INSTRUCTIONS

Detoxi-Gel™ Endotoxin Removing Gel

0415.5

Number	Description
20339	**Detoxi-Gel Endotoxin Removing Gel,** 10mL settled resin, supplied as a 50% slurry in 25% ethanol
20340	**Detoxi-Gel Endotoxin Removing Gel,** 1L settled resin, supplied as a 50% slurry in 25% ethanol
20344	**Detoxi-Gel Endotoxin Removing Columns,** 5 × 1mL settled resin, supplied in 25% ethanol, and prepacked in columns having a bottom twist-off tab and an accessory pack containing five white tips

Support: Crosslinked, 6% beaded agarose (wet bead diameter 45-165µm)

Fractionation range: 10,000-4,000,000 for proteins

Capacity: 1mL of resin removes ≥ 9995 EU (endotoxin units) from a 5mL challenge containing 10,000 EU

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

Introduction

The Thermo Scientific Detoxi-Gel Endotoxin Removing Gel uses immobilized polymixin B to bind and remove pyrogens from solution. The polymixins are a family of antibiotics that contain a cationic cyclopeptide with a fatty acid chain. Polymixin B neutralizes the biological activity of endotoxins by binding to the lipid A portion of bacterial lipopolysaccharide. Studies performed by Kluger *et al*. indicate that the immobilized polymixin B inactivates some but not all endotoxins.[1]

The immobilized polymixin B gel is a stable affinity matrix that resists leaching of ligand into the valuable preparation. Making use of an affinity support permits easy cleanup of buffers, cell culture media, solutions containing macromolecules such as proteins, and pharmacologically important components. Detoxi-Gel Endotoxin Removing Gel also has been used to remove endotoxin from nucleic acid (DNA) samples.[2]

Important Product Information

- Good chromatographic technique must be used to obtain optimal performance. Much higher efficiencies of endotoxin removal will result if Detoxi-Gel Resin is used in a column format rather than a batch method.
- Nonspecific binding may occur, especially when hydrophobic molecules are present. To reduce nonspecific binding, buffer all solutions at physiological pH. To decrease weak ionic interactions with the affinity ligand, use a final concentration of 0.1-0.5M NaCl. If the purified sample is to be lyophilized as a salt-free powder, it is convenient to use a volatile buffer such as 0.1M ammonium bicarbonate, pH 7.8.
- Chaotropes (urea and guanidine) and detergents interfere with binding to the polymixin B. Some proteins, such as BSA, bind tightly to endotoxin, reducing the ability of the endotoxin to interact with and bind to polymixin B. This reduction in binding sometimes can be overcome by increasing the volume of resin to endotoxin. Some proteins bind tightly to endotoxin without inhibiting its ability to bind to the support and will remain bound to the resin with the endotoxin.
- The column flow rate can vary widely depending on column dimensions. Gravity-flow chromatography is superior to pumping a solution under pressure as it allows sufficient contact time of the solution with the immobilized ligand and, therefore, better endotoxin removal. Additionally, increasing contact time by stopping the column flow or multiple passes through the resin will result in greater efficiency. Centrifuge-ready columns (see Related Thermo Scientific Products) can be used in a combination of ways, including in batch or gravity-flow mode for binding steps and then centrifuge mode for sample collection.

Additional Materials required

- Empty columns suitable for the amount of Detoxi-Gel Resin and sample to be used (see Related Thermo Scientific Products)

  Note: Detoxi-Gel Columns are pre-packed and, therefore, empty columns are not necessary.

- 1% Sodium deoxycholate (Product No. 89904 or 89905)

  Note: Sodium deoxycholate must be used. Other detergents and free deoxycholic acid cannot be substituted.

- Pyrogen-free buffer or water

Procedure for Endotoxin Removal from a Solution

Notes:

- Detoxi-Gel Resin must be regenerated before each use, including first use.
- Use only pyrogen-free solutions to prevent introducing additional endotoxin into the sample.
- Degas all solutions before applying to the column to prevent air bubbles from clogging the column and reducing flow.
- Detoxi-Gel Resin may be used at least 10 times without loss of activity.
- Equilibrate all solutions and resin to room temperature before use.
- If using Detoxi-Gel Columns, proceed to Step 3. The supplied column has a twist-off bottom tab, which can be replaced with a supplied white tip.

1. To degas the Detoxi-Gel Resin, place slurry in the bottom of a suction filter flask with a magnetic stirrer. While stirring the slurry, use an aspirator to create a vacuum within the flask. Degas for approximately 15 minutes.
2. Pack the appropriately sized column with degassed slurry; allow the resin to settle for 30 minutes.
3. Regenerate the Detoxi-Gel Resin by washing with five resin-bed volumes of 1% sodium deoxycholate, followed by 3-5 resin-bed volumes of pyrogen-free buffer or water to remove the detergent. Regenerate the resin before each use.
4. Equilibrate the Detoxi-Gel Resin with 3-5 resin-bed volumes of a suitable pyrogen-free buffer or water.
5. Apply sample to the column. Add aliquots of pyrogen-free buffer or water and collect the flow-through. With a gravity-flow column, the sample will begin to emerge from the column about 90% of the bed volume has been collected. For greater efficiency, stop column flow after sample has entered the resin bed, and incubate the column for one hour before collecting the sample.

   Caution: Use extreme caution to prevent sample contamination from dust or dirty glassware subsequent to endotoxin removal. Store solutions frozen or assay them before use to ensure sterility. Bacterial contamination does not occur in lyophilized samples, as the environment is not conducive to growth.
6. Repeat Step 3 to remove any bound endotoxin and regenerate the resin. Store columns in 25% ethanol at 2-8°C.

Related Thermo Scientific Products

88270	**Pierce® High Capacity Endotoxin Removal Resin,** 10mL
88282	**Pierce LAL Chromogenic Endotoxin Quantitation Kit**
69705	**Pierce Spin Columns – Screw Cap,** Kit, 25/pkg
89896	**Pierce Centrifuge Columns,** 2mL, 25/pkg
89897	**Pierce Centrifuge Columns,** 5mL, 25/pkg
89898	**Pierce Centrifuge Columns,** 10mL, 25/pkg

Cited References

1. Kluger, M.J., *et al.* (1985). Polymixin B use does not ensure endotoxin-free solution. *J Immunol Meth* **83:**201-7.
2. Wicks, I.P., *et al.* (1995). Bacterial lipopolysaccharide copurifies with plasmid DNA: Implications for animal and human gene therapy. *Human Gene Therapy* **6:**317-23.

General References

Issekutz, A.C. (1983). Removal of gram negative endotoxin from solution by affinity chromatography. *J Immunol Meth* **61:**275-81.

Morrison, D.C. and Jacobs, D.M. (1976). Binding of polymixin B to the lipid A portion of bacterial polysaccharide. *Immunochemistry* **13:**813-18.

Adam, O. *et al*. (1995). A nondegradative route for the removal of endotoxin from exopolysaccharides. *Anal Biochem* **225(2):**321-327.

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