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Removal of Endotoxin from Monoclonal Antibodies using Vivapure® Q Membrane Adsorbers

Brendan Fish, Karen Bannister, Emma Tribbeck,
Cambridge Antibody Technology, Milstein Building, Granta Park, Cambridge, CB1 6GH, UK

Correspondence
E-Mail: john.cashman@sartorius.com

Abstract

Endotoxins are known to have negative effects in both cell culture and animal models. It is desirable to minimize endotoxins in purified protein preparations prior to their use in cell-based assays. However, conventional approaches to endotoxin removal using resin-based chromatography columns are labor intensive and often result in dilution of the protein sample. In this study, we demonstrate the benefits of Vivapure® centrifugal anion exchange membrane devices, which can remove endotoxin from research grade monoclonal antibody solutions simply, and with high protein recovery.

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Introduction

Endotoxins are lipopolysaccharides present in the cell wall of most Gram-negative bacteria, and are frequently present as contaminants in protein solutions purified in research environments. They have profound biological effects and thus must be minimized prior to use of such preparations in cell-based assays. The term EU is used to describe the activity of endotoxins, and typically the limit for endotoxin is set at 50 EU/mg for bioactive proteins destined for cell-based assays.

Achieving this low level is often a challenge in research, since endotoxins are robust molecules, which persist even through surviving extremes of temperature and pH. Endotoxins are negatively charged under conditions commonly encountered during protein purification. This negative charge facilitates the use of anion exchange chromatography for their removal. If the binding of endotoxin can be achieved under conditions at which the protein of interest carries a net positive charge (i.e. at a pH below it's isoelectric point) then the protein will be repelled from the positively charged matrix. flowing through with the mobile phase, in what is often termed negative mode chromatography (Figure 1). However, this will often result in dilution of the protein, which may call for an additional concentration step.

Furthermore, packing small chromatography columns and maintaining them sanitary is time consuming, and requires specialist knowledge and equipment. Centrifugal ion exchange membrane spin columns offer an alternative to traditional chromatographic removal of endotoxin. They avoid the development of lengthy procedures with expensive equipment and rapidly yield high levels of endotoxin-free protein.

Here, we tested the use of Vivapure® centrifugal anion exchange membrane devices for the removal of endotoxin from research grade antibody samples.

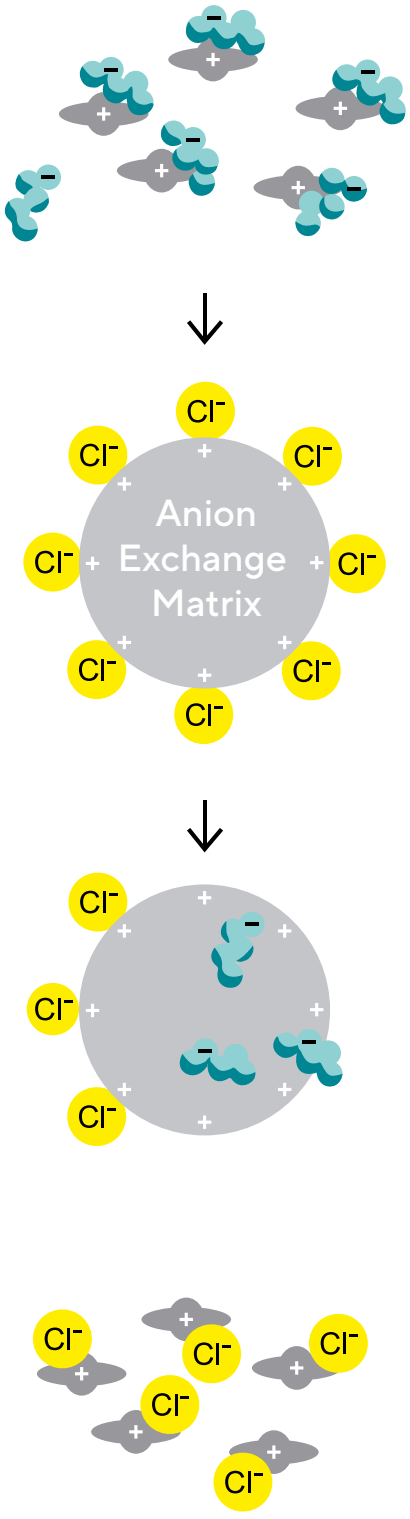

Figure 1: Schematic representation of endotoxin removal by anion exchange. A sample containing the positively charged protein of interest and negatively charged endotoxin is applied to a chromatography matrix. Endotoxin displaces counter ions, adsorbing to the solid phase, whilst the protein remains in the mobile phase.

