Version 221130
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Quality Control

The performance of the Spin Columns for Affinity Chromatography is monitored routinely on a lot-to-lot basis.

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

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Empty Spin Columns for Affinity Chromatography

Product	Cat#	Package size
Empty Spin Columns (500µL) for Affinity Chromatography	S5354.5050	50 columns
Empty Spin Columns (500µL) for Affinity Chromatography	S5354.5100	100 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 (500µL) for expression trials, small-scale screening, and other small-volume purification needs; or Midi Plus for volumes of up to 20mL.

Supplied material

The product comes as fully assembled Spin Columns for Affinity Chromatography inserted in 2.0mL receiver tube with lid. They can be used to purify proteins using 100-200µL of affinity purification matrix of your choice in a bench-top mini centrifuge with a rotor suitable for 2mL tubes.

Up to 600µL of lysate, wash or elution buffer can be loaded in each centrifugation step.

Protocol for Mini Spin Columns (500µL)

Note: The following spin speeds and times are appropriate for 100µL 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 2mL tube adjusted with distilled water**.**

Pre-Equilibration

1. Pipet the appropriate resin slurry into the spin column, which is placed into the receiver tube. 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. Genaxxon Ni-NTA, for example is supplied as a 50% suspension in buffer containing 20% ethanol.

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

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 2mL receiver tube and place the spin column containing the equilibrated purification resin back into it. Load the required volume of filtered sample. The maximum sample volume is 600µL. Close the lid and vortex for 15 seconds to mix the sample and the resin. Repeat the vortexing every 15 minutes for the first 1 hour.

   Note: In some circumstances, more than 1 hour batch incubation may be required. Repeat the vortexing every 30 minutes – 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 with up to 600µL wash buffer and spin at 12.000 x 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 280nm should be <0.1!

Elution

9. Elute the target protein by adding 50-600µL elution buffer and centrifuging at 12.000 x 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. 1.5mL centrifuge tube) and determine the protein concentration of each fraction by measuring absorbance at 280nm and 260nm.

   Optional: Use a fresh 2mL tube for the elution step to avoid contamination with the previous was 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.