

**Protocol 105_LPS removal using detoxiGel
Endotoxin-removal pre-packed columns**

Reagents

Detoxi-Gel Endotoxin Removing Columns – Pierce/Thermo 20344

Up to 1ml sample

Expect reduction from 10,000 EU/ml to 5EU per ml. Columns can be regenerated and re-used more than 10 times with deoxycholate in molecular grade water.

See following URL for detailed instructions

<http://www.piercenet.com/Objects/View.cfm?type=File&ID=0415>

Procedure:

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 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.
6. Repeat step 3 to remove any bound endotoxin and regenerate the resin. Store columns in 25% ethanol at 2-8°C.

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.