Proteus 1-Step Batch Plus Spin Columns
Protocol

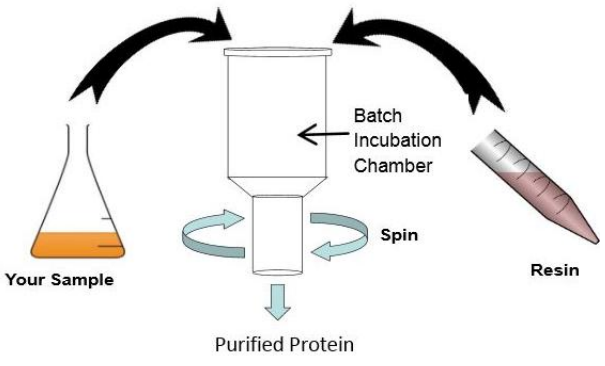
Materials Supplied in the Kit:

- 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
- 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

Protein Purification Protocol:



Recommended Protocol

The following spin speeds and times are appropriate for a 0.25 – 1 ml 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 by centrifuging at 750 x g for 5 min (with the CLEAR SPIN PUSH CAP). 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 resin 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 filter (e.g. 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 up to 20 ml of your filtered sample into batch incubation chamber containing the resin (maximum sample volume 20 ml). Tightly screw on the YELLOW batch incubation cap and invert 2-3 times to mix the sample and the resin. Place the column on a standard tube roller and mix for 1-3 hours.

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).

WASHING STEPS

6. Transfer the spin column barrel to a fresh 50 ml centrifuge tube and load 20 ml of wash buffer. Add the CLEAR SPIN PUSH CAP to the end and wash off the unbound protein at 750g for 5 min. Remove the filtrate and repeat this step.

PURIFIED SAMPLE

7. Transfer the spin column barrel into a fresh 50 ml centrifuge tube. Add up to 10 ml elution buffer to the upper reservoir and elute the purified protein at 750 x g for 5 min using the CLEAR SPIN PUSH CAP. The eluate containing your target protein is now ready for further downstream analyses.

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

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