Materials and Methods

Vivapure® Q Mini spin columns

The monoclonal antibody used in this experiment had an isoelectric point of 7.5. All reagents and containers described below were supplied or prepared endotoxin free. Additionally, pH meter probes and magnetic stirrer bars were depyrogenated according to the manufacturer instruction or by soaking in 0.5 M sodium hydroxide for 1 hour. Vivapure® Q Mini spin columns were washed sequentially with 0.5 mL of water for irrigation (WFI, Baxter), 0.5 mL of 0.5 M sodium hydroxide, 2 × 0.5 mL of WFI and 0.5 ml Dulbecco's phosphate buffered saline, pH 7.2 (PBS, Gibco) by loading each solution into the device and centrifuging at 2,000 *g* for 5 minutes.

The monoclonal antibody (115 mg in 1.3 mL PBS) was divided equally between four Vivapure® Q Mini spin columns and centrifuged as above. The flow through from each column was then filtered through a 0.2 µm sterilizing centrifugal filtration device (Corning, Costar Spin-X, 2,000 *g* for 5 minutes) and pooled.

Residual monoclonal antibody was recovered by washing each Vivapure® Q Mini column twice with 0.5 mL of phosphate buffered saline as above, collecting and combining the washes. Antibody concentration was measured in all samples using UV-vis absorbance measurements at 280 nm and the known extinction coefficient. All volumes were estimated by weight assuming the density of the solutions to be 1 g/mL. Endotoxin (EU) was measured using a kinetic turbidimetric assay (Charles River Endosafe) following the manufacturer instructions.

Vivapure® Q Maxi spin columns

The monoclonal antibody used in this experiment had an isoelectric point of 6.0. Vivapure® Q Maxi spin columns were washed sequentially with 17 mL of water for irrigation (WFI, Baxter), 17 mL of 0.5 M sodium hydroxide and 3 × 17 mL of WFI, followed by 17 mL Dulbecco's PBS (Gibco, previously adjusted to pH 5.5 with the addition of concentrated hydrochloric acid) by loading each solution into the device and centrifuging at 500 *g* for 5 minutes.

The monoclonal antibody (150 mg in 48 mL of PBS) was adjusted to pH 5.5 (i.e. below its pI) by the slow addition of dilute hydrochloric acid with constant mixing. This was then divided equally between four Vivapure® Q Maxi spin columns and centrifuged as above. The flow through from each column was pooled and adjusted to pH 7.2 by the addition of 0.5 M sodium hydroxide. The pH-adjusted sample was sterile filtered (0.2 µm Sartolab® RF, Sartorius) and stored at 4 °C. Residual monoclonal antibody was recovered from the Vivapure® devices by washing each with 15 mL of PBS (pH 5.5), collecting and combining the washes. The concentration of monoclonal antibody and endotoxin levels in all samples was measured as described above.

Results

Recoveries in excess of 90% were attained for both the basic and acidic antibodies tested (Tables 1 and 2). Very high clearance of endotoxin was also detected, with a reduction from 30 to 1.2 EU/mg and >300 to 1.3 EU/mg, respectively. The basic antibody product remained at constant concentration and was suitable for its intended use. The acidic antibody product was slightly reduced in concentration due to dilution on pH adjustment, but also remained suitable for its intended use.

Conclusions

Vivapure® centrifugal anion exchange membrane adsorbers were effective in removal of endotoxin from research grade monoclonal antibody solutions. The clearance of endotoxin was maintained in a high conductivity buffer, PBS, preventing the need for any diafiltration into low salt buffers prior to the anion exchange. This method was also applicable to acidic proteins by simple pH adjustment prior to application to the charged membrane. In addition to the high protein recovery, the starting concentration of the antibody solution was maintained, obviating the need for any further processing. The membrane adsorber technology in Vivapure® provides a rapid, low cost and straightforward method for reduction of endotoxin in protein solutions. Additionally, it allows for convenient processing of multiple samples in parallel, owing to the centrifugal mode of operation.

Table 1: Basic monoclonal antibody recovery and endotoxin level following purification using Vivapure® Q Mini

Sample	Total antibody	Antibody recovery	Endotoxin
Start material	115 mg	–	3,450 EU
Vivapure® Q Mini Flow through	93 mg	81%	112 EU
Vivapure® Q Mini Wash #1	11 mg	10%	ND
Vivapure® Q Mini Wash #2	1 mg	1%	ND

Table 2: Acidic monoclonal antibody recovery and endotoxin level following purification using Vivapure® Q Maxi

Sample	Total antibody	Antibody recovery	Endotoxin
Start material	150 mg	–	45,500 EU
Vivapure® Q Maxi Flow through	125 mg	83%	159 EU
Vivapure® Q Maxi Wash	12 mg	8%	ND EU

References

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Germany
Sartorius Lab Instruments GmbH & Co. KG
Otto-Brenner-Strasse 20
37079 Goettingen
Phone +49 551 308 0

USA
Sartorius Corporation
565 Johnson Avenue
Bohemia, NY 11716
Phone +1 631 254 4249
Toll-free +1 800 635 2906

For further information, visit
www.sartorius.com