ELISA PRODUCT INFORMATION & MANUAL

Endotoxin Removal Kit

NBP2-49796

Enzyme-linked Immunosorbent Assay for quantitative detection. For research use only.
Not for diagnostic or therapeutic procedures.

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1. **Overview**

Endotoxin Removal Kit (Rapid)(NBP2-49796) 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.

The kit quickly and effectively eliminates endotoxins to < 0.05 EU/ml and is ideal for processing small-scale solution sample (0.1-0.5 mL).

2. **Protocol Summary**

Regenerate Rapid Endotoxin Removal Spin Column (including before first use).

Equilibrate Rapid Endotoxin Removal Spin Column in Endotoxin Removal Equilibration Buffer.

Place Rapid Endotoxin Removal Spin Column in an Endotoxin-free Collection Tube and apply sample (0.1-0.5 mL). Allow sample to pass through by gravity.

Repeat sample loading / flow-through 3 – 5 times (alternatively, incubate for 1 hour with end-to-end mixing). Quick spin to collect sample.

Determine the endotoxin concentration of the final eluate.

Repeat the procedure if the endotoxin concentration needs to be further reduced.

3. **General guidelines, precautions, and troubleshooting**

- Please observe safe laboratory practice and consult the safety datasheet.
- For typical data produced using the assay, please see the assay kit datasheet on our website.

4. **Materials Supplied, and Storage and Stability**

- Store kit at 4°C immediately upon receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.
- Endotoxin-free water and buffers expire 2 months after opening.
- Do not freeze reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Rapid Endotoxin Removal Spin Column	5 units	4°C	4°C
Endotoxin-free Collection Tube	10 units	4°C	N/A
Rapid Removal Wash Buffer	10 mL	4°C	N/A
Rapid Endotoxin Removal Regeneration Buffer	2 x 10 mL	4°C	N/A
Endotoxin Removal Equilibration Buffer	10 mL	4°C	N/A

5. **Materials Required, Not Supplied**

These materials are not included in the kit, but will be required to successfully perform this assay:

- Adjustable pipettes and sterile, endotoxin-free (or pyrogen-free) tips.
- Centrifuge for 1.5 - 2 ml microcentrifuge tubes.

6. Reagent Preparation

- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Prepare only as much reagent as is needed on the day of the experiment.

6.1 Rapid Endotoxin Removal Spin Column

Ready to use as supplied. Contains 100 µL of Rapid Endotoxin Removal Agarose. Do not let the resin dry out at any time. Store in Endotoxin Equilibration Buffer containing 20% ethanol after use. Each column can be re-used up to 5 times, providing the separate buffers are purchased (Endotoxin Removal Equilibration Buffer (ab278802), Rapid Endotoxin Removal Regeneration Buffer (ab269281), Rapid Removal Wash Buffer (ab278801)).

6.2 Endotoxin-free Collection Tube

Ready to use as supplied.

6.3 Rapid Removal Wash Buffer

Ready to use as supplied.
Expires 2 months after opening.

6.4 Rapid Endotoxin Removal Regeneration Buffer

Ready to use as supplied.
Expires 2 months after opening.

6.5 Endotoxin Removal Equilibration Buffer

Ready to use as supplied.
Expires 2 months after opening.

7. **Assay Procedure**

- To prevent endotoxin contamination from dust, solution or dirty labware, only use endotoxin-free solutions and tubes and proceed with extra caution.

ΔNote: Rapid Endotoxin Removal Spin Columns must be regenerated by using Rapid Endotoxin Removal Regeneration Buffer before each use, including the first use.

ΔNote: Quick Spin means centrifuge the spin column at 15,000 x *g* for 5-10 seconds.

ΔNote: Ensure the pH of the sample is in the range pH6-8 (ideally, pH 7-8) to ensure efficient endotoxin removal. Adjust the sample pH if necessary.

ΔNote: The maximum endotoxin binding capacity of the spin columns is 0.9 x 10^8 EU/0.1 mL. If the endotoxin levels in the sample are very high aliquot the sample between several columns to avoid exceeding the endotoxin-binding capacity. Alternatively, repeat the regeneration and loading steps several times until endotoxin is reduced to the desired level; if required, a final detox step can be performed using a fresh column.

ΔNote: In cases of low protein sample recovery, increase the NaCl concentration up to 20 mg/mL in the sample buffer and the Endotoxin Removal Equilibration Buffer.

7.1 Snap off the bottom plug and remove the cap (save both plug and cap for step 14). Place the column in an Endotoxin-free Collection Tube. Quick Spin to remove the storage solution. Discard the solution.

7.2 Add 0.5 mL Endotoxin Removal Equilibration Buffer. Quick Spin to remove the solution. Discard the solution.

7.3 Regenerate the column by adding 0.5 mL Rapid Endotoxin Removal Regeneration Buffer. Quick Spin to remove the solution. Discard the solution.

7.4 Repeat Step 3 once (perform step 3 twice in total).

7.5 Add 0.5 mL Rapid Removal Wash Buffer. Quick Spin to remove the solution. Discard the solution.

7.6 Repeat Step 5 once (perform step 5 three times in total).

7.7 Equilibrate agarose by adding 0.5 mL Endotoxin Removal Equilibration Buffer. Quick Spin to remove the solution. Discard the solution.

7.8 Repeat Step 7 once (perform step 7 twice in total).

7.9 Place column in a new Endotoxin-free Collection Tube.

7.10 Apply sample (0.1 - 0.5 mL) to the column. Let the sample pass through the column by gravity.

7.11 Repeat load flow-through several times (3-5 times).

Optional: Incubate the column at room temperature or 4 C for 1 hour with gentle end-over-end mixing with cap and plug on.

7.12 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.

7.13 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 step 3 four times (total five times) to de-contaminate endotoxin completely.

ΔNote: A fresh column should be used for fresh sample to prevent cross contamination.

7.14 Place plug and cap back and store the column in 200 µL Endotoxin Removal Equilibration buffer with 20% ethanol at 4 C.

8. **Typical Data**

Data provided **for demonstration purposes only**.

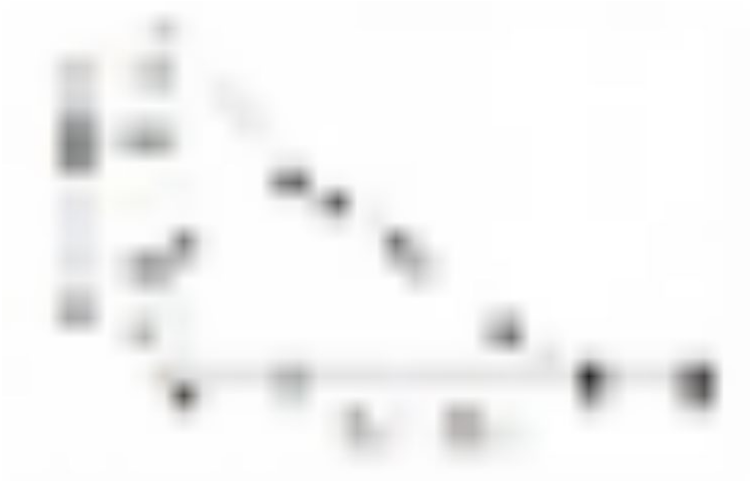

Figure 1. Endotoxin Removal Kit (Rapid)(NBP2-49796) removes >90% of endotoxin from a protein solution sample.

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 capacity is 9.99 x 10^8 EU/mL from the first cycle of detox. The average efficiency of 5 cycles of detox is 94.5% and the average protein recovery from 5 cycles of detox is 89.9%.

9. **Notes**