Proteus NoEndo™ S (Standard) and NoEndo™ HC (High Capacity) Spin Column Kits

Protocol

Materials Supplied in the Kit:

- Proteus spin column plugs containing Proprietary NoEndo™ Endotoxin Binding resin.
- Proteus spin column devices (20 ml capacity in a swing bucket rotor).
- Insertion Tool.
- 50 ml centrifuge tubes.

Additional Materials Required:

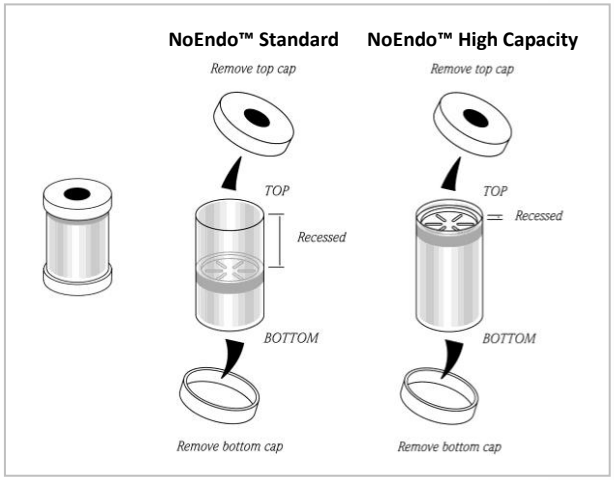
- Filter units: 0.2 and 1.2 µm syringe filters for clarification.
- Low endotoxin pre-equilibration buffer (PBS recommended).
- 50 ml centrifuge tubes.
- A bench-top centrifuge with swing bucket rotor capable of handling 50ml centrifuge tubes. (The preferred rotor is a swing bucket rotor).
- Quartz cuvettes for UV absorbance measurements.
- UV/VIS spectrophotometer.
- Pyrogen-free test tubes, pipettes and buffer for Endotoxin Assay.

Loading the Media Plug into the Spin Column:

1. Unwrap the sealing film from both ends of the plug.

2. Remove the top and bottom caps.

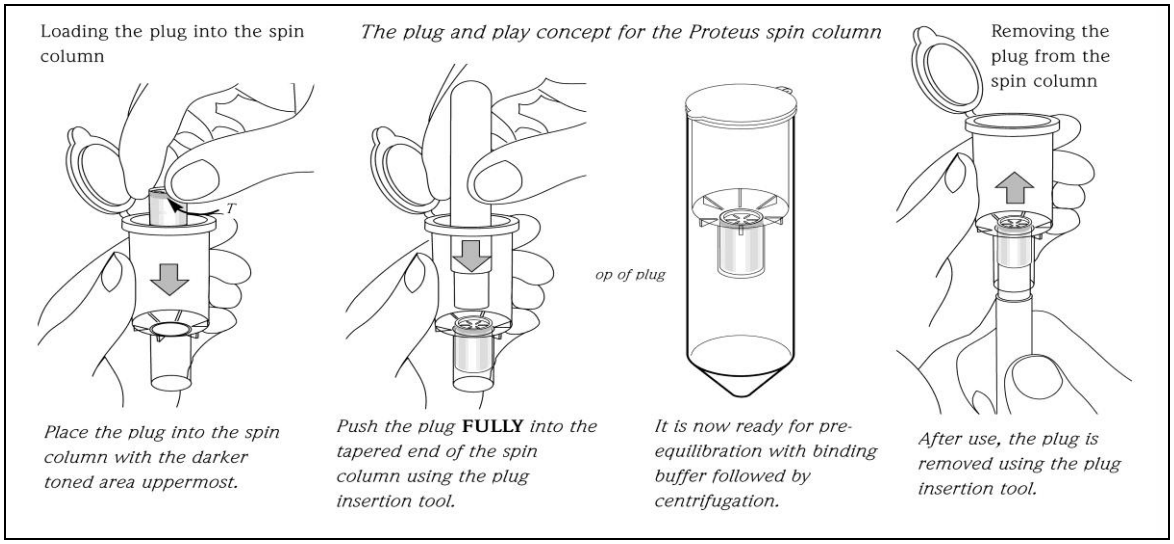
NOTE: Once the caps are removed, the **top** end can be identified because it is recessed.
(12 mm deep recess – Standard,
4 mm deep recess - High Capacity)



3. Insert the plug into the spin column with the top (recessed) end uppermost.

4. Push the plug **fully** into the tapered end of the spin column using the insertion tool.

NOTE: To remove the plug from the spin column, insert the tool into the bottom of the spin column and push upwards.



Recommended Protocol:

After loading the plug into the spin column and placing the spin column into a centrifuge tube, follow the procedure below.

PRE-EQUILIBRATION

1. Pre-equilibrate the Proteus NoEndo™ spin column with 10 ml equilibration buffer by centrifuging the spin column at 500 x g for 3 min.

NOTE: If using one spin column, ensure that the spin column is counterbalanced with a unit of equal weight (adjusted with distilled water; without a plug).

CLARIFICATION OF SAMPLE

2. Pre-filter 20-25 ml sample through a single 1.2 µm (25 mm diameter) syringe filter to remove any cellular debris. Following this, filter the partially clarified 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

3. Pipette 20 ml sample into the spin column. Centrifuge the spin column at 100 x g for 30 min. Ideal sample loading conditions are obtained using a flow rate of less than 1 ml/min. It may be necessary to increase the spin time or spin speed if any sample remains on the top of the plug. Spin speeds as high as 1,500 x g have no damaging

effect upon the NoEndo™ resin. A flow rate slower than expected may be indicative of a partially clogged plug resulting in incomplete filtration of the sample.

NOTE: When necessary, perform a further wash step by reloading 1-2 ml (NoEndo™ Standard) or 2-4 ml (NoEndo™ High Capacity) equilibration buffer for maximum protein recovery.

4. For maximal removal of endotoxin, reload the sample into a new spin column (pre-equilibrated as per step 1) and repeat the centrifugation step (100 x g for 30 min). Increase the spin time or speed if any sample remains above the plug.

NOTE: If using one spin column, ensure that the spin column is counterbalanced with a unit of equal weight (adjusted with distilled water; without a plug).

PURIFIED SAMPLE

5. 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™ S and HC spin column kits is available for download.

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

Telephone: +44 (0) 33 33 44 20 25
FAX: +44 (0) 33 33 44 20 25
Email: info@proteinark.com