**STANDARD OPERATING PROCEDURE**

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<th>Procedure:</th>
<th>Protein purification</th>
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<td>School/Department:</td>
<td>School of Molecular Bioscience</td>
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<td>SOP prepared by:</td>
<td>Nick Coleman</td>
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<td>Version:</td>
<td>SMB023.3</td>
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### Section 1 - Personal Protective Equipment

1. Lab coat or lab gown
2. Gloves when handling biohazards, toxics or corrosives
3. Safety glasses when handling biohazards, toxics or corrosives, or where squirt/splash/spray risk is present
4. Proper enclosed footwear
5. Hair tied back if long

### Section 2 – Potential Hazards + Safety precautions

1. Solvents used to precipitate proteins may be flammable – keep these away from naked flames e.g. Bunsen burners, and dispose of wastes as hazardous waste
2. Solvents (e.g. methanol) used for protein precipitation or chromatography may be toxic – do not allow these to be inhaled, ingested or spilled on skin, and dispose of wastes as hazardous waste
3. Some reagents used to stabilise proteins (e.g. protease inhibitors like PMSF) are highly toxic. Take precautions as above.
4. Some reagents used to stabilise proteins (e.g. dithiothreitol and mercaptoethanol) have a powerful stench; these are irritants or toxins if inhaled, and must be handled in a fume hood.
5. High-pressure chromatography systems contain liquids under pressure, which can cause eye injury if they escape. Wear safety glasses!
6. Electrical equipment used for high-pressure liquid chromatography may cause electrocution if faulty or used incorrectly. Ensure all equipment is functioning correctly before starting work, and consult your supervisor if unsure.
7. Proteins and the DNA constructs / cells used to make them may be biohazards, either due to their toxicity, potential for infection, or their recombinant DNA nature. Handle with care, in a PC2 lab or biosafety cabinet if necessary, and dispose of by autoclaving.
8. Related tasks that are potentially hazardous include using the French Press or Sonicator for cell lysis, Centrifugation, Polyacrylamide gel electrophoresis, Flammables, Toxic chemicals, HPLC, Disposing of chemical wastes, Handling Risk group 2 microorganisms, Using human tissues, Using animal tissues (see separate risk assessments for these).
9. Workers with pre-existing medical conditions (e.g. allergy, immunocompromised state, chemical sensitivity) and workers who are pregnant or expecting pregnancy must consult with their supervisor AND medical specialist AND the university’s WHS services before performing this procedure. If there are any serious concerns expressed by any of these individuals, this task must not be performed.

### Section 3 – Procedure

1. Read and understand this SOP and the risk assessment for Protein Purification, along with any MSDS sheets
2. Put on PPE as described above
3. Know the location of spill kits, eyewashes, safety showers, fire extinguishers and fire blankets before starting work
4. Work in designated PC2 or OGTR PC2 areas and obey PC2 rules if dealing with recombinant or otherwise dangerous biologicals.
5. Download and read the MSDS of all the reagents you use, to familiarize yourself with the hazards. Keep the SDS in a known location so it can be found easily.
6. If using acid or other corrosive agent to clean the protein purification column, wear safety glasses! and beware of excessive pressure or poor-fitting junctions – these can cause acid to spray out.
7. Ensure equipment e.g. HPLC is kept in good working order via routine maintenance as part of service contract or via SMB workshop for items out of warranty. Beware of electrical hazards with machines such as HPLC – get machine serviced immediately if there is any suspicion of
electrical malfunction.
8. Dispose of waste solvents appropriately (to Room 225, not down the sink). Consult waste disposal officer if in doubt.
9. Don’t use flammable solvents near ignition sources, especially naked flames. It is recommended that a 3 m gap exists between solvent use/storage areas and possible ignition sources (these include fridges and freezers and other machines with exposed electricals)
10. Use reagents that make flammable or very smelly vapours (e.g. mercaptoethanol) in the fume hood.
11. Know the risks of all machinery you use, e.g. the equipment used for cell lysis (sonicator, French press) is potentially hazardous – obtain and read the risk assessments for these pieces of equipment or do the risk assessments yourself.
12. Many diverse procedures are required for protein purification, and no single SOP can cover them all. Read any specific instruction manuals and consult any equipment or room custodians before starting work. If you have any concerns or uncertainties, talk to your supervisor.

Section 4 – Disposal / Spills / Incidents
1. Depending on the nature of the spill, clean up as described in SOPs for Biohazard Spills, Flammables, Corrosives, or Toxic Substances.
2. Depending on the nature of the materials used, dispose of wastes as described in SOPs for Biohazard Spills, or Chemical Wastes.
3. Any large spills of hazardous materials (>1L) or incidents resulting in injury must be reported to your supervisor immediately and via the online incident report form within 24 h. Near misses (dangerous situations not leading to an incident) should also be reported.

Section 5 – Repairs / Certification / Validation
1. Ensure any equipment used is kept in good repair. Consult the manufacturer or the SMB service centre if in doubt.

Section 6 – Relevant Material safety data sheets
1. Due to the diversity of protein purification methods, many different SDS may be appropriate, e.g. methanol, acetonitrile, butanol, mercaptoethanol, PMSF. Make sure you know the identity and risks of the chemicals you are using and have read and understood the appropriate SDS.

Section 7 – References
1. Essential: Risk assessment for Protein Purification
2. As needed: Risk assessment and SOP for French Press (SMB051), Sonicator (SMB030), Centrifugation (SMB007), Polyacrylamide gel electrophoresis (SMB028), Flammables (SMB013), Toxic chemicals (SMB034), HPLC (SMB019), Disposing of chemical waste (SMB008), Handling Risk group 2 microorganisms (SMB026), Using human tissues (SMB047), Using animal tissues (SMB002).

SOP Consultation, Training and Approval
Print names and enter signatures and dates to certify that the persons named in this section have been consulted/trained in relation to the development and implementation of this Standard Operating Procedure. WHS Representative (WHS Committee) certifies that consultation has taken place.

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