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A Geno Technology, Inc. (USA) brand name

# **EndotoxinOUT**<sup>TM</sup> **Resin**

## INTRODUCTION

Endotoxin $OUT^{\text{TM}}$  consists of 6% cross-linked agarose covalently linked to polymyxin B to bind and remove harmful pyrogens from a solution. Polymyxin B is a family, polymyxin B1 and B2, of antibiotics that bind to the negatively charged site of the lipid A portion of bacterial lipopolysaccharide layer neutralizing the endotoxic activity.

The covalent coupled agarose and polymyxin B is a stable matrix that resists leaching. An ideal product for the clean up of buffers, cell culture media, protein solutions, nucleic acid (DNA) samples and pharmacological components.

#### ITEMS SUPPLIED

Cat. #	Description	Size*
786-367	EndotoxinOUT <sup>™</sup>	10ml resin
786-368	EndotoxinOUT <sup>™</sup>	1L resin
786-369	EndotoxinOUT <sup>™</sup>	5 x 1ml columns

<sup>\*</sup>EndotoxinOUT resin is supplied as a 50% slurry with 0.02% sodium azide as a preservative.

## STORAGE CONDITIONS

It is shipped at ambient temperature. Upon arrival, store refrigerated at 4°C, **DO NOT FREEZE**. This product is stable for 1 year at 4°C.

# **SPECIFICATIONS**

- Capacity: ≥9995 endotoxin units (EU) removed by 1ml resin from 5ml test containing 10,000EU. ≥99.95% removal.
- Fractionation Range: 10-4,000kDa for proteins
- Bead Structure: 6% cross-linked agarose

## IMPORTANT INFORMATION

- Non specific binding of hydrophobic molecules may occur. To limit non-specific binding buffer all solutions to physiological pH. To inhibit ionic interactions, use a final concentration of 0.1-0.5M NaCl.
- Avoid the use of chaotropes and detergents as this interfere with binding to polymyxin sulfate.
- Some proteins, including BSA, bind tightly to endotoxins reducing their interaction and removal by the polymyxin sulfates. Increasing the volume of EndotoxinOUT<sup>TM</sup> resin to endotoxin containing sample may help.
- In some cases, the interaction between endotoxin and polymyxin sulfate is not inhibited, resulting in immobilization of the protein to the resin.
- Optimal performance of the EndotoxinOUT<sup>™</sup> is best achieved under gravity flow chromatography, with an incubation period, allowing for more time for endotoxins to be in contact with polymyxin sulfate.

## ADDITIONAL ITEMS

- Columns (optional): G-Biosciences offers columns for a large range of resin volumes (Cat. # 786-718 to 786-724)
- Regeneration Solution: 1% sodium deoxycholate in endotoxin free water. Do not substitute a different detergent.
- Endotoxin-Free Water (Cat. # 786-670, 786-671)



## **PROCEDURE**

- Regenerate the EndotoxinOUT<sup>™</sup> before and after every use.
- Use endotoxin free solutions to prevent further endtoxin contamination.
- Degas all solutions to prevent introducing air bubbles that inhibit column flow.
- For 786-369, EndotoxinOUT<sup>™</sup> columns, skip the degassing step.
- 1. Allow the resin and reagents to equilibrate to room temperature.
- 2. Degas the EndotoxinOUT<sup>™</sup> slurry by placing an appropriate volume in a suction filter flask with a magnetic stirrer. Apply a vacuum and gently stir the resin for 15 minutes.
- 3. Add an appropriate volume of degassed, homogenous EndotoxinOUT<sup>™</sup> resin to a suitable column and allow to settle for 30 minutes.
- 4. Regeneration: Wash the resin with 5 resin volumes of Regeneration Solution, then wash with 5 resin volumes of Endotoxin-Free Water.
- 5. Equilibrate the column with 5 resin volumes of an appropriate endotoxin-free buffer or water.
- 6. Apply the sample to the column and add the bottom then top cap. Incubate at room temperature for 30-60 minutes.
- 7. Elute the sample with appropriate aliquots of endotoxin-free buffer or water. Repeat the elution 3-6 times and monitor elutions by absorbance at 280nm or an appropriate assay.
  - NOTE: Use extreme caution when handling samples to prevent contamination from poor handling, dirty glassware, etc.
- 8. Repeat step 4 to regenerate the column and store in 25% ethanol at 4°C. The resin can be regenerated at least 10 times.

## RELATED PRODUCTS

- 1. **Endotoxin Free Water** (Cat. # 786-670, 786-671). Endotoxin Free Water is free of endotoxins and enzymes, including proteases. Certified tested by the Limulus amebocyte lysate (LAL) test for endotoxins and determined to be <0.0050EU/ml.
- 2. **SpinOUT™ Columns** (Cat. # 786-170 to 786-173, 786-703 to 786-708). The SpinOUT™ GT-600 and GT-1200 columns are versatile, spin-format columns for the desalting and buffer exchange of protein solutions ranging from 5ul through to 4ml sample volumes.
- 3. **Empty Columns** (Cat. # 786-718 to 786-727). A selection of spin and gravity flow columns for small scale purifications.
- 4. Tube-O-DIALYZER™ (Cat. # 786-610 to 786-624) Allows dialysis of small samples without having to take the sample out of the tube thus eliminates loss (Medi & Micro size available with 1kDa, 4kDa, 8kDa, 15kDa & 50kDa MW cut off limits).

For related products, visit www.GBiosciences.com.

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