PRODUCT INFORMATION & MANUAL

Endotoxin Removal Kit

NBP2-49796

For research use only.
Not for diagnostic or therapeutic procedures.

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Rapid Endotoxin Removal Kit

(Cat # NBP2-49796, 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 (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.

Novus Rapid Endotoxin Removal Kit 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 10^8 EU/ml resin
- High Sample Recovery: ~90% recovery with protein solution samples
- Column Content: 5 x 100 µl Rapid Endotoxin Removal Agarose
- Resin Content: affinity matrix of polymyxin B, supplied as 50% slurry in 20% ethanol
- Each column can be reused up to 5 times
- Rapid removal: only require 5 minutes for each removal cycle

III. Applications:

- Quickly and effectively eliminate endotoxins to < 0.05 EU/ml
- Ideal for processing small scale solution samples (0.1 ml - 0.5 ml)

IV. Kit Contents:

Components	NBP2-49796
Rapid Endotoxin Removal Spin Column Endotoxin-free	5 columns
Collection Tube	10 tubes
Rapid Removal Wash Buffer	10 ml
Rapid Endotoxin Removal Regeneration Buffer	10 ml X 2
Endotoxin Removal Equilibration Buffer	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.
  - Rapid Removal Wash Buffer
  - Rapid Endotoxin-free Regeneration Buffer
  - Endotoxin Removal Equilibration Buffer
  - Endotoxin Removal Collection Tube

VI. Storage and Handling:

- 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.
- Read entire protocol before performing the experiment.
- Do not let the resin dry anytime. Store the resin in 200 µl Endotoxin Removal Equilibration Buffer with 20% ethanol after use.

VII. Endotoxin Removal Protocol:

Notes:

- Rapid Endotoxin Removal Spin Column must be regenerated by 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).
- *Quick Spin* means to centrifuge the spin column ~15000 × g for 5-10 seconds.

Procedure:

1. Snap off the bottom plug and remove the cap (save both plug and cap for step 14). 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 Removal **Equilibration Buffer**. Quick Spin to remove the solution. Discard the solution.

3. Regenerate the column by adding 0.5 ml Rapid Endotoxin Removal **Regeneration Buffer**. *Quick Spin* to remove the solution. Discard the solution.
4. Repeat Step 3 once (total two times).
5. Add 0.5 ml **Rapid Removal Wash Buffer**. *Quick Spin* to remove the solution. Discard the solution.
6. Repeat Step 5 two times (total 3 times).
7. Equilibrate agarose by adding 0.5 ml Endotoxin Removal **Equilibration Buffer**. *Quick Spin* to remove the solution. Discard the solution.
8. Repeat Step 7 once (total two times).
9. Place column in a new Endotoxin-free **Collection tube**
10. Apply sample (0.1 - 0.5 ml) to the column. Let the sample pass through the column by gravity.
11. Repeat load flow-through several times (3-5 times)
    Optional: Incubate the column at room temperature or 4℃ for 1 hour with gentle end-over-end mixing with cap and plug on.
12. *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.
13. 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 3 to step 12). Each column can be reused up to 5 times. However, to re-use the column, the used column must repeat the step 3 four times (total five times) to de-contaminate endotoxin completely.
    Note: A fresh column should be used for fresh sample to prevent cross contamination.
14. Pace plug and cap back and store the column in 200 µl Endotoxin Removal **Equilibration buffer with 20% ethanol** at 4℃.

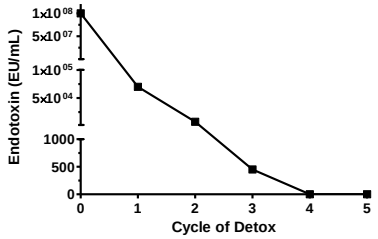

Figure 1. Rapid Endotoxin Removal Kit removes >90% endotoxin from protein solution sample. Endotoxin capacities and endotoxin efficiencies were determined by challenging 0.1 ml resin with 1 x 10^8 EU/ml LPS in 200 µl BSA (10 mg/ml). By reloading samples to the repeatedly regenerated column, the endotoxin spike is reduced to <0.05 EU/ml in the BSA solution. The highest endotoxin binding capacity is 9.99 x 10^8 EU/ml from the first cycle of detox. The average detox efficiency of 5 cycles of detox is 94.5% and the average protein recovery of 5 cycles of detox is 89.6%.

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 6)
	• 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 • For extremely high level endotoxin sample, do 4-5 cycles detox by using one column and then do final cycle detox by using another fresh column.
	• 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