Proteus NoEndo™M (Mini) Spin Column Kits

Protocol

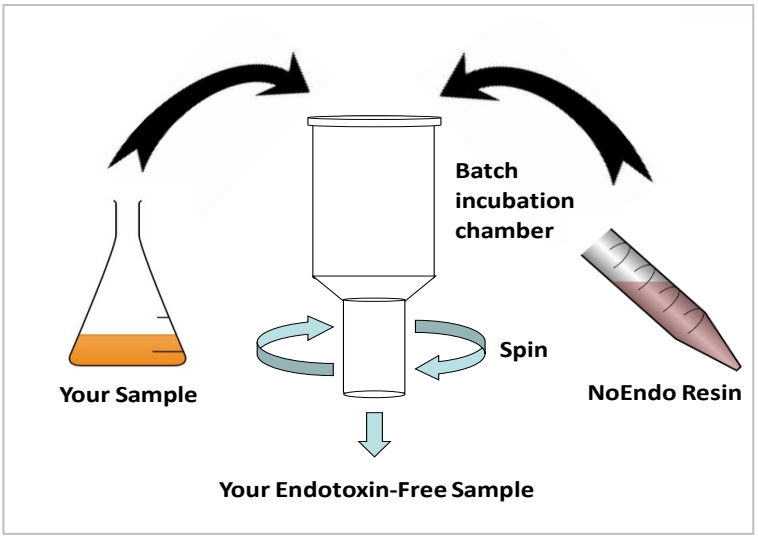
Materials Supplied in the Kit:

- 0.5 ml vials containing NoEndo™ resin (50% NoEndo slurry: 0.25 ml resin).
- Proteus spin column (20 ml capacity in a swing bucket rotor).
- Two caps:
  1. Clear spin push cap for all centrifugation steps.
  2. Yellow screw cap for the batch incubation step only.
- 50 ml centrifuge tubes.

Additional Materials Required:

- 0.2 µm syringe filters for clarification.
- Low endotoxin pre-equilibration buffer (PBS recommended).
- 50 ml centrifuge tubes.
- A bench-top centrifuge with swing bucket rotor capable of handling 50ml centrifuge tubes. (The preferred rotor is a swing bucket rotor).
- Quartz cuvettes for UV absorbance measurements.
- UV/VIS spectrophotometer.
- Pyrogen-free test tubes, pipettes and buffer for Endotoxin Assay.

Endotoxin Removal Protocol:



Recommended Protocol:

PRE-EQUILIBRATION

1. Remove the CLEAR spin push cap and pipette 0.5 ml NoEndo™ resin slurry (50% NoEndo slurry: 0.25 ml resin) into the batch incubation chamber of the spin column barrel. Wash the resin at 500 x g for 5 min.

2. Pre-equilibrate the NoEndo™ Mini spin column with 15 ml equilibration buffer by centrifuging the spin column (with the CLEAR SPIN PUSH CAP) at 750 x g for 5 min. It is critical that you repeat this step one more time with a further 15 ml fresh equilibration buffer.

NOTE: If using one spin column, ensure that the spin column is counterbalanced with a unit of equal weight (adjusted with distilled water).

CLARIFICATION OF SAMPLE

3. Pre-filter the sample through a single 0.2 µm (25 mm diameter) syringe filter.

NOTE: As with all forms of chromatography, it is critical that the sample is filtered through a final 0.2 µm syringe filter immediately before loading it on the spin column. Optimal performance of these devices will depend on these instructions being rigorously followed.

SAMPLE LOADING

4. Transfer the spin column barrel to a fresh 50 ml centrifuge tube and load your required volume of filtered sample. The maximum sample volume is 20 ml. Tightly screw the yellow batch incubation cap and invert 2-3 times to mix the sample and the NoEndo™ resin. Place the column on a standard tube roller and mix for 1-3 hours. To achieve final endotoxin loads < 0.1 EU/ml from starting loads of 300 EU/ml, we recommend a 2-3 hour batch incubation.

5. After batch incubation, replace the yellow cap with the CLEAR spin push cap. Centrifuge the column at 750 x g for up to 10 min and collect the eluate.

NOTE: If using one spin column, ensure that the spin column is counterbalanced with a unit of equal weight (adjusted with distilled water).

PURIFIED SAMPLE

6. The eluate contains the target analyte largely depleted of endotoxin and is now ready for further downstream analyses.

Technical Support:

The complete user guide for the Proteus NoEndo™ Mini spin column kits is available for download.

For further information please visit the website **www.proteinark.com** or contact us via:

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