High Capacity Endotoxin Removal Kit

rev 03/17

(Catalog Number: NBP2-49797; 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.

High Capacity Endotoxin Removal Kit can effectively decrease high concentration to levels lower than < 0.05 EU/mlwithout using toxic buffers while maintaining the protein recovery rate at >97%. 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 x 10^9 EU/ml resin
- High Sample Recovery: ~97% recovery with protein solution samples
- Columns Content: 5 x 100 µl High Capacity Endotoxin Removal Agarose
- 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	NBP2-49797
High Capacity Endotoxin Removal Spin Column Endotoxin-free Collection Tube	5 columns 10 tubes
Endotoxin-free Water	10 ml
High Capacity Endotoxin Removal Regeneration Buffer High Capacity Endotoxin Removal Wash Buffer Endotoxin Removal Equilibration Buffer	10 ml 10 ml 10 ml

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.
  - Endotoxin-free Water
  - High Capacity Endotoxin Removal Regeneration Buffer
  - High Capacity Endotoxin Removal Wash Buffer
  - Endotoxin Removal Equilibration Buffer
  - Endotoxin-free Collection Tube

VI. Storage and Handling:

- Read entire protocol before performing the experiment.
- Store kit and components at 4°C. Do not freeze.
- ™ 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 Endotoxin Removal Equilibration Buffer with 20%ethanol after use.

VII. Endotoxin Removal Protocol:

Notes:

- High Capacity Endotoxin Removal Spin Column must be regenerated by 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 × 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 Endotoxin-free Collection Tube. Quick Spin to remove the storage solution. Discard the solution.

2. Add 0.5 ml ™ Endotoxin-free **Water** and invert the column 3 times. Quick Spin to remove the solution. Discard the solution.
3. Close the column with the bottom plug. Regenerate the column by adding 0.5 ml High Capacity Endotoxin Removal **Regeneration Buffer**. Incubate the column with gentle end-over-end mixing at room temperature for 1~2 hours.
4. Loosen the cap and remove the bottom plug. *Quick Spin* to remove the solution. Discard the solution.
5. Add 0.5 ml 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.
6. Equilibrate resin by adding 0.5 ml Endotoxin Removal **Equilibration Buffer** and invert the column 3 times. *Quick Spin* to remove the solution. Discard the solution. Repeat Step 6 once.
7. Place column in a new 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 ℃ for 1 hour with gentle end-over-end mixing.
8. 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 Endotoxin Removal **Equilibration Buffer** to the column. *Quick Spin* to elute more sample from the spin column.
9. 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.
10. Place the bottom plug back and store columns in 200 µl Endotoxin Removal **Equilibration buffer with 20% ethanol** at 4 ℃.

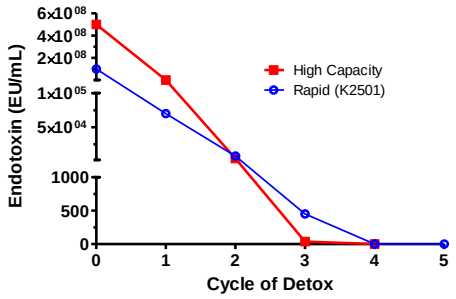

Figure. High Capacity Endotoxin Removal Kit removes >99% endotoxin from protein solution sample (Red square). Endotoxin capacities and endotoxin efficiencies were determined by challenging 0.1 ml resin with 5 x 10^8 EU/ml LPS in 300 µl BSA (10 mg/ml). By reloading samples to the repeatedly regenerated 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 x 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 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 Endotoxin Removal **Equilibration buffer**
	• Endotoxin binds to target components, such as proteins	• Optimize the pH and salt concentration of sample buffer to reduce aggregation