

# ToxOut™ High Capacity Endotoxin Removal Agarose

02/16

(Cat # 7942-5, 5 ml; Store at 4°C; Do not freeze)

## I. Introduction:

Endotoxin is the lipopolysaccharide (LPS) complex located in the outer membrane of gram-negative bacteria. A single *E.coli* bacterium contains ~2 million LPS molecules (2 – 20 fg/cell). During experimental procedures, large amount of endotoxins are shed and can easily contaminate labware, buffers and downstream products. *In vitro*, endotoxin causes a variety of problems in cell-based research. *In vivo*, endotoxin may cause various side effects, including inflammatory response, organ failure or septic shock in host organisms. Therefore, it is critical to remove endotoxin from samples and products.

BioVision's ToxOut™ High Capacity Endotoxin Removal Agarose can effectively reduce high concentration endotoxin to levels lower than 0.05 EU/ml in solutions containing proteins or pharmacologically important components without using toxic buffers while maintaining a protein recovery that is higher than 97%. BioVision's ToxOut™ High Capacity Endotoxin Removal Agarose is utilizing poly-ε-lysine as affinity ligand. It is a safe, nontoxic food preservative known for capturing endotoxin and preventing toxic effects.

## II. Product Features:

- High Binding Capacity: up to  $1.5 \times 10^9$  EU/ml resin
- High Sample Recovery: ~97% recovery with protein solution samples
- Resin Content: affinity matrix of poly-ε-lysine, supplied as 50% slurry in 20% ethanol
- ToxOut™ High Capacity Endotoxin Removal Agarose can be reused up to 5 times

## III. Applications:

- Effectively reduce high concentration endotoxin to levels lower than 0.05 EU/ml

## IV. Contents:

- 5 ml settled resin, supplied as a 50% slurry in 20% ethanol

## V. User Supplied Reagents and Equipment:

- Adjustable pipettes and sterile, endotoxin-free (or pyrogen-free) tips
- Centrifuge for 1.5 - 2 ml microcentrifuge tubes
- Endotoxin-free components are available at BioVision:
  - ToxOut™ Endotoxin-free Water (Cat. No. 7938-50)
  - ToxOut™ High Capacity Endotoxin Removal Regeneration Buffer (Cat. No. 7945-50)
  - ToxOut™ High Capacity Endotoxin Removal Wash Buffer (Cat. No. 7946-50)
  - ToxOut™ Endotoxin Removal Equilibration Buffer (Cat. No. 7940-50)
  - ToxOut™ Endotoxin-free Collection Tube (Cat. No. 7937-50)

## VI. Storage and Handling:

- Store resin at 4°C. Do not freeze.
- To prevent endotoxin contamination from dust, solution or dirty labware, only use endotoxin-free solutions and tubes and proceed with extra caution.
- Read entire protocol before performing the experiment.
- Do not let the resin dry at anytime. Store the resin in endotoxin-free buffer or water with 20% ethanol after use.

## VII. Endotoxin Removal Protocol:

### Notes:

- ToxOut™ High Capacity Endotoxin Removal Agarose must be regenerated by ToxOut™ High Capacity Endotoxin Removal **Regeneration Buffer**, followed by washing using ToxOut™ High Capacity Endotoxin Removal **Wash Buffer** before each use, **including first use**.
- Sample recovery rate may be increased by optimizing NaCl concentration or pH level of samples and equilibration buffer. Additional information is available in "Troubleshooting" section (section VIII).
- Protocol of ToxOut™ High Capacity Endotoxin Removal Kit (K2502-5) provides details for small scale purification using spin columns.

### Procedure:

1. Pack the appropriately sized column with ToxOut™ High Capacity Endotoxin Removal Agarose.
2. Wash resin with 1 resin-bed volume of ToxOut™ Endotoxin-free **Water**.
3. Close the column with a stopper. Regenerate the column by 3-5 resin-bed volumes of ToxOut™ High Capacity Endotoxin Removal **Regeneration Buffer**. Incubate the column with gentle end-over-end mixing at room temperature for 1-2 hours.
4. Remove the stopper and wash the column with 3-5 resin-bed volumes of ToxOut™ High Capacity Endotoxin Removal **Wash Buffer**.
5. Equilibrate agarose by washing with 5 resin-bed volumes of Endotoxin Removal **Equilibration Buffer**.  
Optional: You may equilibrate resin with buffers of your choice, but the buffer must be endotoxin-free or at least prepared with endotoxin-free water. ToxOut™ High Capacity Endotoxin Removal Agarose performs best at neutral pH without extreme salt concentration. Test the detox cycles with small amount of sample if switching to alternative equilibration buffer.
6. Apply sample to the equilibrated column.  
Optional: For greater efficiency, incubate sample with resin at room temperature or 4 °C for 1 hour with gentle end-over-end mixing.
7. Flow through and collect the sample.

