Application Note

Vivaspin® 500
High-cell-density Cultivation of Ultrafiltration: a substitute to lyophilization

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Summary

Lyophilization, the removal of the majority of the water in a sample under conditions of low temperature and vacuum, is a widely used technique in the areas of Protein purification, protein reagent preparation, and the manufacture of protein biomolecules for therapeutic and diagnostic applications. It can be performed on a sample in order to increase stability, reduce the volume, storage, transport and various other applications. Proteins are highly diverse and whereas a single chain protein with a highly ordered tertiary structure may freeze dry with little difficulty, a multimeric protein with multiple domains and hydrophobic proteins will pose a far more demanding challenge to achieve successful Lyophilization. Freeze drying (Lyophilization) is split into three process stages, freezing, primary drying at lower temperatures when most of the water is removed, and secondary drying at ambient or higher temperatures to minimize the final unbound water content. During the freezing process, water crystallizes to ice and the excluded excipient salts concentrate to local concentrations far higher than those in the original liquid state. This may in itself have implications for the stability of the protein(s) present, which may be destabilized and denature because of the change in ionic strength. In addition, if buffer salts, such as mixed phosphate buffer are present, the selective crystallization or precipitation of one of these salts at a higher temperature to the other may result in localized pH shifts, which gain may induce denaturation of the proteins. Such denaturation may lead to exposure of normally buried residues and an increase in aggregation, some of which may be irreversible on reconstitution. Other proteins may be satisfactorily immobilized in the lyophilized state but undergo changes that result in aggregation on reconstitution. Membrane bound proteins may pose special problems when undergoing Lyophilization, cell membranes are particularly prone to disruption during the dehydration process and so membrane-associated proteins will also be at risk.

Ultrafiltration separates dissolved particles and molecules according to size and configuration by flowing a solution that contains these molecules through a membrane under a driving force. The driving force mostly applied is centrifugal; unlike certain applications may use static force, positive pressure etc. The membrane will retain most particles and molecules above its retention rating and will allow smaller molecules, along with the solvent to pass through the membrane. Because some UF membranes have the ability to retain larger macromolecules, they have been historically characterized by a molecular weight cut off (MWCO) rather than by a particular pore size. The concept of the MWCO expressed in Daltons is a measure of the removal characteristic of a membrane in terms of atomic weight rather than size. This, UF membranes with a specified MWCO are presumed to act as a barrier to compounds or molecules with a molecular weight exceeding the MWCO. Various membranes have been commercialised for Ultrafiltration based on their efficiency to perform*viz*, Polyethersulfone, Cellulose triacetae, Stabilized Cellulose etc; each one having their own importance. Where Polyethersulfone membrane is recommended for fastest concentrations, Cellulose Triacetate is chosen for Protein Removal. Stabilised cellulose serves special application when highest recovery with lg fractions is expected. The membrane pore size (measured in MWCO) should be selected at least 50% smaller than the size of the molecule to be retained. Ultrafiltration is preferred over other methods when high & easy recovery of protein is required without causing any structural or functional changes; in short time.

Suggested Method

1. Select a Vivaspin 500 of MWCO 5,000 as the protein of interest is around 12,000 Dalton.
2. Fill the Vivaspin 500 up to 500 µl volumes ensuring the screw closure is fully sealed.
3. Centrifuge for the recommended amount of time at an appropriate speed for your MWCO membrane and centrifuge with fixed angle rotor. (10,000 rpm for 20 min)
4. Empty the filtrate container and refill the concentrator with additional sample if required.
5. Centrifuge again as before (repeat until entire sample has been loaded).
6. Centrifuge until sample reaches the desired volume.
7. Recover the concentrate from the insert with a pipette.

Results and Discussion

The recombinant mutated lectin purified through Ni-NTA (nickel nitrilotriacetic acid) column was concentrated using Vivaspin 500 in parallel to Lyophilization. The degree of concentration was analyzed in 15% SDS-PAGE stained with Coomassive brilliant blue. Both the concentrated samples were run in 15% SDS-PAGE along with the Ni-NTA column purified protein. Equal volume of sample was loaded in each lane. Clear band of 12 kDa was observed in each lane of SDS-PAGE (Figure 1).

The result (Figure 1) clearly indicates the fundamental advantage offered by ultrafiltration over Lyophilization. The protein was much more concentrated by Vivaspin centrifugal units with minimal loss (Lane 3) in comparison to Lyophilization. Moreover Lyophilization took a long time when compared to ultrafiltration which took not more than 20 minutes for each run. This clearly indicates the efficiency of ultrafiltration over Lyophilization.

Test sample

- Cell Line: BL21 (Invitrogen, CA, USA)
- Media: LB Broth (Luria Bertini)
- Protein of Interest: Recombinant mutated lectin.
- Size of protein: 12kDa

Equipment

- Sartorius Vivaspin 500, 5,000 MWCO
- Sorvall MC12V Centrifuge, fixed angle rotor
- Lab scale Operon Freeze dryer (-55°C)
- Standard Eppendorf pipettes and tips
- Biorad SDS Gel apparatus

Testimonial

"Ultrafiltration is a smarter, one step process to concentrate protein sample in less time, with good yield, in comparison to Lyophilization. I can directly store my protein sample at -80°C with no further processing for future use. I will prefer this technique for my further protein work" – Prithwi Ghosh, Senior Research Fellow (SRF), Plant Biology Division, Bose Institute.

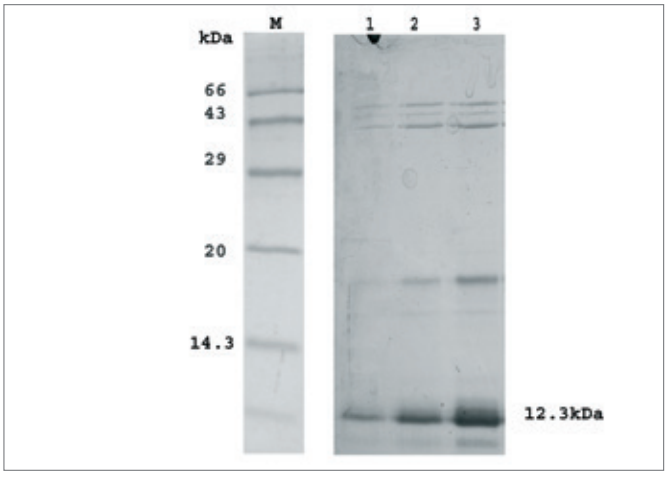

Figure 1: SDS-PAGE profile for evaluating ultrafiltration and Lyophilization: Lane M, molecular weight marker; lane 1, Ni-NTA column purified protein; lane 2, Lyophilized protein; lanes 3, Ultrafiltered protein. The molecular weights (kDa) of the protein markers are sown at the left. Label indicates the recombinant protein band.)

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