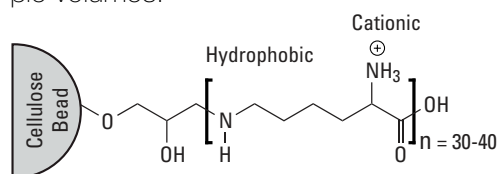


# easily eliminate endotoxin

## Endotoxin Removal Resin

### High-Capacity Endotoxin Removal

Endotoxin contamination is a common problem with recombinant proteins purified from gram-negative bacteria such as *E. coli*. **Thermo Scientific Pierce High Capacity Endotoxin Removal Resin** selectively binds and removes endotoxins from protein, peptide and antibody samples using a modified  $\epsilon$ -poly-L-lysine [poly( $\epsilon$ -lysine)] affinity ligand. Endotoxin levels in biological samples are reduced by  $\geq 99\%$  in as fast as 1 hour using our spin column format, and protein recovery is  $\geq 85\%$ . Pierce® High Capacity Endotoxin Removal Resin is available as a slurry to pack a custom column or in convenient pre-packed, single-use spin columns optimized for different sample volumes.



Poly( $\epsilon$ -lysine) Resin

High Capacity Endotoxin Removal Resin

#### Highlights:

- **High capacity** – bind up to 2,000,000 EU/mL<sup>†</sup> to eliminate  $>99\%$  of endotoxins
- **Durable** – reuse resin up to 10 times
- **Selective** – recover  $\geq 85\%$  of your protein sample
- **High performance** – complies with FDA guidelines by reducing final EU concentration to  $<5$  EU/mL
- **Fast** – our new spin column format enables endotoxin depletion within 1 hour
- **Clean** – single-use spin columns avoid cross contamination of samples
- **Optimized** – spin columns are optimized for different sample volumes
- **Economical** – large-volume discounts available

**Figure 1. The poly( $\epsilon$ -lysine) affinity ligand binds endotoxins through both ionic and hydrophobic interactions.** The multiple  $\epsilon$ -aminobutyl groups impart both a positive charge via the primary amines as well as a hydrophobic characteristic via the butyl spacer between primary amines. The hydrophilic nature of the porous cellulose base-matrix is masked by thorough derivatization of its interior and exterior surfaces with the poly( $\epsilon$ -lysine) ligand.

Table 1. Benefits of Thermo Scientific Pierce High Capacity Endotoxin Removal Resin over traditional methods.

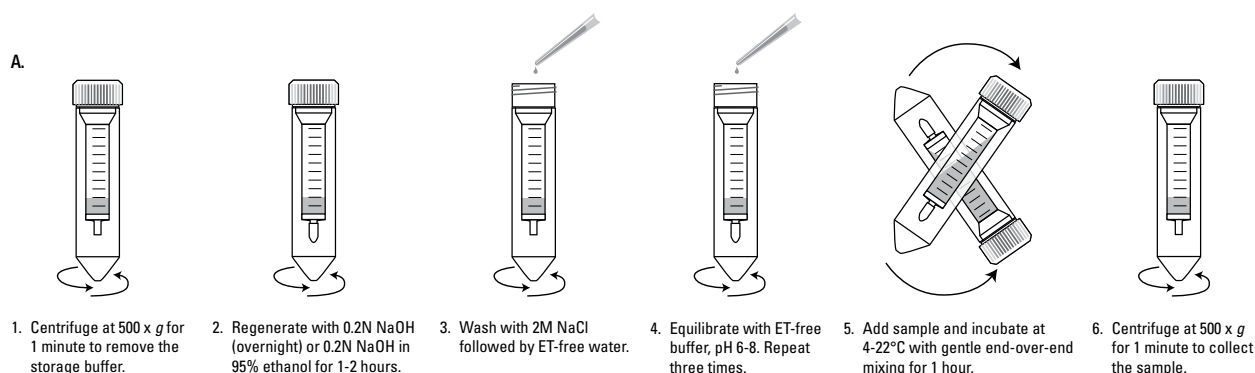
Traditional endotoxin removal method	Limitations	Benefit from Pierce High Capacity Endotoxin Removal Resin
Anion-exchange chromatography	Loss of negatively charged protein	Successfully process proteins across a range of isoelectric points
Ultrafiltration	Only removes large endotoxin aggregates, so it is compatible only with low-molecular-weight proteins. Endotoxin bound to toxin will not be effectively removed. Technique also exerts strong physical forces on the protein	Successfully process proteins ranging in molecular weight from 12 to 150kDa
Membrane-based chromatography	Reduced endotoxin binding capacity compared to resin-based methods; non reusable	Resin binds up to 2,000,000 EU/mL and can be reused up to 10 times with no loss in performance
Polymyxin B affinity ligand	Ligand exhibits neurotoxicity and sodium deoxycholate buffers cause renal tubular necrosis, making it unsuitable for therapeutics	Poly( $\epsilon$ -lysine) is a safe, nontoxic polymer of the natural amino acid lysine and is commonly used as a food preservative

<sup>†</sup> One endotoxin unit/mL (EU/mL) equals approximately 0.1 ng endotoxin/mL of solution.

