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Application Note

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Concentration of a Low Molecular Weight Peptide – Concentration of an 8 kDa Peptide Fragment with Vivaspin 6 Ultrafiltration Devices

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Introduction

An 8 kDa fragment, 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. A Vivaspin concentrator, with a 5000 MWCO PES membrane was used to concentrate the protein and exchange the buffer. The buffer was successfully exchanged to enable proteolytic cleavage of the fusion tag, the protein was brought to sufficient concentration 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 protein was subjected to a Vivaspin 6 concentrator with a 5000 MWCO PES membrane for several concentration/buffer exchange cycles at ~3,000 g in a swing-out bucket rotor, or at 7,500 g in an SS-34 fixedangle rotor. Centrifugation times ranged from ~1 hour to over 2 hours for a 30-fold concentration, depending on the concentration of protein. Prior to use, the membrane was pre-blocked as follows: The Vivaspin devices were loaded with buffer and

centrifuged for 20 min. Following this, the devices were loaded with 1 mg/ml of lysozyme and centrifuged for another 20 min.

Two centrifugations with buffer were subsequntly performed in order to cleanse the membrane, followed by several washes with buffer. The blocked 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 performed 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 show that the protein can be concentrated to the desired concentration using the Vivaspin device. Non-specific protein bands that co-eluted with the recombinant peptide were also concentrated with the device. Blocking the filters with lysozyme increased the retention efficiency of the 8 kDa protein to >50 % (results not shown). BSA and ethanolamine were also tried as blocking agents instead of lysozyme and showed similar retention efficiency.

Conclusions

Concentration of the protein using the Vivaspin 6 ml concentrators successfully exchanged the buffer and concentrated the protein for the removal of the His-tag fusion. In addition, the high concentration factor allowed us to detect the presence of protein contaminants, which could not be detected prior to concentration, indicating that a further purification is necessary before spectroscopic analysis can be performed.

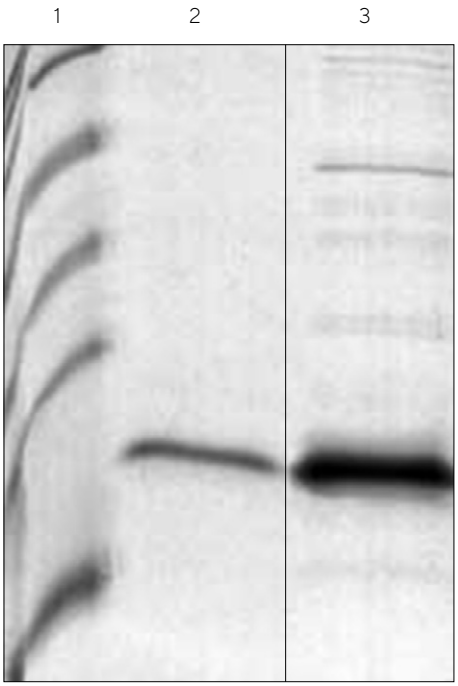

Fig.1: A 400 ml bacterial culture was grown, and the soluble fraction was passed through an affinity column. The eluate was subjected to concentration and buffer exchange using the Vivaspin 6 concentrator device. A standard protein marker is shown in lane 1. The eluted and subsequently concentrated fractions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

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