# Practical Guide: Selecting the Optimal Resins for Endotoxin Depletion in Process Purification

Payal Khandelwal, PhD
Bio-Rad Laboratories, Inc., 6000 James Watson Drive, Hercules, CA 94547

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## Effectively Remove Endotoxins with Our Versatile Process Resins

Endotoxins are one of the most common pyrogenic (fever-inducing) impurities found in therapeutic protein preparations. They are negatively charged complex aggregates of lipopolysaccharides (LPS) present in the cell walls of gram-negative bacteria. Endotoxins are released by the shedding of viable cell walls or the breakdown of dead bacteria. The presence of even miniscule amounts of such pyrogens in purified proteins can cause major upheaval in downstream purification. In therapeutic proteins, such contaminants can lead to septic shock, tissue injury, and even death in certain cases. In research samples, endotoxins can hinder accurate data screening and immunological readouts. Therefore, it is essential that endotoxins are removed from all purified proteins, especially the ones destined for therapeutic uses such as drugs, injectables, and other pharmaceutical products.

Endotoxins are extremely thermally stable and insensitive to pH changes. This makes efficient and cost-effective removal of endotoxins very challenging. In fact, the removal of endotoxins is one of the most difficult tasks in downstream protein processing. Bio-Rad has provided a progressive selection of chromatography resins for process-scale purification of proteins for more than 50 years. This guide highlights the resins that have been successfully used for endotoxin removal.

## Anion Exchange (AEX) Chromatography Resins

Since endotoxins are negatively charged, AEX chromatography is often the ideal choice for removing them.

### Macro-Prep® High Q Resin

This is a strong AEX resin ideal for the binding of acidic biomolecules. It is an excellent choice for rapid, cost-effective purification and provides high-resolution separations at high flow rates. It has a hydrophobic backbone that offers strong interaction with the LPS of endotoxins and thus enhances the efficiency of endotoxin removal. The superior mechanical and chemical stability of Macro-Prep High Q makes it the preferred choice over other commercially available anion (Q) resins for rapid processing of large-volume feedstreams. We have successfully depleted endotoxins from different protein products using this resin.

Protein X (MW 15.4 kD, pI 6.5) was purified from yeast and generated a positive signal in Limulus amebocyte lysate (LAL) testing, indicating the presence of endotoxins. However, chromatographic resins that had previously been successful in removing endotoxins from proteins failed to do so for this sample. Hence, we tested a panel of 20 different resins to identify the best one for endotoxin elimination (bulletin 2204). The six resins that exhibited good removal efficiency are shown in Figure 1. Purification using Macro-Prep High Q resulted in the lowest ratio of endotoxin to protein X relative to the other five commercially available resins.

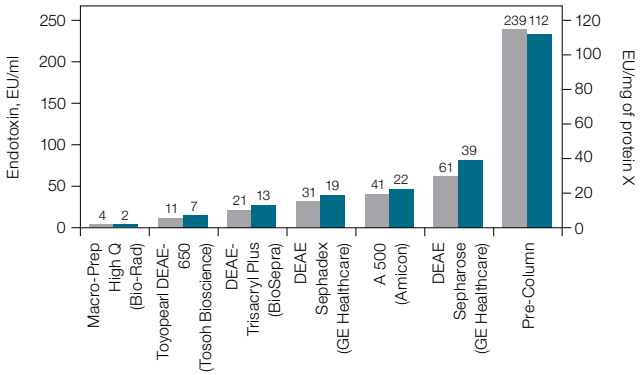


**Fig. 1. Evaluation of six AEX resins for endotoxin removal.** Endotoxin, EU/ml (■); EU/mg of protein X (■).

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Protein Z (MW 28.3 kD, pI ~6) is a lipophilic protein that shows a high affinity for endotoxins, making it difficult to purify. Traditional organic solvent extraction methods reduced the endotoxin level by only 97%. Macro-Prep High Q purification of protein Z helped fractionate the protein based on the bound endotoxin level. The protein Z molecules containing low endotoxin levels were eluted at low salt concentrations, whereas the molecules with high levels of endotoxins were eluted later in the gradient. Overall, 77% of the total protein Z was recovered with 99.99% reduction in the endotoxin content (Table 1) (bulletin 2204).

**Table 1. Results from endotoxin reduction of protein Z using Macro-Prep High Q Resin under denaturing conditions.**

| Sample | Protein Z, mg | EU/ml x $10^3$ | EU/mg Protein Z |
|---|---|---|---|
| Load | 857 | 35,100 | 40,960 |
| Unbound pool | 758 | 29 | 38.3 |
| Gradient pool | ~20 | <1 | – |
| 2 M NaCl strip | <5 | 6,680 | >1.3 x $10^6$ |
| Final lyophilized preparation | 662 | 3.5 | 5.3 |

Bio-Rad also offers two other AEX resins that can be used for endotoxin removal during protein purification.

