

Pierce™ High-Capacity Endotoxin Removal Resin

Catalog Numbers 88270, 88271, 88272, 88273, 88274, 88275, 88276, and 88277

Doc. Part No. 2162373 Pub. No. MAN0016351 Rev. B



WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from [thermofisher.com/support](https://www.thermofisher.com/support).

Product description

The Thermo Scientific™ Pierce™ High Capacity Endotoxin Removal Resin contains porous cellulose beads that have been surface modified with covalently attached, modified ϵ -poly-L-lysine. Modified polylysine has a high affinity for endotoxins with the affinity ligand eliminating toxicity associated with alternative technologies using polymyxin B ligands and sodium deoxycholate buffers. The binding capacity of 2,000,000 Endotoxin Units (EU)/mL allows endotoxin levels to be reduced by 99% in samples containing 10,000 EU/mL; typical protein samples processed with the resin have a final endotoxin concentration below 5 EU/mL. The resin is offered in a slurry format for custom packing of endotoxin removal columns for gravity flow or continuous flow (10–15 mL/hour) applications or a spin column format for the fast, single-use batch method. Removal of endotoxins from differing volumes of biological samples can be done in ≥ 1 hour (see Table 1).

Endotoxins consist of lipopolysaccharides (LPS), which are biologically active, structural components of the outer cell membrane of all gram-negative bacteria. Small amounts of endotoxin in recombinant protein preparations can cause side effects, including endotoxin shock, tissue injury and death in host organisms; therefore, it is essential to remove endotoxins from drugs, injectables, and other biological products.

One milliliter of this resin can reduce the endotoxin concentration from a 1 mL sample containing 10,000 EU to ≤ 5 EU.

Required materials not supplied

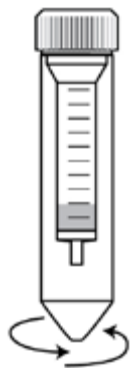
- Endotoxin-free, ultrapure water
 - Endotoxin-free 15 mL conical collection tubes (for 0.25 mL and 0.5 mL spin columns)
 - Endotoxin-free 50 mL conical collection tubes (for 1.0 mL spin columns)
 - Regeneration buffer: 0.2 N NaOH for overnight incubation at room temperature **OR** 0.2 N NaOH and 95% ethanol for 1–2 hours of incubation at room temperature
- Note:** Regenerate the column before the first use and after each subsequent use.
- 2 M NaCl solution: If possible, use ready-made endotoxin-free NaCl solution; alternatively, dissolve pure sodium chloride crystals in endotoxin-free water
 - Variable-speed centrifuge with rotor and carriers capable of handling 15 mL and 50 mL conical collection tubes

Contents and storage

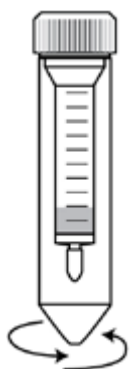
Item	Cat. No.	Amount	Storage
Pierce™ High-Capacity Endotoxin Removal Resin	88270	10 mL of settled resin supplied as 50% slurry in 20% ethanol	Store at 4°C.
Pierce™ High-Capacity Endotoxin Removal Resin	88271	100 mL of settled resin supplied as 50% slurry in 20% ethanol	
Pierce™ High-Capacity Endotoxin Removal Resin	88272	250 mL of settled resin supplied as 50% slurry in 20% ethanol	
Pierce™ High-Capacity Endotoxin Removal Spin Column, 0.25 mL	88273	5 columns, each column contains 25% slurry in 20% ethanol	
Pierce™ High-Capacity Endotoxin Removal Spin Column, 0.50 mL	88274	5 columns, each column contains 25% slurry in 20% ethanol	
Pierce™ High-Capacity Endotoxin Removal Spin Column, 0.50 mL	88275	25 columns, each column contains 25% slurry in 20% ethanol	
Pierce™ High-Capacity Endotoxin Removal Spin Column, 1.0 mL	88276	5 columns, each column contains 25% slurry in 20% ethanol	
Pierce™ High-Capacity Endotoxin Removal Spin Column, 1.0 mL	88277	25 columns, each column contains 25% slurry in 20% ethanol	

Procedure summary

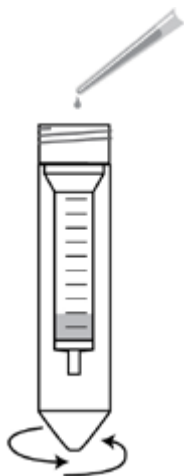
1. Centrifuge at 500 x *g* for 1 minute to remove the storage buffer.



