Deep Science Publishing
https://doi.org/10.70593/978-93-49307-52-0_28

Chapter 28

Isolation and purification of recombinant proteins: Principles, techniques, and biotechnological applications

Yashdeep Srivastava[1], Keshawanand Tripathi[1*], Narendra Kumar[2]
[1] *Department of Biotechnology, Invertis University, Bareilly, Uttar Pradesh, India.*
[2]*School of Biotechnology and Bioengineering, Institute of Advanced Research, Gandhinagar, Gujarat, India.*
[*]Email: tripathikn009@gmail.com

1. Introduction:

In biotechnology and molecular biology, isolating and purifying recombinant proteins are crucial for various applications such as therapeutic drug development, industrial enzyme production, and structural biology studies (Janson, and Rydén, 1998; Yadav et al., 2021). The process begins with expressing the target protein in a different host system, like bacteria, yeast, insect cells, or mammalian cells, using recombinant DNA technology (Tripathi et al., 2018). Subsequently, separating the recombinant protein from host cell components and contaminants becomes essential (Smith, and Johnson, 1988; Wingfield, 2016).

Affinity chromatography is a widely used method for isolating recombinant proteins, exploiting specific interactions between the target protein and a ligand immobilized on a chromatography matrix. Affinity tags such as polyhistidine (His-tag), glutathione S-transferase (GST), or maltose-binding protein (MBP) are often fused to aid purification. After cell lysis, the lysate passes through a chromatography column housing the immobilized ligand, allowing the target protein to selectively bind while nonspecific contaminants are washed away. Elution with a competitive ligand or alteration in pH or salt concentration releases the purified protein. Other purification techniques like ion exchange chromatography, size exclusion chromatography, and hydrophobic interaction chromatography utilize differences in charge, size, or hydrophobicity for separation. Further purification steps include dialysis, ultrafiltration, or high-performance liquid chromatography (HPLC) to enhance purity and concentration (Srivastava et al., 2022), Tiwari et al., 2023). Successful isolation and purification rely on meticulous selection and optimization of strategies tailored to the target protein and expression system, ensuring high-quality proteins for various research and industrial applications (Scopes, 1993).

2. Procedure:

1. **Collection of *E. coli* Cells:**
2. **Cell Harvesting:**
   a. Utilize a centrifuge to spin the cell culture in 1 L bottles (2 bottles per culture) for 15 minutes at 14,000 x g at 4 °C.
   b. Gently pour off the supernatant and carefully transfer the bacterial pellets to two 50 ml tubes using a slim spatula. Optionally, perform an additional brief centrifugation step to consolidate the pellet.
   c. Note: Each 1.8 L culture yields two bacterial pellets, each stored in a 50 ml centrifuge tube.
3. **Storage:**
   a. Freeze the pellets at -80 °C until required for further processing in protein isolation.
   b. Isolation and Purification of Recombinant Protein:
   c. Method Selection: If the protein is soluble, utilize the "Native Isolation" method. For insoluble proteins, employ "Nonnative Isolation" procedures.
4. **Cell Lysis - Nonnative Isolation:**
   a. Thaw the *E. coli* pellet from the -80 °C freezer and add 20 ml of Lysis/Wash Buffer to each centrifuge tube.
   b. Mix thoroughly until the pellet dissolves into the buffer, then incubate overnight. The protein will denature, resulting in a thick slurry by morning.
   c. Centrifuge the mixture at 20,500 x g for 30 minutes at room temperature. Transfer the supernatant to a new tube, discarding any solid residue.
5. **Cell Lysis - Native Isolation:**
   a. Retrieve the *E. coli* pellet from the freezer and add Lysis Buffer to achieve a final volume of 30 ml.
   b. Agitate the tube to dislodge the pellet, then clean the sonicator probe with ethanol and water.
   c. Sonicate the pellet on ice for 5 minutes, with intervals totaling 10 minutes at 30% amplitude.
   d. Ensure thorough disruption of the pellet by gently agitating the tube during sonication. The mixture should resemble a gelatinous substance afterward.
   e. Clean the sonicator probe with ethanol and water before and after each use, as well as for storage purposes.
   f. Centrifuge the mixture at 20,500 x g for 30 minutes at 4 °C. Transfer the supernatant to a new tube, discarding any solid residue.

