

# ToxOut™ Rapid Endotoxin Removal Agarose

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(Store at 4°C; Do not freeze)

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**Cat. No.**

**7941-5 ToxOut™ Rapid Endotoxin Removal Agarose, 5 ml**

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**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. Presence of endotoxin in any of these has a detrimental effect on culturing of cells and can lead to generation of irreproducible or negative experimental data. Therefore, it is critical to remove endotoxin from samples and products.

**BioVision's ToxOut™ Rapid Endotoxin Removal Agarose** can quickly and effectively eliminate endotoxins to < 0.05 EU/ml in solutions containing proteins or pharmacologically important components via the immobilized polymyxin B, which is known for capturing endotoxin and preventing toxic effects.

**II. Product Features:**

- High Binding Capacity: up to 9 x 10<sup>8</sup> EU/ml resin.
- High Sample Recovery: ~90% recovery with protein solution samples.
- Resin Content: affinity matrix of polymyxin B, supplied as 50% slurry in 20% ethanol.
- ToxOut™ Rapid Endotoxin Removal Agarose can be reused up to 5 times.

**III. Applications:**

- Quickly and effectively eliminate endotoxins to < 0.05 EU/ml.

**IV. Contents:**

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

**V. User Supplied Reagents and Equipment:**

- ToxOut™ Endotoxin-free Water (BioVision Cat. No. 7938-50)
- ToxOut™ Rapid Endotoxin Removal Regeneration Buffer (BioVision Cat. No. 7939-50)
- ToxOut™ Endotoxin Removal Equilibration Buffer (BioVision Cat. No. 7940-50)
- ToxOut™ Endotoxin-free Collection Tube (BioVision Cat. No. 7937-50)
- Endotoxin-free Column
- Adjustable pipettes and sterile, endotoxin-free (or pyrogen-free) tips
- Centrifuge for 1.5 - 2 ml microcentrifuge tubes

**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 anytime. Store the resin in endotoxin-free buffer or water with 20% ethanol after use.

**VII. Endotoxin Removal Protocol:**

**Notes:**

- ToxOut™ Rapid Endotoxin Removal Agarose must be regenerated by ToxOut™ Rapid 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).
- Protocol of ToxOut™ Rapid Endotoxin Removal Kit (K2501-5) provides details for small scale purification using spin columns.

**Procedure:**

1. Pack the appropriately sized column with ToxOut™ Rapid Endotoxin Removal Agarose.
2. Regenerate the resin by washing with 5 resin-bed volumes of ToxOut™ Rapid Endotoxin Removal **Regeneration Buffer**.
3. Wash the resin with 3-5 resin-bed volumes of ToxOut™ Endotoxin-free **Water**.
4. 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™ Rapid Endotoxin Removal Agarose performs best at neutral pH without extreme salt concentration. Test the detox cycles with small amount of sample before changing the equilibration buffer.
5. Apply samples to the equilibrated resin.  
Optional: For greater efficiency, incubate samples with resin at room temperature or 4°C for 1 hour.
6. Flow through and collect samples in new ToxOut™ Endotoxin-free collection Tubes.

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

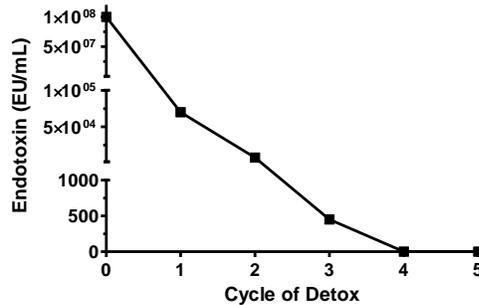
Optional: Higher sample recovery rate may be achieved by adding another 2-4 resin-bed volumes of ToxOut™ Endotoxin Removal **Equilibration Buffer** or buffer of your choice to elute more sample.

7. 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 6). ToxOut™ Rapid Endotoxin Removal Agarose can be reused up to 5 times.

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

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

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



**Figure 1. 100 µl ToxOut™ Rapid Endotoxin Removal Agarose removes >90% endotoxin from protein solution sample.** Endotoxin capacities and endotoxin efficiencies were determined by challenging 0.1 ml resin with 1 x 10<sup>8</sup> EU/ml LPS in 200 µl BSA (10 mg/ml) using the ToxOut™ Rapid Endotoxin Removal Kit (Cat. No. K2501-5). 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 is 9.99 x 10<sup>8</sup> EU/ml from the first cycle of detox. The average detox efficiency of 5 cycles of detox is 95% and the average protein recovery of 5 cycles of detox is 90%.

#### VIII. Troubleshooting:

Problem	Cause	Solution
<b>Low detox efficiency</b>	<ul style="list-style-type: none"> <li>The pH of the sample is not between pH 6-8</li> </ul>	<ul style="list-style-type: none"> <li>Adjust the sample to neutral pH (best range: pH 7-8)</li> </ul>
	<ul style="list-style-type: none"> <li>The contacting time between sample and the resin is too short</li> </ul>	<ul style="list-style-type: none"> <li>Adjust incubation time according to sample condition (Optional step 6)</li> </ul>
	<ul style="list-style-type: none"> <li>Endotoxin concentration is high in sample</li> </ul>	<ul style="list-style-type: none"> <li>Use more resin to avoid overloading endotoxin amount</li> <li>Repeat regenerating and reloading steps until endotoxin concentration reduces to desired value</li> </ul>
	<ul style="list-style-type: none"> <li>External endotoxin contamination</li> </ul>	<ul style="list-style-type: none"> <li>Use endotoxin-free solutions and labware</li> </ul>
<b>Low Sample/Protein Recovery</b>	<ul style="list-style-type: none"> <li>Non-specific binding of sample to the resin</li> </ul>	<ul style="list-style-type: none"> <li>Increase NaCl concentration in the sample buffer and ToxOut™ Endotoxin Removal <b>Equilibration buffer</b> (up to 0.4 M)</li> </ul>
	<ul style="list-style-type: none"> <li>Endotoxin binds to target components, such as proteins</li> </ul>	<ul style="list-style-type: none"> <li>Optimize the pH and salt concentration of sample buffer to reduce aggregation</li> </ul>
<b>Protein Cross Contamination</b>	<ul style="list-style-type: none"> <li>Same resin are used for different samples</li> </ul>	<ul style="list-style-type: none"> <li>Always use new resin for fresh samples</li> </ul>

#### IX. Related Products:

ToxOut™ Rapid Endotoxin Removal Kit (Cat. No. K2501-5)  
 ToxOut™ High Capacity Endotoxin Removal Kit (Cat. No. K2502-5)  
 ToxOut™ High Capacity Endotoxin Removal Agarose (Cat. No. 7942-5)  
 ToxOut™ Rapid Endotoxin Removal Regeneration Buffer (Cat. No. 7939-50)  
 ToxOut™ Endotoxin Removal Equilibration Buffer (Cat No. 7940-50)  
 ToxOut™ Endotoxin-free Collection Tube, 2.0 ml (Cat. No. 7937-50)  
 ToxOut™ Endotoxin-free Water (Cat. No. 7938-50)  
 ToxOut™ Endotoxin-free PBS (Cat. No. 7943-50)  
 ToxOut™ Endotoxin-free Glass Vial (Cat. No. 7944-2, -10)

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