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Application Note

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Concentration of Low Molecular Weight Peptides - Concentration of Peptides with Vivaspin 500 Ultrafiltration Devices

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Introduction

It is often useful and desirable to reduce the volume of a protein sample in order to carry out further analysis. The are many applications which require the protein or peptide to be contained in a small volume. For example, many protein purification experiments require the eluate from a particular method to be concentrated in order to enable the sample to be tested for activity in some form of tissue culture assay. Similarly, column eluates to be run on PAGE gels must be concentrated to a small volume for loading. Other applications may require the recovery and concentration of peptides, such as the products of protein proteolysis experiments in which the peptides are required for mass spectroscopic microsequence analysis. In this application note, we have used a 3 kDa MWCO PES membrane incorporated into Vivaspin 500 ultrafiltration devices to concentrate two peptides.

Methods

Four milligrams of Insulin A chain peptide (Molecular weight 2.5 kDa) and 4 mg of thyrocalcitonin (Molecular weight 3,593 Da) were made up in 4 ml of 20 mM phosphate buffer. 500 µl of each 1 mg/ml peptide solution was added to 3× Vivaspin 500 ultrafiltration devices incorporating a 3 kDa MWCO PES Molecular weight cut-off membrane. The samples were centrifuged at 13,000 rpm for 1 hour at room temperature or until the retentate was equal in volume to 100 µls.

Protein Estimation

The protein content of both the filtrate and retentate for both Insulin A chain peptide and thyrocalcitonin were measured using an Advanced Protein assay.

Results

The protein content of the filtrate and retentate for both Insulin A chain peptide and thyrocalcitonin following concentration of both proteins in the Vivaspin 500 are shown in Table 1. The percentage of recovered protein in the retentate is also shown.

Conclusions

It is evident from the results presented here that the Vivaspin 500 is both a reliable and efficeint method for the concentration of peptides. These devices are very useful for the concentration of and/or exchange of buffer any sample that requires such manipulations is precious since the recovery from the retentate of the Vivaspin 500 is excellent.

The new 3 kDa MWCO membrane incorporated into the Vivaspin 500 is ideally suited to handling peptides with molecular weights as low as 2.5–3 kDa. Interestingly, almost 100 % recovery of Insulin A chain was observed when using 3 kDa MWCO membrane from Vivascience. This suggests that the, nominally assigned 3 kDa MWCO membrane could be successfully used to concentrate peptides less than 2.5 kDa.

Sample	Protein (mg) in Filtrate	Protein (mg) in Retentate	Recovery of protein (%)
Insulin A Chain	0.02	3.9	98
Thyrocalcitonin	0.005	3.97	99

Table 1: Protein recovery from the concentration of peptides in the Vivaspin 500.

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