**FOR RESEARCH USE ONLY! Not to be used on humans.**

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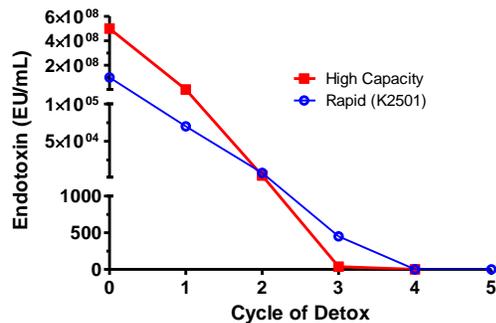
**Optional:** Higher sample recovery rate may be achieved by adding another 2-4 resin-bed volumes of ToxOut™ Endotoxin Removal **Equilibration Buffer** or buffer of your choice to elute more sample.

- Determine the endotoxin concentration of the processed sample. If the final endotoxin concentration is above the desired endotoxin concentration, repeat the endotoxin removal procedure (step 2 to step 8). ToxOut™ High Capacity Endotoxin Removal Agarose can be reused up to 5 times.

**Caution:** Handle processed sample with extra caution to prevent sample contamination.

A fresh resin should be used for fresh sample to prevent cross contamination.

- Store resin in ToxOut™ Endotoxin Removal **Equilibration buffer** or buffer of your choice **with 20% ethanol** at 4 °C.



**Figure 1. ToxOut™ High Capacity Endotoxin Removal Agarose removes more than 99% endotoxin from protein solution sample (Red square).** Endotoxin capacities and endotoxin efficiencies were determined by challenging 0.1 ml resin with 5 × 10<sup>8</sup> EU/ml LPS in 300 µl BSA (10 mg/ml). By reloading samples to the repeatedly regenerated column, the endotoxin spike is reduced to <0.05 EU/ml in the BSA solution. The highest endotoxin binding capacity of High Capacity Endotoxin Removal Agarose is 1.5 × 10<sup>9</sup> EU/ml from the first cycle of detox and its average detox efficiency of 4 cycles of detox is 99.3% and the average protein recovery of 4 cycles of detox is 97.5%. The endotoxin binding capacities of ToxOut™ Rapid Endotoxin Removal Kit (Cat No.K2501) is presented in blue circle.

### VIII. Troubleshooting:

Problem	Cause	Solution
<b>Low detox efficiency</b>	• The pH of the sample is not between pH 6-8	• Adjust the sample to neutral pH (best range: pH 7-8)
	• The contacting time between sample and the resin is too short	• Adjust incubation time according to sample condition (Optional step 8)
	• Endotoxin concentration is high in sample	• Aliquot the sample to several columns to avoid overloading endotoxin amount to one column • Repeat regenerating and reloading steps until endotoxin concentration reduces to desired value
	• External endotoxin contamination	• Use endotoxin-free solutions and labware
<b>Low Sample/Protein Recovery</b>	• Non-specific binding of sample to the resin	• Increase NaCl concentration up to 20 mg/ml in the sample buffer and ToxOut™ Endotoxin Removal <b>Equilibration buffer</b>
	• Endotoxin binds to target components, such as proteins	• Optimize the pH and salt concentration of sample buffer to reduce aggregation

### IX. Related Products:

- ToxOut™ High Capacity Endotoxin Removal Kit (Cat. No. K2502-5)
- ToxOut™ High Capacity Endotoxin Removal Regeneration Buffer (Cat. No. 7945-50)
- ToxOut™ High Capacity Endotoxin Removal Wash Buffer (Cat. No. 7946-50)
- ToxOut™ Rapid Endotoxin Removal Kit (Cat. No. K2501-5)
- ToxOut™ Rapid Endotoxin Removal Agarose (Cat. No. 7941-5)
- ToxOut™ Endotoxin Removal Equilibration Buffer (Cat No. 7940-50)
- ToxOut™ Endotoxin-free Water (Cat. No. 7938-50)
- ToxOut™ Endotoxin-free PBS (Cat. No. 7943-50)
- ToxOut™ Endotoxin-free Collection Tube, 2.0 ml (Cat. No. 7937-50)
- ToxOut™ Endotoxin-free Glass Vial (Cat. No. 7944-2, -10)

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