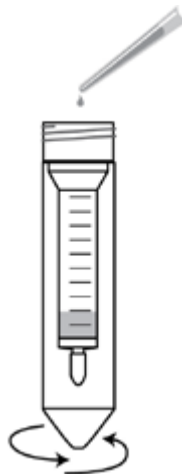
2. Regenerate with 0.2 N NaOH (overnight) or 0.2 N NaOH in 95% ethanol for 1–2 hours.



3. Wash with 2 M NaCl followed by ET-free water.



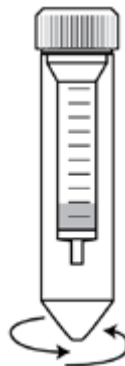
4. Equilibrate with ET-free buffer, pH 6–8. Repeat 3 times.



5. Add sample and incubate at 4–22°C with gentle end-over-end mixing for 1 hour.



6. Centrifuge at 500 x *g* for 1 minute to collect the sample.



Important product information

- All materials (for example, containers, buffers and pipette tips) must be endotoxin-free. Use endotoxin-free water to prepare buffers and other solutions.
- Endotoxin binding to the resin occurs at pH 6–8; Equilibrate the resin with an endotoxin-free buffer at neutral pH that includes 10–50 mM sodium phosphate buffer or Tris-HCl buffer containing 0.1–0.2M NaCl. Check the sample pH and adjust to pH 6–8 with 0.1 M NaOH or 0.1 M HCl.
- The speed of sample application and wash depends on the sample endotoxin state. Free endotoxin can bind quickly to the resin, but protein-bound endotoxin may require longer incubation, slower flow rates and/or multiple recycling of the sample through the column. Tightly bound endotoxin may be incubated overnight at room temperature or 4°C in batch mode with gentle shaking.
- Resins can be used a minimum of 5 times without loss of endotoxin-removal efficiency.
- Sample volumes vary depending on the chosen pre-loaded column size (see Table 1).

Table 1 Sample volumes for the Pierce™ High-Capacity Endotoxin Removal Columns.

Column size	Sample volume
0.25 mL	0.5-1.0 mL
0.50 mL	1.0-4.0 mL
1.0 mL	2.0-10.0 mL

Remove endotoxin using the column method

- Regenerate the resin before the first use and after each subsequent use.
 - Equilibrate all solutions and the resin to room temperature before use.
 - Degas the resin slurry before applying to the column to prevent air bubbles from clogging the column and reducing flow.
1. To degas the resin, place slurry in a suction filter flask and degas with gentle stirring.
 2. Pour the degassed resin slurry into an appropriately sized column and allow the resin to settle.
 3. Regenerate the resin by washing with 5 resin-bed volumes of 0.2 N NaOH overnight at room temperature **OR** 5 resin-bed volumes of 0.2 N NaOH in 95% ethanol for 1–2 hours at room temperature.
 4. Wash with 5 resin-bed volumes of 2 M NaCl.
 5. Wash with 5 resin-bed volumes of endotoxin-free, ultrapure water.
 6. Equilibrate with 5 resin-bed volumes of endotoxin-free buffer.
 7. Apply sample to the column at a flow rate of 10–15 mL/hr and collect the flowthrough (see “Important product information” on page 3).

8. Elute the protein by adding endotoxin-free buffer and collecting fractions; one or two resin-bed volume elutions are sufficient.
9. Determine the endotoxin concentration of the processed sample. **Use caution to prevent sample contamination from dust or contaminated tubes subsequent to endotoxin removal.** Store solutions frozen or assay them before use to ensure sterility.
10. Regenerate the resin as described in step 3 through step 5 and store the column in 20% ethanol at 2–8°C.