Table 1: Major components used in isolation and purification of proteins

S. No.	Isolation Technique	Buffer Used	Components
1	Non-native	Lysis/Wash	6M GuHCl, 100mM H_2NaPO4, 10mM Tris Base, 10mM imidazole; pH=8.0
2	Elution	Elution	6M GuHCl (Guanidine hydrochloride), 200mM Glacial Acetic Acid (17.4 M)
3	Dialysis (Renaturation)	Phosphate Buffer Saline (PBS)	21.7 mM NaH_2PO4, 15.3 mM Na_2HPO4, 149 mM NaCl
4	Buffer 1	PBS	0.2 M GuHCl, 1.99 mM dithiothreitol (DTT) in 4 L ultrapure water; pH=7.4
5	Buffer 2	PBS	in 4 L ultrapure water; pH=7.4
	Native	Lysis	50mM NaH_2PO4, 300mM NaCl, 10mM imidazole; pH=8.0

4. Precautions:

1. Choose a suitable expression system and optimize culture conditions for protein expression.
2. Use appropriate buffers and detergents for cell lysis and protein extraction, following manufacturer instructions.
3. Handle cells and cell lysates gently to preserve protein structure and avoid degradation.
4. Use affinity chromatography or other purification techniques suitable for the protein's characteristics.
5. Monitor purification steps carefully to maximize protein yield and purity.
6. Perform endotoxin removal steps if necessary, especially for proteins intended for biological assays or therapeutic use.
7. Use validated assays (e.g., SDS-PAGE, Western blotting) to verify the presence and purity of the recombinant protein.
8. Store purified protein in appropriate conditions (e.g., at -80°C) to maintain stability and functionality.
9. Dispose of cell cultures, purification buffers, and contaminated materials properly according to biohazard guidelines.

5. References:

1. Scopes, R. K. (1993). *Protein purification: Principles and practice* (3rd ed.). Springer.
2. Wilson, K., & Walker, J. (2010). *Principles and techniques of biochemistry and molecular biology* (7th ed.). Cambridge University Press.
3. Janson, J. C., & Rydén, L. (1998). *Protein purification: Principles, high-resolution methods, and applications* (2nd ed.). Wiley-VCH.
4. Smith, D. B., & Johnson, K. S. (1988). Single-step purification of polypeptides expressed in *Escherichia coli* as fusions with glutathione S-transferase. *Gene, 67*(1), 31–40.
5. Wingfield, P. T. (2016). Overview of the purification of recombinant proteins. *Current Protocols in Protein Science, 80*(1), 6.1.1–6.1.35.
6. Yadav, R. K., Thagela, P., Tripathi, K., & Abraham, G. (2016). Physiological and proteomic analysis of salinity tolerance of the halotolerant cyanobacterium *Anabaena* sp. *World Journal of Microbiology and Biotechnology, 32*(1), 1–11.
7. Tripathi, K., Kumar, N., & Abraham, G. (Eds.). (2018). *The role of photosynthetic microbes in agriculture and industry.* Nova Science Publishers.
8. Yadav, R. K., Tripathi, K., Varghese, E., & Abraham, G. (2021). Physiological and proteomic studies of the cyanobacterium *Anabaena* sp. acclimated to desiccation stress. *Current Microbiology, 78*(6), 2429–2439.
9. Tiwari, P., Srivastava, Y., Sharma, A., & Vinayagam, R. (2023). Antimicrobial peptides: the production of novel peptide-based therapeutics in plant systems. *Life, 13*(9), 1875.
10. Srivastava, Y., Tripathi, S., Mishra, B., & Sangwan, N. S. (2022). Cloning and homologous characterization of geranylgeranyl pyrophosphate synthase (GGPPS) from Withania somnifera revealed alterations in metabolic flux towards gibberellic acid biosynthesis. *Planta, 256*(1), 4.