

# ToxOut™ High Capacity Endotoxin Removal Kit

rev 03/16

(Cat # K2502-5; 5 kits; 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. 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 Kit can effectively decrease high concentration to levels lower than < 0.05 EU/ml without using toxic buffers while maintaining the protein recovery rate at >97%. BioVision's ToxOut™ High Capacity Endotoxin Removal Agarose in this kit is using poly-ε-lysine as affinity ligand. It is a safe, nontoxic food preservative known for capturing and removing endotoxin from samples.

## 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
- Columns Content: 5 x 100 µl ToxOut™ High Capacity Endotoxin Removal Agarose (Cat. No. 7942-5)
- Resin Content: affinity matrix of poly-ε-lysine, supplied as 50% slurry in 20% ethanol
- Each column can be reused up to 5 times (The buffers provided in the kit is sufficient for single use.)
- Final products are compatible with potential therapeutic applications as ligand and buffers are nontoxic.

## III. Applications:

- Effectively remove endotoxins to levels lower than < 0.05 EU/ml
- Ideal for processing small scale solution samples (0.1 ml - 0.5 ml)

## IV. Kit Contents:

Components	K2502-5	Part Number
ToxOut™ High Capacity Endotoxin Removal Spin Column	5 columns	K2502-5-1
ToxOut™ Endotoxin-free Collection Tube	10 tubes	K2502-5-2
ToxOut™ Endotoxin-free Water	10 ml	K2502-5-3
ToxOut™ High Capacity Endotoxin Removal Regeneration Buffer	10 ml	K2502-5-4
ToxOut™ High Capacity Endotoxin Removal Wash Buffer	10 ml	K2502-5-5
ToxOut™ Endotoxin Removal Equilibration Buffer	10 ml	K2502-5-6

## V. User Supplied Reagents and Equipment:

- Adjustable pipettes and sterile, endotoxin-free (or pyrogen-free) tips
- Centrifuge for 1.5 - 2 ml microcentrifuge tubes
- Each kit contains buffers and tubes sufficient for at least 5 reactions. Additional 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:

- Read entire protocol before performing the experiment.
- Store kit and components at 4°C. Do not freeze.
- ToxOut™ Endotoxin-free water and buffers expire 2 months after opening.
- To prevent endotoxin contamination from dust, solution or dirty labware, only use endotoxin-free solutions and tubes and proceed with extra caution.
- Do not let the resin completely dry anytime. Store the resin in 200 µl ToxOut™ Endotoxin Removal Equilibration Buffer with 20% ethanol after use.

## VII. Endotoxin Removal Protocol:

### Notes:

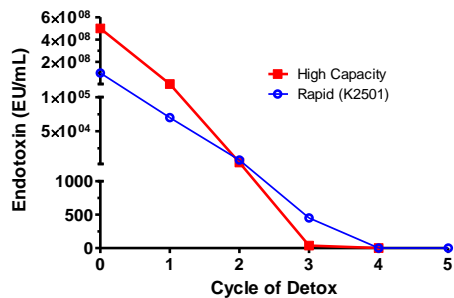
- ToxOut™ High Capacity Endotoxin Removal Spin Column must be regenerated by ToxOut™ High Capacity Endotoxin Removal **Regeneration Buffer** before each use, **including first use**.
- Sample recovery rate may be increased by optimizing NaCl concentration or pH level of samples and equilibration buffer. Additional information is available in "Troubleshooting" section (section VIII).
- *Quick Spin* means to centrifuge the spin column  $<150 \times g$  for 10 seconds.

### Procedure:

1. Snap off the bottom plug (save it for step 3 and future reuse procedure). Slightly loosen the top cap. Place the column in a ToxOut™ Endotoxin-free Collection Tube. Quick Spin to remove the storage solution. Discard the solution.

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

- Add 0.5 ml ToxOut™ Endotoxin-free **Water** and invert the column 3 times. Quick Spin to remove the solution. Discard the solution.
- Close the column with the bottom plug. Regenerate the column by adding 0.5 ml ToxOut™ High Capacity Endotoxin Removal **Regeneration Buffer**. Incubate the column with gentle end-over-end mixing at room temperature for 1–2 hours.
- Loosen the cap and remove the bottom plug. *Quick Spin* to remove the solution. Discard the solution.
- Add 0.5 ml ToxOut™ High Capacity Endotoxin Removal **Wash Buffer** and invert the column 3 times. *Quick Spin* to remove the solution. Discard the solution. Repeat Step 5 once.
- Equilibrate resin by adding 0.5 ml ToxOut™ Endotoxin Removal **Equilibration Buffer** and invert the column 3 times. *Quick Spin* to remove the solution. Discard the solution. Repeat Step 6 once.
- Place column in a new ToxOut™ Endotoxin-free **Collection Tube**. Apply sample (0.1 - 0.5 ml) to the column.  
Optional: For greater efficiency, place the bottom plug back to the column before applying sample to resin. Apply sample, tighten the cap and incubate column at room temperature or 4 °C for 1 hour with gentle end-over-end mixing.
- Loosen the cap and remove the bottom plug. *Quick Spin* to collect the sample.  
Optional: Higher sample recovery rate may be achieved by adding another 0.2 - 0.4 ml ToxOut™ Endotoxin Removal **Equilibration Buffer** to the column. *Quick Spin* to elute more sample from the spin column.
- 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 10). Each column can be reused up to 5 times.  
Caution: Handle processed sample with extra caution to prevent sample contamination.  
A fresh column should be used for fresh sample to prevent cross contamination.
- Place the bottom plug back and store columns in 200 µl ToxOut™ Endotoxin Removal **Equilibration buffer with 20% ethanol** at 4 °C.



**Figure. ToxOut™ High Capacity Endotoxin Removal Kit removes >99% endotoxin from protein solution sample (Red square).** Endotoxin capacities and endotoxin efficiencies were determined by challenging 0.1 ml resin with  $5 \times 10^8$  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  $1.5 \times 10^9$  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%. (Blue Circle: Rapid (K2501), the endotoxin binding capacities of ToxOut™ Rapid Endotoxin Removal Kit)

#### VIII. Troubleshooting:

Problem	Cause	Solution
Low detox efficiency	• 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 9)
	• 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
Low Sample/Protein Recovery	• 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 Agarose (Cat. No. 7942-5)
- 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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