissues may contain pathogens. Manipulation of cells should be undertaken in a biosafety cabinet in a BSL-2 laboratory. Biohazardous waste must be autoclaved before disposal.
* **Environmental Hazards:** Improper disposal of chemical or biological waste can lead to contamination.

1. **Training**

Ensure all personnel have received proper training on their hazards and safe handling techniques. Undergo medical surveillance and register as a biohazard worker prior to start of work if needed.

* MC06 Biological Safety
* MC03 Chemical Safety II / Hazardous Waste Management
* MC07 Chemical Safety I / Chemical Safety for Laboratory Users

1. **Procedures**
2. Preparation

* Read and understand the risks associated with protein purification and all other safety data sheets (SDS) associated with any chemicals that are to be manipulated. The SDS should be placed in a location that is easily accessible.
* PPE should be put on as described above.
* The closest location of spill kits, safety showers, eyewashes, fire extinguishers and fire blankets in relative to the working location should be known before start of work.
* Precautions should be taken when working with recombinant biologicals. If necessary, BSL-2 practices should be enforced.
* Safety glasses should be worn when cleaning the protein purification column with acids or other corrosives.
  + **NOTE:** User should be aware of poor-fitting junctions or excessive pressure generated during operation and acid may spray out in these cases.
* Liquid waste should be disposed of in waste disposal containers and **DO NOT** pour down the drain or sink.
* Maintain a 3 metre gap between solvents use or storage areas and potential ignition sources, including refrigerators, freezers, and other equipment with exposed electrical components. Do not use flammable solvents near ignition sources, particularly for open flames.
* When working with flammable reagents or reagents with a strong smell (e.g. mercaptoethanol), it should be conducted in the fume hood.
* Many diverse procedures and protocols are to be utilised in protein purification and involve more than one SOP. Read and understand all specific instruction manuals and consult equipment custodian prior to start of work. Should there be any concerns, talk to your supervisor.

1. Maintenance of Equipment

* Ensure equipment (e.g. HPLC) is maintained properly through routine servicing under a service contract.
* Beware of electrical hazards associated with machinery. Promptly arrange for servicing if there are any signs of electrical issues.

**6) Disposal, Spills or Incident Reporting**

* SOPs should be consulted for Biohazard Spills, Flammables, Corrosives, or Toxic Substances depending on the type of spill that occurred.
* In case of a spill, follow the laboratory’s spill response procedures.
* Promptly notify the Principal Investigator (PI) and/or the departmental safety officer (DSO) of any accidents, spills, or equipment malfunctions.
* For serious incidents, contact the Security Unit immediately by calling the 24-hour hotline on **2358 8999**.

**7) References**

* Nikolic, A. (2016). *SOP\_SMB023: Protein Purification.* Risk Assessment. The University of Sydney.
* Coleman, N. (2016). *SOP SMB023.2 (NC 0714): Protein Purification.* Standard Operating Procedure. The University of Sydney.
* Safety and Environmental Protection Manual *- Chapter 7: General Laboratory Safety | Health, Safety and Environment Office - the Hong Kong University of Science and Technology*
* Safety and Environmental Protection Manual *- Chapter 9: Biological Safety | Health, Safety and Environment Office - the Hong Kong University of Science and Technology*
* HKUST Emergency Procedures – *Hong Kong University of Science and Technology*