INSTRUCTIONS

PIERCE
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Detoxi-Gel™ Endotoxin Removing Gel

20339 20340 20344

0415.1

Number	Description
20339	**Detoxi-Gel™ Endotoxin Removing Gel,** 10 ml settled gel, supplied as a 50% slurry in 25% ethanol
20340	**Detoxi-Gel™ Endotoxin Removing Gel,** 1,000 ml settled gel, supplied as a 50% slurry in 25% ethanol
20344	**Detoxi-Gel™ AffinityPak™ Pre-packed Columns,** 5 x 1 ml settled gel, supplied in 25% ethanol

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

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

Capacity: 1 ml of gel removes ≥9,995 EU (endotoxin unit) from a 5 ml challenge containing 10,000 EU

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

Introduction

The 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™ Endotoxin Removing Gel 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 0.1-0.5 M NaCl. If the purified sample is to be lyophilized as a salt-free powder, it is convenient to use a volatile buffer such as 0.1 M 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 gel to endotoxin. Some proteins bind tightly to endotoxin without inhibiting its ability to bind to the support and will remain bound to the gel 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 gel will result in greater efficiency.

Materials required

- Empty chromatography columns: For 0.5-2.0 ml gel bed volumes use Product No. 29920; for 1-5 ml gel bed volumes use Product No. 29922; for 2-10 ml gel bed volumes use Product No. 29924. The Column Trial Pack (Product No. 29925) contains two each of three column sizes.

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

- 1% Sodium deoxycholate

  Note: Sodium deoxycholate (deoxycholic acid, sodium salt) 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:

- The column must be regenerated before each use, including first time use.
- Use only pyrogen-free solutions to prevent introducing any 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™ Endotoxin Removing Gel may be used at least 10 times without loss of activity.
- Equilibrate all solutions and gel to room temperature before use.
- If using Detoxi-Gel™ AffinityPak™ Columns, proceed to Step 3.

1. To degas gel, 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 gel to settle for 30 minutes.
3. Regenerate gel by washing with five column volumes of 1% sodium deoxycholate; followed by 3-5 column volumes of pyrogen-free water to remove detergent. The gel must be regenerated before each use, including first time use.

   Note: Sodium deoxycholate (deoxycholic acid, sodium salt) must be used. Other detergents or free deoxycholic acid cannot be substituted at this step.
4. Equilibrate gel with 3-5 column 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 flow-through. The sample will begin to emerge from the column after the void volume has been collected, which is 94% of the bed volume. For greater efficiency, replace bottom and top caps after sample has entered the gel bed. Incubate column for at least one hour, remove top and bottom caps sequentially, and add pyrogen-free buffer or water to collect 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 regenerate the column and remove any bound endotoxin. Store columns in 25% ethanol at 2-8°C.

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.