INSTRUCTIONS

Pierce® High-Capacity Endotoxin Removal Resin

2373.1

Number	Description
88270	**Pierce High-Capacity Endotoxin Removal Resin,** 10mL of settled resin supplied as 50% slurry in 20% ethanol
88271	**Pierce High-Capacity Endotoxin Removal Resin,** 100mL of settled resin supplied as 50% slurry in 20% ethanol
88272	**Pierce High-Capacity Endotoxin Removal Resin,** 250mL of settled resin supplied as 50% slurry in 20% ethanol
88273	**Pierce High-Capacity Endotoxin Removal Spin Column, 0.25mL,** 5 columns, each column contains 25% slurry in 20% ethanol
88274	**Pierce High-Capacity Endotoxin Removal Spin Column, 0.50mL,** 5 columns, each column contains 25% slurry in 20% ethanol
88275	**Pierce High-Capacity Endotoxin Removal Spin Column, 0.50mL,** 25 columns, each column contains 25% slurry in 20% ethanol
88276	**Pierce High-Capacity Endotoxin Removal Spin Column, 1.0mL,** 5 columns, each column contains 25% slurry in 20% ethanol
88277	**Pierce High-Capacity Endotoxin Removal Spin Column, 1.0mL,** 25 columns, each column contains 25% slurry in 20% ethanol

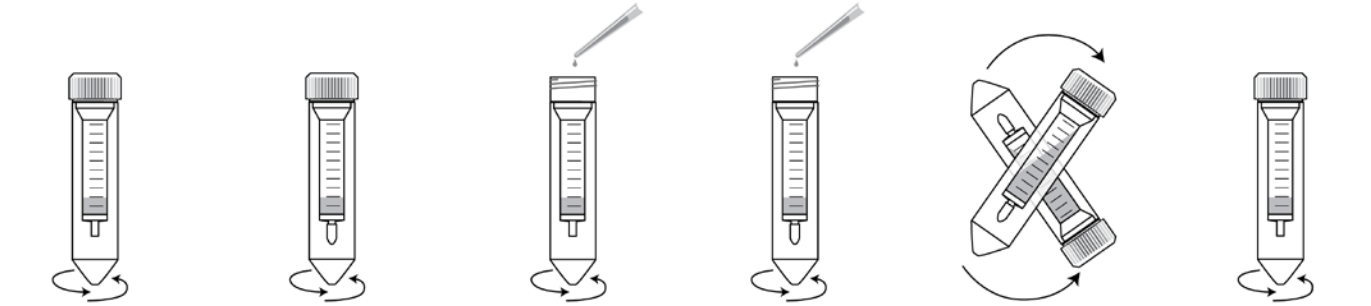
Storage: Upon receipt store at 4°C. Product shipped at ambient temperature.

Introduction

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 polymixin 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 10,000EU/mL; typical protein samples processed with the resin have a final endotoxin concentration below 5EU/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.

Procedure Summary



1. Centrifuge at 500 x *g* for 1 minute to remove the storage buffer.
2. Regenerate with 0.2N NaOH (overnight) or 0.2N NaOH in 95% ethanol for 1-2 hours.
3. Wash with 2M NaCl followed by ET-free water.
4. Equilibrate with ET-free buffer, pH 6-8. Repeat three 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 (e.g., 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 10-50mM sodium phosphate buffer or Tris-HCl buffer containing 0.1-0.2M NaCl. Check the sample pH and adjust to pH 6-8 with 0.1M NaOH or 0.1M 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 five 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 Thermo Scientific Pierce High-Capacity Endotoxin Removal Columns.

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

Additional Materials Required

- Endotoxin-free, ultrapure water
- Endotoxin-free 15mL conical collection tubes (for 0.25mL and 0.5mL spin columns)
- Endotoxin-free 50mL conical collection tubes (for 1.0mL spin columns)
- Regeneration buffer: 0.2N NaOH for overnight incubation at room temperature **OR** 0.2N 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.
- Variable-speed centrifuge with rotor and carriers capable of handling 15mL and 50mL conical collection tubes

Endotoxin Removal Procedure 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 the 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 five resin-bed volumes of 0.2N NaOH overnight at room temperature **OR** five resin-bed volumes of 0.2N NaOH in 95% ethanol for 1-2 hours at room temperature.
4. Wash with five resin-bed volumes of 2M NaCl.
5. Wash with five resin-bed volumes of endotoxin-free, ultrapure water.
6. Equilibrate with five resin-bed volumes of endotoxin-free buffer.
7. Apply sample to the column at a flow rate of 10-15mL/hr and collect the flow-through (see the Important Product Information Section).
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.

   Note: 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 Steps 3-5 and store the column in 20% ethanol at 2-8°C.

    Note: One milliliter of resin can reduce the endotoxin concentration from a 1mL sample containing 10,000EU to $\leq$ 5EU.

Endotoxin Removal Procedure using the Batch Method with Spin Columns

- Regenerate the resin before the first use and after each subsequent use. For regeneration and equilibration (steps 1-10), use regeneration buffer in 2mL volumes for the 0.25mL column, 3.5mL volumes for the 0.5mL column and 8mL volumes for the 1mL column.
- Wear appropriate gloves while handling the spin columns.

  Note: 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 and loosen the top cap. Place the spin column into a collection tube. Centrifuge the column at 500 × *g* for 1 minute to remove the storage solution. Discard the storage solution.
3. Remove the column cap and insert the bottom plug. To regenerate, add 0.2N 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 0.2N 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 plug. Place the column in a collection tube and centrifuge at 500 × *g* for 1 minute to remove the solution. Discard the solution.
5. Remove the cap and insert the bottom plug. Add 2M 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 plug. Place the column in a collection tube and centrifuge at 500 × *g* for 1 minute to remove the solution. Discard the solution.
7. Remove the cap and insert the bottom plug. 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 plug. Place the column in a collection tube and centrifuge at 500 × *g* for 1 minute to remove the solution. Discard the solution.
9. Remove the cap and insert the bottom plug. 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 plug. Place the column in a collection tube and centrifuge at 500 × *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 plug. 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 the Important Product Information Section).
13. Loosen the cap and remove the bottom plug. Place column in a collection tube and centrifuge at 500 × *g* for 1 minute to collect the sample.
14. Determine the endotoxin concentration of the processed sample.

    Note: Use caution to prevent sample contamination after endotoxin removal.

    Note: Store solutions at -20°C or assay before use to ensure sterility.

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 0.4M
	Nonspecific binding of sample to the resin	

Related Thermo Scientific Products

88282 **Pierce LAL Chromogenic Endotoxin Quantitation Kit**

89896 **Pierce Centrifuge Columns, 2mL,** 25/pkg

89897 **Pierce Centrifuge Columns, 5mL,** 25/pkg

89898 **Pierce Centrifuge Columns, 10mL,** 25/pkg

23225 **Pierce BCA Protein Assay Kit**

22660 **Pierce 660nm Protein Assay**

Current product instructions are available at *www.thermoscientific.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.

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