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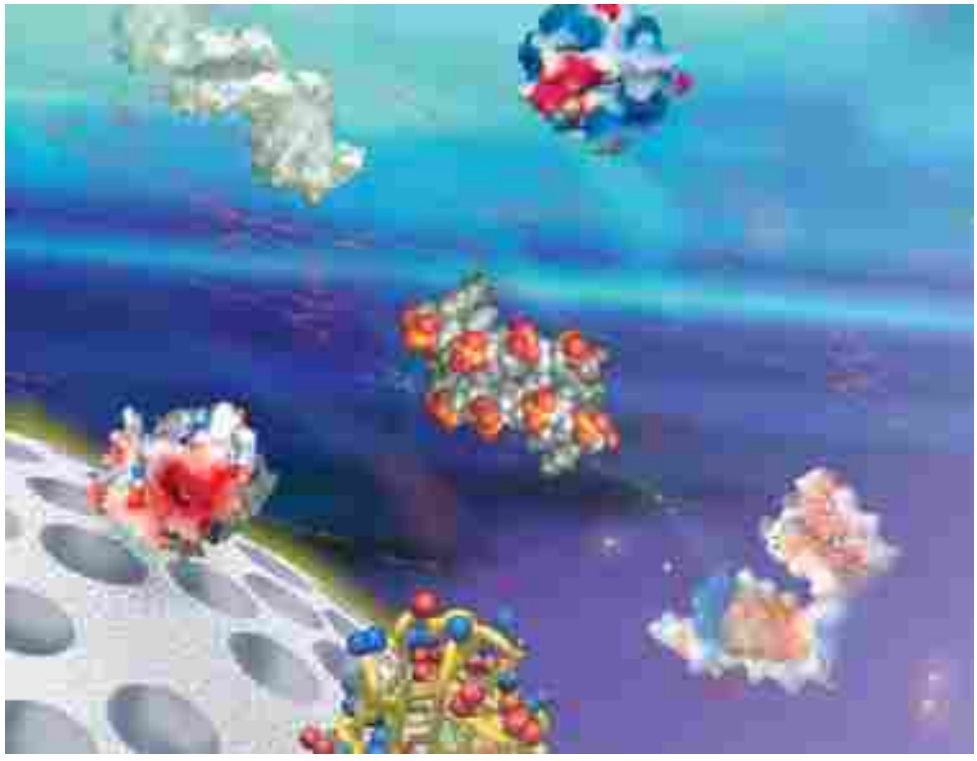
Removal of Endotoxin from Monoclonal Antibodies



Application Note

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turning science into solutions

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It is desirable to minimise endotoxins in purified protein preparations prior to their use in cell-based assays. Vivapure® centrifugal anion exchange membrane devices can remove endotoxin from research grade monoclonal antibody solutions simply with high protein recovery.

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 minimised 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 as endotoxins are robust molecules 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 and flow through with the mobile phase, in what is often termed negative chromatography mode (Figure 1). However, this will often result in dilution of the protein, which may call for an additional concentration step.

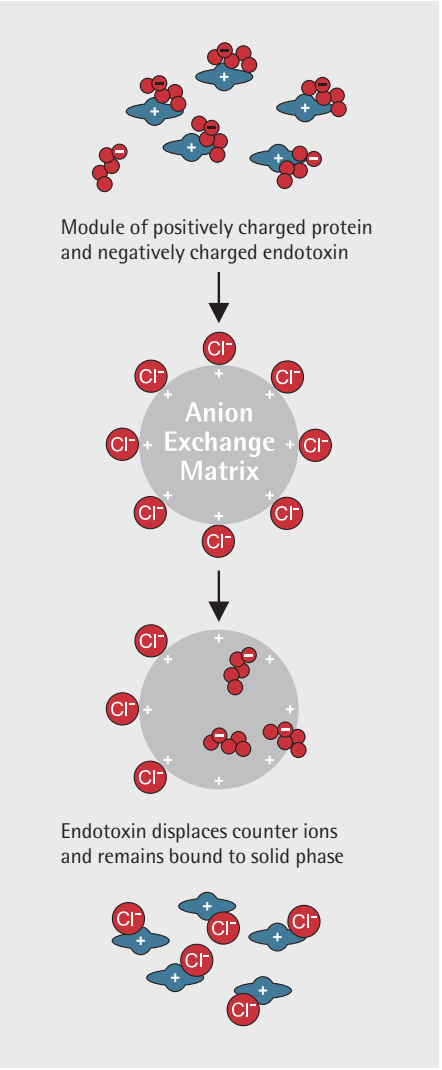

Fig. 1: Anion exchange of for endotoxin removal

Also, 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 potentially could rapidly yield high levels endotoxin-free protein.

In this report we tested the use of centrifugal anion exchange membrane devices for the removal of endotoxin from research grade antibody solutions.

Absorption of endotoxin from a basic monoclonal antibody

Vivapure® Mini Q spin columns
The monoclonal antibody used in this study has 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 manufacturers instruction or by soaking in 0.5 M sodium hydroxide for 1 hour. Vivapure® Mini Q 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 followed by centrifugation at 2,000 × g for 5 minutes.

The monoclonal antibody (115 mg in 1.3 ml PBS) was divided equally amongst four mini spin columns and centrifuged as above. The flow through from each column was then filtered through a 0.2 µm sterilising centrifugal filtration device (Corning, Costar Spin-X, 2,000 xg for 5 minutes) and pooled.

Residual monoclonal antibody was recovered by washing each Vivapure® 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 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 manufacturers instructions.

Vivapure® Maxi H spin Q columns
The monoclonal antibody used in this study has an isoelectric point of 6.0. Vivapure® Maxi H spin Q 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 and 17 ml Dulbecco's phosphate buffered saline, (Gibco, previously adjusted to pH 5.5 with the addition of concentrated hydrochloric acid) by loading each solution into the device followed by centrifugation 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 amongst four Vivapure® maxi spin columns and centrifuged as above. The flow through from each column was then pooled and adjusted to pH 7.2 by the addition of 0.5 M sodium hydroxide. The pH-adjusted pool was then filtered through a 0.2 µm sterilising filter (Millipore Stericup or Sartorius Satorlab) and stored at 4°C. Residual monoclonal antibody was recovered from the Vivapure® maxi columns by washing each with 15 ml of PBS adjusted to pH 5.5 as above, collecting and combining the washes. The concentration of monoclonal antibody and endotoxin levels in all samples was measured as described above.

Results and discussion
High recovery of antibody was achieved, for both the basic and acidic antibodies; 92 % and 91 % respectively (Tables 1 and 2). Very high clearance of endotoxin was also seen, with the levels being reduced to 1.2 and 1.3 EU/mg for both antibodies (Tables 1 and 2). 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 remained suitable for its intended use.

Conclusions
Vivapure® centrifugal anion exchange membrane devices 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. This method is a trouble-free method for reduction of endotoxin in protein solutions and would allow for easy processing of multiple samples over a short period.

Sample	Total antibody (mg)	Antibody recovery (%)	Endotoxin (EU)
Start material	115	–	3450
Vivapure® Mini Q Flow through	93	81	112
Vivapure® Mini Q Wash #1	11	10	ND
Vivapure® Mini Q Wash #2	1	1	ND

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

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

ND = Not determined

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

References
Petsch, D. and Anspach, F.B. (2000)
Endotoxin removal from protein solutions.
Journal of Biotechnology, 76, 97–199.

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