### Nuvia™ Q Resin

This is an ultra high capacity next-generation AEX resin. It delivers high binding capacity over a range of pH and flow rates, providing a wide experimental design space for process developers. Its unique design gives it best-in-class dynamic binding capacity (bulletin 6129).

### UNOsphere™ Q Resin

This is a strong anion exchanger. The highly macroporous nature of UNOsphere Q provides high binding capacities in the linear velocity range of 150–1,200 cm/hr (bulletin 2724).

## Cation Exchange (CEX) Chromatography Resins

Nuvia™ cPrime™ and other CEX resins are also effective endotoxin removal tools, as target proteins bind to the resin while negatively charged endotoxins are eliminated in the flowthrough.

## Mixed-Mode Chromatography

Mixed-mode chromatography resins offer unique separation properties, such as unparalleled selectivity and resolution, for a variety of molecules.

### CHT™ Ceramic Hydroxyapatite Media

CHT is a mixed-mode chromatography media with a long history of applications in antibody purification. It can retain solutes by AEX with positively charged calcium, metal affinity with calcium, CEX with phosphate groups, and/or hydrogen bonding with crystal hydroxyl groups. Endotoxins are highly acidic due to a high content of phosphoryl and carboxyl residues, which have strong affinity for the calcium ions in CHT.

During gradient studies, it was found that a high concentration of phosphate in the buffer resulted in the co-elution of endotoxins and the target chimeric mAb. This problem was overcome by maintaining buffers with low phosphate concentration and using an NaCl gradient, which resulted in the retention of endotoxins on the CHT column and the elution of the mAb in the early fractions (Figure 2). Thus, CHT can be used to optimize the buffer composition to reach a balance between the elution of a pure product and the elimination of contaminants. Table 2 shows the drastic reduction in endotoxin levels after NaCl gradient purification (bulletin RP0033).

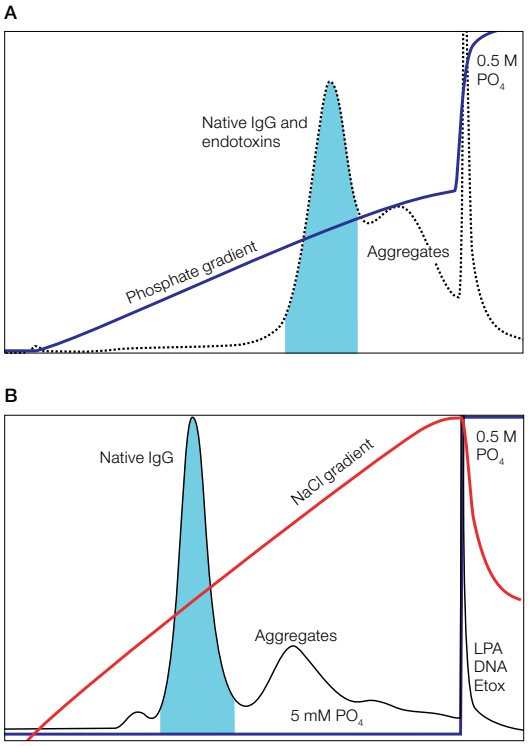


**Fig. 2. Elution characteristics of IgG in various buffers. A**, distribution of native IgG and impurities in a phosphate gradient from 5 to 300 mM. **B**, distribution of native IgG and major contaminant classes in a sodium chloride gradient at 5 mM phosphate. LPA, lysophosphatidic acid; Etox, endotoxins.

**Table 2. Comparison of endotoxin clearance between phosphate and sodium chloride gradients.**

| | Endotoxin, EU/ml |
|---|---|
| Starting material | >500 |
| Phosphate pool | 16.6 |
| NaCl pool | <0.05 |

### MPC™ Ceramic Hydroxyfluoroapatite Media

This media is a composite of hydroxyapatite and fluoroapatite that retains the unique separation properties of CHT. It also possesses high pH stability, providing additional benefits to process economics (bulletin 6432).

### CFT™ Ceramic Fluoroapatite Media

This is a composite of fluoroapatite and hydroxyapatite prepared by chemically converting hydroxyapatite nanocrystals to fluoroapatite with a fluorine reagent. CFT can be used under stringent chromatography conditions to separate acidic proteins requiring buffering at a pH as low as 5.6 (bulletin 3111).

The details provided here can help you design an endotoxin elimination strategy for recombinant protein or antibody purification. For technical/product support or to request a quote, email your regional Bio-Rad representative at process@bio-rad.com or contact our customer service at 1-800-4-BIORAD (1-800-424-6723).

Explore our extensive selection of process-scale chromatography resins and their performance characteristics and applications (bulletin 6713). For process optimization of your endotoxin elimination workflow, request a sample.

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