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PureCube 1-step batch Mini Columns

Product	Catalog No.	Package size
PureCube 1-step batch Mini Columns (50)	**63104**	**50 columns**
PureCube 1-step batch Mini Columns (100)	**63107**	**100 columns**

Product Description

Designed for small-scale protein purification, the PureCube 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. PureCube 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 PureCube 1-step batch Mini Columns featuring the SelfSeal Technology. They can be used to purify proteins using 100 – 200 µL of affinity purification matrix of your choice in a bench-top mini centrifuge with a rotor suitable for 2 mL centrifuge tubes. Up to 600 µL 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 100 µL 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 2 mL tube adjusted with distilled water.

Note: Detailed protocols with information on recommended buffer volumes and compositions, incubation times and other useful information for a range of affinity purification resins are available at www.cube-biotech.com/protocols.

PRE-EQUILIBRATION

1. Pipet the appropriate resin slurry into the batch incubation chamber of the spin column barrel. Wash the resin at 12.000 – 14.000 x g for 20 sec.
   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. PureCube Agarose is provided as 50% suspension in buffer containing 20% ethanol.

2. Pre-equilibrate the Mini spin column with 600 µL equilibration buffer by centrifuging the spin column at 12.000 – 14.000 x g for 20 sec.

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 2.2 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 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.
6. Centrifuge the column at 12.000 – 14.000 x g for 20 sec and collect the flow-through. **Note:** Keep an aliquot of the flow-through fraction for subsequent SDS-PAGE analysis.

WASH

7. Load the spin column barrel with up to 600 µL of wash buffer and spin at 12.000 – 14.000 x g for 20 sec. Remove the flow-through. Note: The flow-through contains the wash fractions. Keep aliquots of the individual wash fractions for subsequent SDS-PAGE analysis.

8. Repeat the wash step for at least two times to ensure removal of unspecifically bound protein. If applicable, check the samples for protein content using a UV-spectrophotometer. Absorbance at 280 nm should be < 0.1.

ELUTION

9. Elute the target protein by adding 50-600 µl elution buffer and centrifuging at 12.000 – 14.000 x g for 20 sec. If necessary, repeat the elution step up to 5 times. Save each eluate 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 2.2 ml tube for the elution step to avoid contamination with the previous wash fractions.

10. 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

Additional Information

For more protein purification protocols, please visit our webpage at: www.cube-biotech.com/protocols.
For affinity purification of His-tagged, GST-tagged, rho-tagged or strep®-tagged proteins, Cube Biotech offers dedicated agarose resins, magnetic beads and prepacked cartridges. Also available are a range of ultrapure detergents and buffers for extraction and purification of membrane proteins.
See www.cube-biotech.com/products for details.