Proteus 1-Step Batch Mini Spin Columns
Protocol

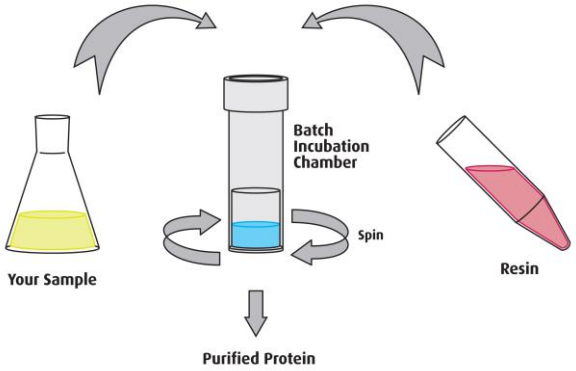
Materials Supplied in the Kit:

- Proteus spin column (600 µl capacity in a swing bucket rotor) (GEN-1SBM-40: 40 pc, GEN-1SBM-100: 100 pc)
- 2.2 ml centrifuge tubes (GEN-1SBM-40: 80 pc, GEN-1SBM-100: 200 pc)

Additional Materials Required:

- 0.2 µm clarification device (recommended Proteus mini-clarification spin columns from Protein Ark (GEN-MSF500) or a syringe filter)
- Microfuge with a fixed angle rotor capable of handling 2.2 ml centrifuge tubes (diameter 11 mm)
- Buffer
- Quartz cuvettes for UV absorbance measurements
- UV/VIS spectrophotometer

Protein Purification Protocol:



Recommended Protocol

The following spin speeds and times are appropriate for a 100 µl resin bed volume. Spin times for each of the following steps may increase with larger bed volumes.

PRE-EQUILIBRATION

1. Pipette the appropriate resin slurry into the batch incubation chamber of the spin column barrel. Wash the resin at 12-14,000 x g for 20 sec. This step is critical to ensure that all ethanol is removed from the resin. Many resins are stored in 20 - 30% ethanol.

NOTE: Ethanol does interfere with sealing properties of the Self Seal™ membrane technology.

2. Pre-equilibrate the Mini spin column with 600 µl equilibration buffer by centrifuging the spin column at 12-14,000 x g for 20 sec. It is **critical** that you repeat this step one more time with a further 600 µl 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 filter using a Proteus Mini clarification spin column (Cat # GEN-MSF500) or appropriate 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. Load the required volume of filtered sample. The maximum sample volume is 600 µl. Close the lid and vortex for 15 seconds to mix the sample and the resin. Repeat the vortexing every 15 min for the first 1 hour. In some circumstances, more than 1 hour batch incubation may be required. Repeat the vortexing every 30 min-1 hour.

5. After batch incubation, centrifuge the column at 12-14000 x g for 20 sec 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).

WASHING STEPS

6. Wash off any unbound protein with 600 µl binding buffer at 12-14,000g for 20 sec. Repeat this step, if necessary, to ensure that all the unbound protein has been removed e.g. A280< 0.1.

PURIFIED SAMPLE

7. Transfer the spin column into a fresh collection tube and then elute the target protein with 50-600 µl elution buffer by centrifuging the spin column at 12-14,000 g for 20 sec. The eluate contains the target protein and is now ready for further downstream analyses.

Please visit **www.proteinark.com** for further information or contact us via:

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