***STANDARD OPERATING PROCEDURE – B005***

**DNA Purification via Phenol or Chloroform Method**

1. **Objectives**

The objective of this document is to establish standard operating procedures for DNA purification via phenol/chloroform method, 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 DNA purification via phenol/chloroform method, 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 or latex gloves prevent direct contact with hazardous materials.
  + **NOTE:** Gloves should be changed if they are or suspected to be contaminated with phenol. Avoid contamination of lab equipment with phenol.
* Face mask to reduce the risk of inhalation of aerosols or particulates.
* If the user has long hair, it should be tied back.

1. **Potential Hazards**

DNA purification via the phenol/chloroform method poses various hazards that must be managed to maintain a safe working environment. These include:

* **Chemical Exposure:** Handling reagents like phenol, chloroform, or ethanol can cause toxicity, irritation, or chemical burns.
  + **Phenol** is corrosive, an irritant, and highly toxic. Extended contact of phenol with the eye may cause **corneal damage or blindness**. Skin contact can lead to inflammation and blistering.
  + **Chloroform** is an irritant, an aesthetic, and a suspected carcinogen. It is hazardous in case of skin / eye contact, or if inhaled or ingested.
  + **Ethanol** is flammable, toxic, and an eye irritant.
* **Biological Hazards:** Handling biological samples (e.g., blood, tissue) may expose individuals to infectious agents.
* **Skin Damage:** Direct contact with phenol or chloroform can cause irritation, burns, or systemic toxicity.
* **Fire or Electrocution Risk:** Centrifuge malfunctions or electrical faults can pose fire or electrocution hazards. Regular rotor inspections and verify proper rotor installation are required and do not exceed maximum speed settings recommended by the manufacturer prior to operation.

1. **Safety Precautions:**

* Accidental Eye Contact with Chloroform / Phenol
* Remove contact lenses if present.
* Flush eyes with running water for at least 15 minutes.
* Seek medical attention immediately.
* Accidental Skin Contact with Chloroform / Phenol
* Remove contact lenses if present.
* Flush skin with running water for at least 15 minutes while removing contaminated clothing and shoes.
* Irritated skin should be covered with emollient (e.g. moisturizing cream).
* Contaminated clothing and shoes should be washed thoroughly before reuse.
* Should phenol burns appear extensive or severe, medical attention should be sought immediately.
* Accidental Inhalation Contact with Chloroform / Phenol
* The Person should be taken to an area with fresh air.
* Artificial respiration should be given to the person if they are not breathing.
* Medical attention should be sought immediately if breathing is absent, a person appears to have difficulty breathing, or nervous symptoms are apparent (e.g. dizziness or loss of consciousness).

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

* Gather all necessary materials and reagents, including spin column kits for DNA or RNA extraction, the sample (tissue, blood, or cultured cells), lysis buffer, wash buffer, elution buffer, ethanol, microcentrifuge tubes, and pipettes with tips.
* Safety data sheet (SDS) should be read and understood prior to work.
* Understand the risk involved or whether the risk can be controlled. If the user is unsure of the risk level, then the procedure should not proceed.
* The location of spill kits, eye washes, safety showers, fire extinguishers, and fire blankets should be known to the user before starting work.

1. During DNA Purification

* Collect biological samples (e.g. blood, tissue, or cell culture) following appropriate protocols for handling and storage.
* Carefully transfer the collected sample into a microcentrifuge tube to prepare for lysis.
* Add an appropriate volume of lysis buffer to the sample in the microcentrifuge tube.
* Vortex the mixture gently to ensure thorough mixing and complete lysis of the sample, facilitating the release of nucleic acids.
* **When using phenol / chloroform, work in a fume hood always (**unless small quantities of less than 100 µL are being used in completely sealed tubes**).**
  + Should a strong smell be apparent, move to work inside a fume hood and/or check whether the hood is in operation.
* **Polypropylene tubes are highly recommended for use during this procedure**. The cap must be tight fitting. Other materials such as glass will risk breakage, and polycarbonate tubes will be dissolved by phenol / chloroform.
* Care must be taken when centrifuging tubes at high speeds to separate mixture in aqueous phase, junk layer, and organic layer. Follow the manufacturer’s instructions on correct use of centrifuges. Should a spill in a centrifuge occur, they should be cleaned up immediately with appropriate PPE.

1. Waste Disposal

* All phenol / chloroform waste should be disposed of as hazardous chemical waste (refer to SOP for chemical waste disposal). These wastes should not be poured down into the sink.

**7) Spills and Accident / Incident Reporting**

* All spills must be cleaned up following Standard Operating Procedure B002 - Cleanup of Chemical Spills.
* Report any incidents / accidents that result in injuries to the PI and/or the departmental safety officer (DSO) immediately.
* For serious incidents, notify the Security Unit immediately by calling the 24-hour hotline on **2358 8999**.

**8) References**

* Hofer, M. (2016). *SOP\_SMB012: DNA purification using phenol/chloroform.* Risk Assessment. The University of Sydney.
* Kant, S., & Coleman, N. (2014). *SOP SMB012.1 (SK NC 0714): DNA purification via phenol/chloroform.* Standard Operating Procedure. The University of Sydney.
* Safety and Environmental Protection Manual *- Chapter 9: Biological Safety | Health, Safety and Environment Office - the Hong Kong University of Science and Technology*
* HEALTH, SAFETY AND ENVIRONMENT OFFICE. (n.d.). Retrieved June 23, 2025, from https://hseo.hkust.edu.hk/sites/default/files/Proper%20Disposal%20of%20Chemical%20Waste.pdf