Application Note

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Sartobind® Lab Membrane Adsorbers Provide Constant Binding Capacities for More Than 100 Cycles

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Introduction

Sartobind® Q membrane adsorbers carry quaternary ammonium groups linked to a cellulose backbone. We performed a test to examine the stability of these units over multiple purification cycles. After 100 cycles, a NaOH regeneration step was performed. Our results show that membrane chromatography can be used for at least 100 cycles with no loss of binding capacity, therefore offering extended consumable lifetime and increased productivity compared to resin columns. An SDS gel proved the function of Sartobind® Q in the final cycles.

Find out more: sartorius.com/en/products/lab-filtration-purification/membrane-chromatography

Materials and Methods

A liquid chromatography system with gradient pumps was used. All buffers were autoclaved at 121 °C for 30 min prior use. Bovine serum (prepared from bovine blood using 0.65 µm cross flow cassettes and stored frozen) was thawed and diluted 1:20 with 0.05 M Tris-HCl pH 8.8, 10 mM NaCl and filtered through a 0.2 µm Minisart®. Only the amount of serum needed for one day was prepared every morning.

A 5 µm Minisart® filter was connected via Luer lock to the inlet of a Sartobind® Q Lab unit with 2.1 mL membrane volume. This filter was replaced when pressure increase was observed. At a flow rate of 10 mL/min, 52.5 mL of sample was loaded so that a two step breakthrough curve could be recorded. The first step was the unbound IgG and minor components, while the second was the BSA (data not shown). The Sartobind® Lab unit was washed with 15 mL buffer at a flow rate of 10 mL/min and BSA was eluted with 10 mL of 1 M NaCl in 0.05 M Tris-HCl pH 8.8 buffer. During elution, only the BSA peak was collected and stored for subsequent analysis.

The Sartobind® Lab unit was washed with 10 mL of equilibration buffer and the next cycle started. After 100 cycles the unit was regenerated by applying 50 mL of 1 M NaOH, incubating for 30 minutes, then washing with buffer to the starting conditions. 200 cycles were performed in less than two working days.

The eluates were diluted 1:5 with elution buffer, mixed thoroughly and protein content determined by UV-vis spectrophotometry, measuring absorbance at 280 nm against elution buffer. Selected eluates were also assessed for purity by PAGE. Samples were diluted 1:50 with Laemmli buffer prior to loading 10-20 µL aliquots onto the gels. Proteins were visualized by coomassie staining after electrophoresis.

Results

The amount of eluted protein was plotted against the cycle number to generate Figure 1. These data demonstrate a highly consistent binding capacity of around 0.6 mg BSA per square centimeter of membrane area, over multiple cycles. Coomassie staining of polyacrylamide gels showed high purity of BSA could be achieved even after hundreds of cycles (Figure 2).

Figure 1: *A constant binding capacity for bovine serum albumin (BSA) is maintained for more than 100 purification cycles with a single Sartobind® Q Lab unit.*

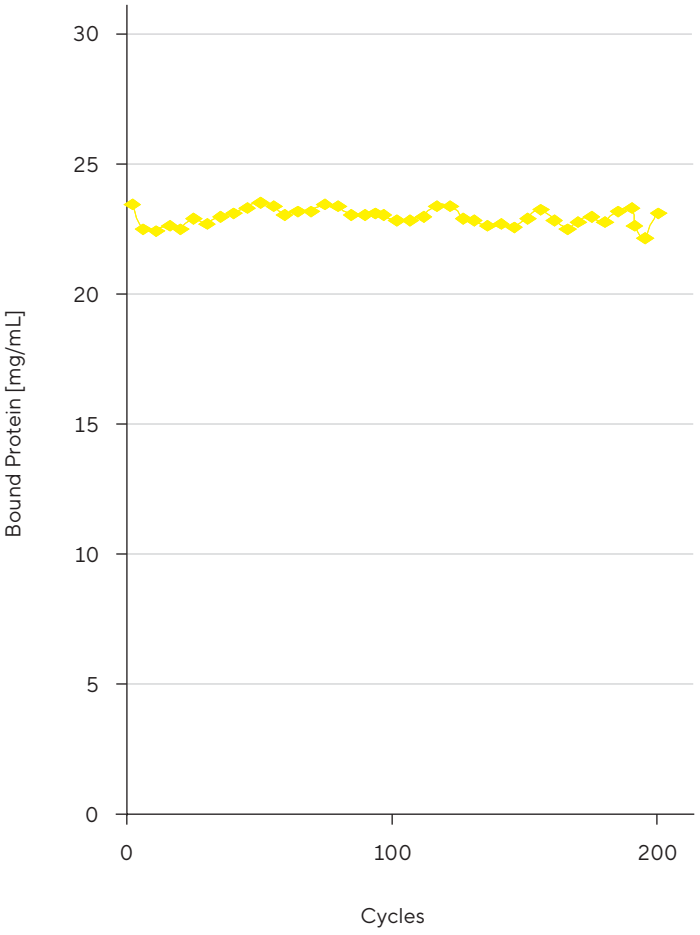
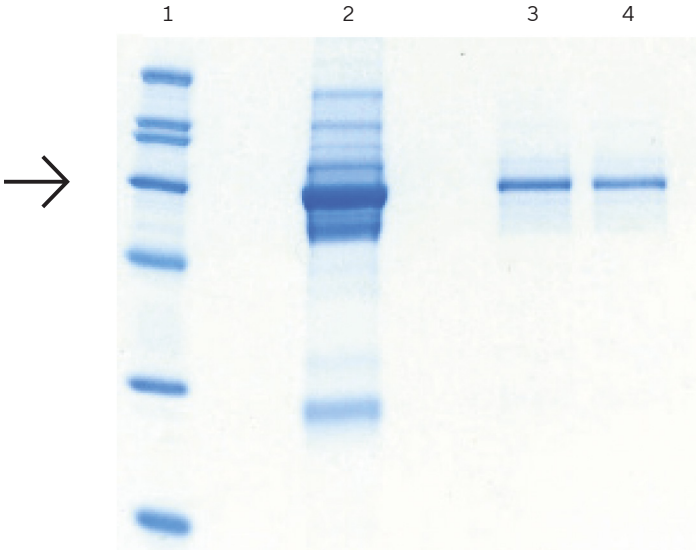


Figure 2: *PAGE analysis of BSA purified following multiple cycles on a Sartobind® Q Lab unit. Lane 1, molecular weight marker (200, 116, 97, 66 (arrow), 45, 31 and 21.5 kDa), lane 2, load, lane 3-4, selected elution peak fractions.*



Conclusion

In this study, we have presented the reusability of Sartobind® Q Lab membrane adsorbers. Our results show that these units exhibit highly consistent binding capacities while yielding high purity eluates, even after more than 100 cycles. This makes Sartobind® Lab an economial choice for any research laboratory to perform frequent, reproducible, and reliable protein purifications.

Note

Literature published up to c.2022 may reference the use of Sartobind® MA, which is a name previously used for the Sartobind® Lab membrane adsorbers. When these products were renamed, there was no change made to fit, form or function. Therefore, results collected and methods established using Sartobind® MA remain valid also for Sartobind® Lab.

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