Protocol

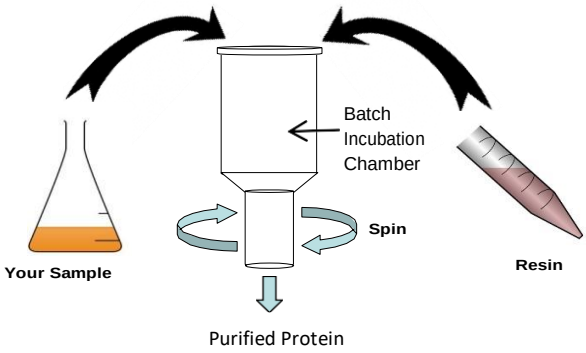
Proteus '1-Step Batch' Midi Plus Spin Columns with the SelfSeal™ Advantage

Materials Supplied in the Kit:

- Proteus spin column (20 ml capacity in a swing bucket rotor)
- Two caps:
  - Clear spin push cap for all centrifugation steps
  - **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



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. Spin the resin at 750 x g for 5 min. **This step is critical to ensure that all ethanol is removed from the resin. Many resins are stored in 20-30% ethanol. N.B. Ethanol does interfere with sealing properties of the Self Seal™ membrane technology.**

2. Pre-equilibrate the Midi spin column with 15 ml equilibration buffer by centrifuging the spin column at 750 x g for 5 min. It is **critical** that you repeat this step <u>one more time</u> with a further 15 ml fresh equilibration buffer to remove any residual ethanol.

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 final 0.2 µm filter (e.g. syringe filter).

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

SAMPLE LOADING

1. Transfer the spin column barrel to a fresh 50 ml centrifuge tube and load the 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 resin. Place the column on a standard tube roller or rotator and mix for 1-3 hours.

4. 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 flow through.
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

2. Wash off any unbound protein with 20 ml binding buffer at 750 x g for 5 min. Repeat this step, sadif necessary, to ensure that all the unbound protein has been removed eg $A_{280} < 0.1$. Transfer the spin column into a fresh collection tube and then elute the target protein with up to 1-10 ml elution buffer by centrifuging the spin column at 750 x g for 5 min. The eluate contains the target protein and is now ready for further downstream analyses.

For any further questions, please contact:
Tel : +33 9 77 40 09 09
Fax : +33 9 77 40 10 11
E-mail : info@neobiotech.com

Post : NeoBiotech, 74 rue des Suisses, 92000 Nanterre, France