Protein Årk

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

- 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 μ m (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.</p>
- 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.

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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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