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# Concentration of Low Molecular Weight 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. There are many applications which require the protein or peptide to be contained in a small volume. For example, protein purification experiments require the eluate from a particular method to be concentrated so that the sample can be tested for activity in some form of tissue culture assay. Similarly, column eluates to be analyzed on polyacrylamide 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 3.6 kDa) were dissolved 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 with 3 kDa MWCO PES 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 µL.

## 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 samples following concentration of both proteins in the with 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 efficient tool for the concentration of peptides. These devices are very useful for the concentration and/or exchange of buffer in any sample that requires such manipulations, since the recovery from the retentate reached rates of between 98 and 99%.

The 3 kDa MWCO membrane incorporated into 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 a 3 kDa MWCO device. This suggests that the 3 kDa nominal MWCO could even be used to concentrate peptides with molecular weights lower than 2.5 kDa.

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

Table 1: Protein recovery following concentration of peptides with Vivaspin® 500.

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