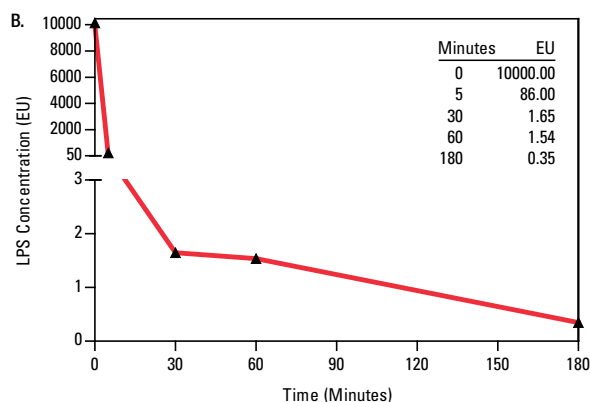
## Fast clean-up

Pierce High Capacity Endotoxin Removal Resin is available as a bulk slurry for custom packing of endotoxin removal columns and can be used with gravity flow systems or automated chromatography systems with flow rates ranging from 10 to 15mL/hour. Alternatively, the pre-packed spin column format is a fast,

single-use method to remove 99% of endotoxins from protein samples in as fast as 1 hour (Figure 2). These spin columns use a batch format to bind and remove endotoxins while allowing for >85% protein recovery. Three pre-packed spin-column sizes are available to process protein samples of different volumes.



**Figure 2. Remove endotoxins from protein samples in 1 hour.** **A.** Protocol for Pierce High Capacity Endotoxin Removal Spin Columns. **B.** Pierce High Capacity Endotoxin Removal Spin Columns, 0.5mL, were challenged for different time intervals with 1mL samples containing 1mg/mL BSA spiked with 10,000EU. The final endotoxin concentration was determined using the Thermo Scientific Pierce LAL Chromogenic Endotoxin Quantitation Kit. A one-hour incubation time provided 99% endotoxin removal, and longer incubation periods accomplished even greater endotoxin removal.



## High binding capacity

**Table 2. Efficiently remove endotoxins from heavily contaminated samples.** Pierce High Capacity Endotoxin Removal Spin Columns, 0.5mL, were challenged for 1 hour with protein (2mL of 1mg/mL BSA) containing 5,000 to 500,000EU/mL from *E. coli* strain 055:B5. Even at the highest endotoxin levels clean-up was >98%.

Initial endotoxin concentration (EU/mL)	Final endotoxin concentration (EU/mL)	Endotoxin removal efficiency	Protein recovery
5,000	<1	99.98%	>90%
12,500	<1	99.99%	>90%
25,000	1.26	99.99%	>90%
50,000	7.1	99.99%	>90%
250,000	32	99.98%	>90%
500,000	9,600	98.08%	>90%



## Compatible with proteins of various size and charges

**Table 3. Clean-up proteins of various molecular weights and isoelectric points with minimal sample.** Pierce High Capacity Endotoxin Removal Spin Columns, 0.5mL, were challenged for 1 hour with different proteins of various molecular weight and charge. In all samples endotoxin removal was >99% and protein recovery was >85%.

Protein (1mg/mL)	Molecular weight (Da)	Isoelectric point (pI)	Initial endotoxin concentration (EU/mL)	Final endotoxin concentration (EU/mL)	Endotoxin removal efficiency	Protein recovery
Cytochrome C	12,000	10.6	10,000	1.35	>99.9%	86%
Myoglobin	17,000	6.8	10,000	3.67	>99.9%	87%
Bovine serum albumin (BSA)	66,000	4.9	10,000	0.80	>99.9%	85%
Bovine gamma globulin (BGG)	150,000	7.4	10,000	4.60	>99.9%	92%

## Superior endotoxin removal

**Table 4. Thermo Scientific Pierce High Capacity Endotoxin Removal Resin delivers the best sample clean-up.** Various endotoxin removal resins were challenged with 2mL of 1mg/mL BSA containing 25,000EU/mL according to the respective manufacturers' procedures. The poly(ε-lysine)-based Pierce High Capacity Endotoxin Removal Resin consistently exhibited the highest endotoxin removal efficiency and the highest protein recovery.

