

Empty Midi Spin Columns for Affinity Chromatography

Product	Cat#	Package size
Empty Midi Spin Columns (20mL) for Affinity Chromatography	S5354.2008	8 columns
Empty Midi Spin Columns (20mL) for Affinity Chromatography	S5354.2048	48 columns

Product description

Empty Spin Columns for Affinity Chromatography are perfectly suited for batch purifications. They can be filled with your affinity resin of choice, and allow for binding, washing, and elution in one single spin column.

Designed for small to mid-scale protein purification, the Empty Spin Columns for Affinity Chromatography 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 tube assembled to the column.

The Genaxxon Spin Columns for Affinity Chromatography are available in two sizes: Mini (S5354.5050/S5354.5100 - 500µL) for expression trials, small-scale screening, and other small-volume purification needs; or Midi for volumes of up to 20mL (S5354.2008/S5354.2048).

Supplied material

This product comes as fully assembled 1-step batch Midi Spin Columns for Affinity Chromatography. They can be used to purify proteins using 0.25 – 1mL of affinity purification matrix of your choice in a bench-top centrifuge with a swing bucket rotor of handling 50mL centrifuge tubes.

Up to 20mL of lysate, wash or elution buffer can be loaded in each centrifugation step

Stability and Storage

Columns can be stored at room temperature. Kit contains no component that has to be stored below RT. If columns are stored in the sealed bag, shelf life is >2 years.

Disclaimer

The Genaxxon bioscience products are intended for molecular biology applications. These products are not intended for diagnostics, prevention, or treatment of a disease.

Related Products

Product	Cat#	Package sizes
Ni-IDA agarose	S5353	10mL, 50mL, 250mL
Ni-NTA agarose	S5377	10mL, 50mL, 250mL
Co-IDA agarose	S5370	10mL, 50mL, 250mL
Co-NTA agarose	S5356	10mL, 50mL, 250mL
Pre-packed Ni-IDA columns	S5353	5mL, 25mL
Pre-packed Co-IDA columns	S5372	5mL, 25mL

Protocol for Midi Spin Columns (20mL)

Note: The following spin speeds and times are appropriate for 0.25 – 1mL 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 50mL 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.

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 Genaxxon bioscience

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 at 400 x g for 5 minutes.

Note: This step is critical to ensure that all ethanol is removed from the resin to avoid interference with the SelfSeal™ membrane. Genaxxon Ni-NTA, for example is supplied as a 50% suspension in buffer containing 20% ethanol.

2. Pre-equilibrate the Midi Spin Column with 15mL equilibration buffer by centrifuging the spin column at 400 x g for 5 minutes.
3. Repeat this step to remove any residual ethanol.

Sample Preparation

4. Immediately before loading re-filter the sample through a 0.2µm 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 50mL 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 20mL. 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 minutes and collect the flow-through.

Wash

6. Load the spin column with up to 20mL 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 280nm should be <0.1!

Elution

8. Elute the target protein by adding 1mL elution buffer and centrifuging at 400 x g for 5 min. **Repeat the elution step 5 times!** Save each eluate fraction in a separate tube (e.g. 1.5mL centrifuge tube) and determine the protein concentration of each fraction by measuring absorbance at 280nm and 260nm.

Optional: Use a fresh 50mL tube for the elution step to avoid contamination with the previous wash fractions.

9. We recommend saving 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.