***STANDARD OPERATING PROCEDURE – B004***

**DNA or RNA Extraction Using Spin Columns**

1. **Objectives**

The objective of this document is to establish standard operating procedures (SOP) for DNA or RNA extraction using spin columns, 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 for DNA or RNA extraction using spin columns, 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 to prevent direct contact with hazardous chemicals.
* Face mask to reduce the risk of inhalation of aerosols or particulates.

1. **Potential Hazards**

The process of DNA or RNA extraction using spin columns poses various hazards that must be managed to maintain a safe working environment. These include:

* **Chemical Exposure:** Handling reagents like phenol, chloroform, ethanol, or chaotropic salts can cause toxicity, irritation, or chemical burns.
* **Biological Hazards:** Handling biological samples (e.g., blood, tissue) may expose individuals to infectious agents.
* **Contamination Risk:** Neglecting aseptic techniques can compromise sample integrity and lead to unreliable results.
* **Environmental Damage:** Incorrect disposal of recombinant DNA or hazardous chemicals can harm the environment.
* **Skin Damage:** Direct contact with chaotropic salts or corrosive reagents can cause irritation or injury.
* **Injury from Centrifuge Use:** Improper use or malfunction of centrifuges can lead to physical injury or equipment damage.
* **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.

All chemicals’ safety data sheet (SDS) used for DNA or RNA extraction should be checked for any hazardous components.

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.
* Review the Safety Data Sheets (SDS) for all reagents to understand their hazards and emergency procedures.
* Ensure the work area is clean, organized, and free of clutter.

1. Sample Preparation

* 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.

1. DNA/RNA Extraction
2. Column Preparation

* Place the spin column into a clean collection tube to facilitate the collection of the flow-through during the extraction process.
* If specified by the manufacturer, add the binding buffer to the spin column to enhance nucleic acid binding.

1. Sample Loading

* Carefully transfer the lysate from the microcentrifuge tube into the spin column.
* Close the lid of the spin column and centrifuge at the recommended speed (typically 10,000 - 14,000 rpm) for 30 seconds to allow the nucleic acids to bind effectively to the column matrix.

1. Washing Steps

* After centrifugation, discard the flow-through liquid and reposition the spin column into the same collection tube.
* Add the specified wash buffer to the spin column as per the kit protocol.
* Centrifuge again at the designated speed for the recommended duration to remove contaminants.
* Repeat the wash step as needed to ensure that all impurities are effectively washed away.

1. Elution

* After the last wash, discard the flow-through and place the spin column in a fresh microcentrifuge tube.
* Add the elution buffer to the center of the spin column membrane for optimal nucleic acid recovery.
* Incubate the column at room temperature for a few minutes, then centrifuge at the specified speed for the recommended time to elute the nucleic acids into the new tube.

1. Post-Extraction Procedures

* Store the extracted DNA or RNA at appropriate temperatures, typically -20°C for DNA and -80°C for RNA, until further analysis is conducted.
* Dispose of used reagents, spin columns, and biological materials following institutional biosafety and hazardous waste disposal guidelines.
* Do not dispose of spin column waste down the sink. Instead, autoclave it if recombinant DNA is present or treat it as hazardous chemical waste.
* Clean the work area with suitable disinfectants to prevent contamination and maintain a safe laboratory environment.

**6) Spills or Incident Reporting**

* All biohazard spills must be cleaned up following Standard Operating Procedure B002 - Cleanup of Biohazard Spills.
* If binding buffer contacts the eyes or skin, flush with running water for at least 15 minutes and seek medical attention as soon as possible.
* If the binding buffer is in contact with skin or eyes, flush with water and wash immediately.
* Report any accidents that result in injuries or near misses 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**.

**7) References**

* Coleman, N. (2016). *SOP\_SMB011: DNA or RNA extraction using spin columns.* Risk Assessment. The University of Sydney.
* Nikolic, A., & Coleman, N. (2014). *SOP SMB011.2 (AN NC 0314): DNA or RNA extraction using spin columns.* 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*