	Initial endotoxin concentration (EU/mL)	Final endotoxin concentration (EU/mL)	Protein recovery
Thermo Scientific Pierce High Capacity Endotoxin Removal Resin	25,000	<1	91%
Thermo Scientific Detoxi-Gel Endotoxin Removal Gel [Thermo Fisher Scientific]	25,000	77	74%
Affi-Prep® Polymyxin Matrix [Bio-Rad]	25,000	13	88%
Polymyxin B-Agarose [Sigma]	25,000	900	63%
ReductEtox™ [Sterogene]	25,000	13,900	92%
EndoTrap® red [Hyglos]	25,000	64	82%

## Compatible with different samples

**Table 5. Thermo Scientific Pierce High Capacity Endotoxin Removal Resin removes endotoxins from various strains of *E. coli*.** Pierce High Capacity Endotoxin Removal Spin Columns, 0.5mL, were challenged with 1mL BSA (1mg/mL) spiked with 10,000EU from different strains of *E. coli*. In all samples removal was performed with >99% efficiency.

<i>E. coli</i> strain	Initial endotoxin concentration (EU/mL)	Final endotoxin concentration (EU/mL)	Endotoxin removal efficiency	Protein recovery
011:B4	10,000	0.36	>99.9%	83%
026:B6	10,000	4.18	>99.9%	88%
0127:B8	10,000	0.87	>99.9%	87%
0128:B12	10,000	5.01	>99.9%	88%

**Table 6. Process protein and antibody samples from a variety of sources.** Protein samples from cell culture supernatants, *E. coli* lysates or human serum were processed with the Pierce High Capacity Endotoxin Removal Spin Columns. In all samples endotoxin levels were reduced to less than 1EU/mL.

Protein source	Initial endotoxin concentration (EU/mL)	Final endotoxin concentration (EU/mL)
Anti-Fractalkine from cell culture supernatant	8.26	<1
His-tag GFP from <i>E. coli</i>	9780	<1
IgG from human serum	78	<1



## Endotoxin Quantitation Kit

### The Thermo Scientific Pierce LAL Chromogenic Endotoxin Quantitation Kit

measures the amount of endotoxin in a protein, peptide or antibody sample using the *Limulus* Amebocyte Lysate (LAL) assay. Designed for up to 50 samples, this quantitation kit is ideal for processing a few samples at a time compared to other high-throughput quantitation kits. The endotoxin concentration in a sample is measured via a

chromogenic signal generated in the presence of endotoxins. Samples can be measured on a microplate absorbance reader at 405nm. A standard curve is created using the *E. coli* endotoxin standard included with each kit to calculate endotoxin levels as low as 0.1EU/mL (Figure 3). Protein and antibody samples can be assayed in as few as 30 minutes.

#### Highlights:

- **Sensitive** – detect as little as 0.1EU/mL
- **Fast** – perform this assay in 30 minutes
- **Economical** – assay requires only 10µL of a protein sample
- **Accurate** – *E. coli* 0111:B4 standard in each kit enables accurate endotoxin quantitation
- **Versatile** – 405nm absorbance reading is compatible with common ELISA plate readers

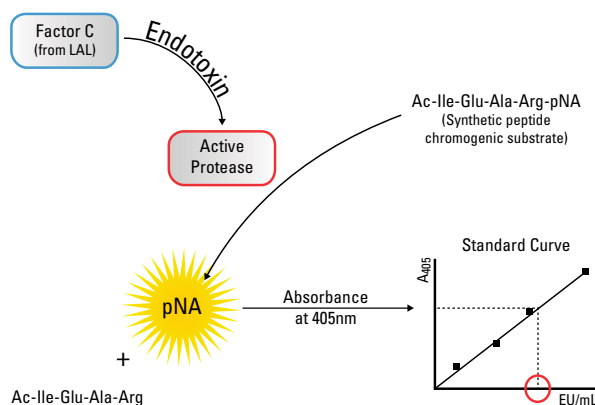


Figure 3. LAL endotoxin quantitation assay overview.

#### Ordering Information

Product #	Description	Pkg. Size
88270	Pierce High Capacity Endotoxin Removal Resin	10mL
88271	Pierce High Capacity Endotoxin Removal Resin	100mL
88272	Pierce High Capacity Endotoxin Removal Resin	250mL
88273	Pierce High Capacity Endotoxin Removal Spin Columns, 0.25mL	5 columns
88274	Pierce High Capacity Endotoxin Removal Spin Columns, 0.5mL	5 columns
88275	Pierce High Capacity Endotoxin Removal Spin Columns, 0.5mL	25 columns
88276	Pierce High Capacity Endotoxin Removal Spin Columns, 1mL	5 columns
88277	Pierce High Capacity Endotoxin Removal Spin Columns, 1mL	25 columns
88282	Pierce LAL Chromogenic Endotoxin Quantitation Kit	50-test kit



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