HighSpec 1-step batch Midi Plus

#Cat: NB-40-00190-1 Size: 8rxns
#Cat: NB-40-00190-2 Size: 48rxns

Product Description

Designed for small to mid-scale protein purification, the HighSpec 1-step batch spin columns save time and pipetting steps. Featuring SelfSeal™ membrane technology, the column retains resin and sample in a chamber for batch incubation. By centrifugation, the membrane pores dilate and the filtered eluate gathers in the collection chamber of the column. HighSpec 1-step batch spin columns are available in two sizes: Mini for expression trials, small-scale screening, and other small-volume purification needs; or Midi Plus for volumes of up to 20 mL.

This product contains fully assembled HighSpec 1-step batch Midi Plus featuring the SelfSeal Technology. They can be used to purify proteins using 0.25-1 mL of affinity purification matrix of your choice in a bench-top centrifuge with swing bucket rotor of handling 50 mL centrifuge tubes. Up to 20 ml of lysate, wash or elution buffer can be loaded in each centrifugation step.

Protocol

Note: The following spin speeds and times are appropriate for a 0.25-1 mL resin bed volume. Spin times may increase with larger bed volumes.

Note: If using only one spin column, ensure that the spin column is counterbalanced with a unit of equal weight, e.g. an empty 50 mL tube adjusted with distilled water.

Note: The clear spin push cap should be used for all centrifugation steps. The yellow screw cap is recommended for the batch incubation steps only.

PRE-EQUILIBRATION

1. Pipet the appropriate resin slurry into the batch incubation chamber of the spin column barrel. Use the clear spin push cap to close the chamber and spin the resin at 400 x g for 5 min.
   Note: This step is critical to ensure that all ethanol is removed from the resin to avoid interference with the SelfSeal membrane technology. E.g. HighSpec Agarose is provided as 50% suspension in buffer containing 20% ethanol.
2. Pre-equilibrate the Midi Plus spin column with 15 mL equilibration buffer by centrifuging the spin column at 400 x g for 5 min.
3. Repeat this step to remove any residual ethanol.

SAMPLE PREPARATION

4. Immediately before loading re-filter the sample through a 0.2 µm filter (e.g. syringe filter) to remove any solid material that might clog the column. Note: It is critical to perform this step immediately before loading the sample on the column to ensure optimal performance.

SAMPLE LOADING

5.Empty the 50 mL centrifuge tube and place the spin column barrel containing the equilibrated purification resin back into it. 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 tube on a standard tube roller or rotator and mix for 1-3 hours. After batch incubation, replace the yellow cap with the clear spin push cap. Centrifuge the column at 400 x g for up to 10 min and collect the flow- through. Note: Keep an aliquot of the flow-through fraction for subsequent SDS-PAGE analysis.

WASH

6. Load the spin column barrel with up to 20 mL of wash buffer and spin at 400 x g for 5 min. Remove the flow-through. Note: The flow-through contains the wash fractions. Keep aliquots of the individual wash fractions for subsequent SDS-PAGE analysis.
7. Repeat the wash step for at least two times to ensure removal of non-specifically bound protein. If applicable, check the samples for protein content using a UV-spectrophotometer. Absorbance at 280 nm should be < 0.1.

ELUTION

8. Elute the target protein 5 times by adding 5 x 1 ml elution buffer and centrifuging at 400 x g for 5 min. Save each elution fraction in a separate tube (e.g. 1.5 ml centrifuge tube) and determine the protein concentration of each fraction by measuring absorbance at 280 and 260 nm. Optional: Use a fresh 50 ml tube for the elution step to avoid contamination with the previous wash fractions.
9. We recommend to save small aliquots of the collected fractions at various steps and analyzing them by SDS-PAGE and Western Blot to assess the efficacy of the purification process.

Shipping & Storage

Shipment and Storage Temperature	Ambient temperature