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Application Note

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Concentration of an 8 kDa Peptide Fragment with Vivaspin® 6 Ultrafiltration Devices

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Introduction

An 8 kDa peptide, corresponding to the trans-lumenal domain of the integral nuclear pore complex protein gp210 was expressed in *E.coli* for the purpose of spectral analysis. The fragment, which includes an N-terminal histidine tag, was purified on an affinity column. Vivaspin® 6 with 5 kDa MWCO PES membrane was used to concentrate the protein and exchange the sample buffer. This diafiltration step enabled subsequent proteolytic cleavage of the fusion tag, while the protein was brought to a concentration which was sufficient for further purification and spectroscopic analysis.

Materials and Methods

The recombinant protein was cloned in the pET-19b vector and expressed in BL-21(DE3)pLys(S) expression hosts (Novagen). Bacteria were grown to an OD_{600} of 0.4 at 37 °C and induced with 0.4 mM IPTG at 25 °C for 12 hours. Lysis of the culture followed, and the lysate was passed through a TALON metal affinity column (Clontech, US). Elution was performed in 50 mM Tris pH 8.0/100 mM NaCl buffer, in the presence of 250 mM imidazole, to a final protein concentration of ~0.05- 0.1 mg/mL.

For buffer exchange and protein concentration for spectral analysis, the eluate sample was applied to a Vivaspin® 6 concentrator with 5 kDa MWCO PES membrane for several concentration/buffer exchange cycles at ~3,000 ***g*** in a swing-out rotor, or at 7,500 ***g*** in an SS-34 fixed angle rotor. Centrifugation times ranged from ~1 hour to over 2 hours for a 30-fold concentration, depending on the starting concentration of protein. Prior to use, the membrane was passivated to block non-specific binding sites, as follows:

Vivaspin® devices were filled with buffer and centrifuged for 20 min. Following this, the devices were filled with 1 mg/mL of lysozyme and centrifuged for another 20 min.

Several washes with buffer were subsequntly performed to remove excess lysozyme. The passivated filters were stored with buffer at 4 °C until use. Products were assayed using tricine-SDS-PAGE and stained with Coomassie brilliant blue (0.025% in 10% acetic acid) overnight, following a 30–60 min fixation with 50 % methanol and 10 % acetic acid (performed to prevent the diffusion of the short peptide fragment from the gel). De-staining was effected with a 10 % acetic acid solution overnight.

Results

To check expression and purification efficiency, an expression and purification protocol was performed on a 400 mL bacterial culture. Selected fractions were analyzed and are shown in figure 1.
The results showed that the protein of interest could be concentrated to the desired level using Vivaspin® 6. Non-specific protein bands that co-eluted with the recombinant peptide were also concentrated with the device. Passivation of the filters with lysozyme prior to sample concentration increased recovery of the 8 kDa protein to >50 % (results not shown). BSA and ethanolamine were also tested as blocking agents instead of lysozyme and showed similar recovery efficiency.

Conclusions

Concentration of the protein using the Vivaspin® 6 enabled exchange of the buffer and concentration of the protein for the removal of the his-tag. In addition, the high concentration factor that was achieved allowed us to detect the presence of protein contaminants, which could not be visualized prior to concentration, indicating that a further purification step was necessary before spectroscopic analysis could be performed.

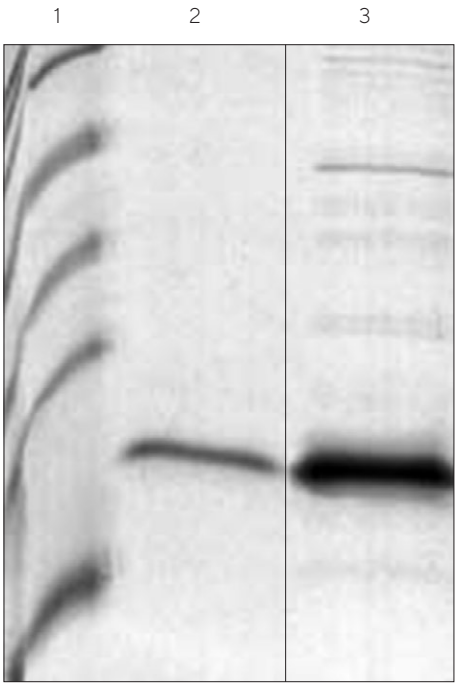

Fig.1: A 400 mL bacterial culture was grown, and the soluble fraction passed through an affinity column. The eluate was subjected to concentration and buffer exchange using Vivaspin® 6. A standard protein marker is shown in lane 1. The eluted and subsequently concentrated fractions are shown in lanes 2 and 3, respectively.

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