

The Steps To A Successful Protein Purification

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DOI: <https://doi.org/10.63363/aijfr.2026.v07i05.8485>

Abstract

Protein purification is a cornerstone of biochemistry and structural biology, enabling researchers to isolate specific proteins of interest from complex cellular lysates. This paper outlines a standard multi-step purification workflow based on laboratory procedures performed. The protocol details sample preparations, cell lysis via sonication, affinity chromatography, size exclusion chromatography as a polishing step, and downstream purity verification using gel staining.

1. Introduction

Isolating a target protein- such as Isocitrate Dehydrogenase 1- requires a combination of selective techniques designed to exploit differences in protein size, charge, binding affinity, and solubility. Typically, purification is executed in sequential stages:

1. Breaking open cellular membranes to release intracellular proteins while protecting them from proteolysis
2. Capturing the target protein based on a specific tag
3. Polishing the sample by separating proteins according to hydrodynamic radius
4. Verifying purity and tracking yield through gel staining

Materials

- Lysis Buffer
- Reducing agents: Beta-mercaptoethonal (BME), Dithiothreitol(DTT), DTAP
- Protease Inhibitors: Phenylmethylsulfonyl Fluoride(PMSF)
- Washing and Elution Buffers

Step-By-Step Purification Protocol

1. Thaw cell pellets containing the expressed protein on ice
2. Resuspend cell pellets thoroughly in lysis buffer supplemented with 5 mM BME and PMSF. Ensure any visible chunks are fully dispersed using a micropipette
3. Transfer the suspension into a metal cup chilled in an ice water bath to prevent thermal denaturation
4. Keep at 20 seconds at 30 amps, repeat 5 times
5. Equilibrate a chromatography column packed with histidine-purifying resin with water to remove storage ethanol and free cations
6. Filter the clarified lysate through 0.8 μ m filter and apply it to the column

7. Wash the the column with wash buffer containing 50 mM imidazole and BME to remove any contaminants
8. Apply the elution buffer and collect 1 mL fractions sequentially
9. Prepare SEC buffer, Filter and degas the buffer using a vacuum filtration assembly with a nitrocellulose membrane
10. Equilibrate the SEC column with buffer for approximately 2 hours, ensuring system pressure remains below 0.28 MPa to protect resin
11. Filter the concentrated protein sample through a 0.2 μ m filter and inject it through the sample loop
12. Collect fractions based on UV absorbance peaks and conductivity readings
13. Mix 15 μ m of each fraction with μ L of 4X reducing sample loading dye
14. Heat samples on a heating block at 98 degrees Celsius for 3 minutes
15. Load samples alongside a molecular weight ladder into a pre-cast gel running in a 1X tank buffer
16. Run at a constant voltage 160V for approximately 45 minutes
17. Stain the gel using dye, destain to clear background, and image using a gel documentation system

Conclusion

Following the structured sequence of cell lysis, immobilized metal affinity chromatography, and size exclusion chromatography yields high-purity protein suitable for structural and functional studies. Tracking each step ensures quality control and process optimization.