Remove endotoxin using the batch method with spin columns

- Regenerate the resin before the first use and after each subsequent use. For regeneration and equilibration (steps 1 on page 3–10 on page 4), use regeneration buffer in 2 mL volumes for the 0.25 mL column, 3.5 mL volumes for the 0.5 mL column and 8 mL volumes for the 1 mL column.
- Wear appropriate gloves while handling the spin columns. Use caution to prevent sample contamination during the endotoxin-removal procedure.

1. Equilibrate the spin column to room temperature.
2. Twist off the column's bottom closure (Save closure for later) and loosen the top cap. Place the spin column into a collection tube. Centrifuge the column at 500 x g for 1 minute to remove the storage solution. Discard the storage solution.
3. Remove the column cap and insert the bottom closure by inverting the twist-off cap and applying directly to the column bottom with gentle pressure. To regenerate, add 0.2 N NaOH, replace the cap, invert the column several times until the resin is suspended in the solution and incubate overnight at room temperature, **OR** add 0.2 N NaOH in 95% ethanol, replace the cap, invert the column several times until the resin is suspended in the solution and incubate 1–2 hours at room temperature.
4. Loosen the cap and remove the bottom closure. Place the column in a collection tube and centrifuge at 500 x g for 1 minute to remove the solution. Discard the solution.
5. Remove the cap and insert the bottom closure. Add 2 M NaCl, replace the cap and invert the column several times until the resin is suspended in the solution.
6. Loosen the cap and remove the bottom closure. Place the column in a collection tube and centrifuge at 500 x g for 1 minute to remove the solution. Discard the solution.
7. Remove the cap and insert the bottom closure. Add endotoxin-free, ultrapure water. Replace the cap and invert the column several times until the resin is suspended in the solution.
8. Loosen the cap and remove the bottom closure. Place the column in a collection tube and centrifuge at 500 x g for 1 minute to remove the solution. Discard the solution.

9. Remove the cap and insert the bottom closure. Add endotoxin-free buffer, replace the cap and invert the column several times until the resin is suspended in the solution.
10. Loosen the cap and remove the bottom closure. Place the column in a collection tube and centrifuge at 500 x *g* for 1 minute to remove the solution. Discard the solution. Repeat steps 9 and 10 two additional times.
11. Remove the cap and insert the bottom closure. Add the sample to the resin, replace the cap and invert the column several times until the resin is suspended in the solution.
12. Incubate the column with gentle end-over-end mixing at room temperature or 4°C for 1 hour. Incubation time can be extended depending on the sample type and requirements (see "Important product information" on page 3).
13. Loosen the cap and remove the bottom closure. Place the column in a collection tube and centrifuge at 500 x *g* for 1 minute to collect the sample.
14. Determine the endotoxin concentration of the processed sample. **Use caution to prevent sample contamination after endotoxin removal.** Store solutions at –20°C or assay before use to ensure sterility.
15. Regenerate the resin as described in step 3 and store the column in 20% ethanol at 2–8 °C.

Troubleshooting

Problem	Possible Cause	Solution
Low endotoxin removal efficiency	Sample pH was not within a neutral range.	Adjust sample to pH 6-8.
	Incubation time was not sufficient.	Increase the incubation time for the batch method.
	Endotoxin was bound to the target protein.	Recycle the sample through the column several times.
	The removal or detection system was contaminated by extrinsic LPS.	Use endotoxin-free labware and buffers.
Low protein/sample recovery	Target protein aggregated with endotoxin and was removed.	Increase NaCl concentration in the sample and equilibration buffer to 0.4 M.
	Nonspecific binding of sample to the resin.	

Related products

Product	Cat. No.
Pierce™ Centrifuge Columns, (2 mL, 5 mL and 10 mL, respectively), 25/pkg	88282
Pierce™ LAL Chromogenic Endotoxin Quantitation Kit	89896–8
Pierce™ BCA™ Protein Assay Kit	23225
Pierce™ 660 nm Protein Assay	22660



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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0016351 B

Revision	Date	Description
B	13 May 2024	Removing bottom caps to correspond with product change.
A.0	17 October 2015	New document for Pierce™ High-Capacity Endotoxin Removal Resin.

The information in this guide is subject to change without notice.

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