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Reg. No. IC 03214 034

Jena Bioscience

Low Density Nickel Agarose
His-Spin Columns

Cat. No.	Amount
AC-304SPC-25	25 empty columns and 3.0 ml resin
AC-304SPC-50	50 empty columns and 5.5 ml resin

For *in vitro* use only
Quality guaranteed for 12 months
Store at 4°C. Do not freeze.

Description

His-spin columns provide a simple way for the purification of His-tagged proteins with no need for any special purification equipment. Pure proteins are obtained quickly and easily with either centrifuge or microcentrifuge tubes or with a syringe. All our chelating resins are available in this format.

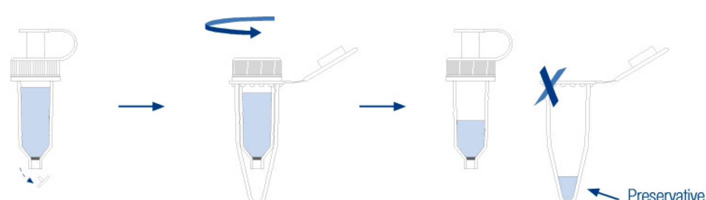
General Procedure for the Purification of His-tagged Proteins

The following procedure describes the purification process of a His-tagged protein under native conditions using a centrifuge. Please see the notes ate the end of this paragraph before working under denaturing conditions. The process is performed in an equivalent way when using a syringe.

1. Filling of the column and removal of the storage solution

Open the top cap of an empty column and fill it with 100 µl of suspended resin (shake slightly before pipetting).

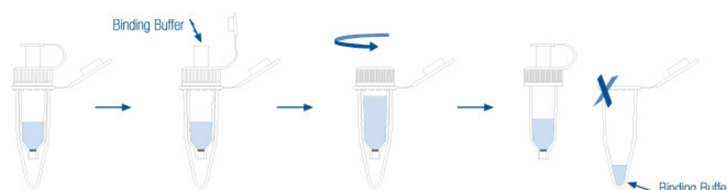
Remove the bottom cap of the spin column, place in a microtube and centrifuge, then discard the storage solution (preservative) collected in the tube.



Please note: In all centrifugation steps carried out in the procedure, normally a mild centrifugation (1,000 - 1,500 rpm) is sufficient.

2. Equilibration of the spin column

Introduce the spin column in a microcentrifuge tube and add binding buffer through the top. Centrifuge



and discard the obtained residue.

The typical binding buffer is 20 mM disodium phosphate, 500 mM NaCl, 10 mM imidazole at pH 7.5.

The choice of binding buffer depends on the particular properties of the protein as well as on the type of chelate used. The most frequently used buffers are acetate (50 mM) or phosphate (10 - 150 mM). The pH of binding buffers is usually close to neutral pH (7.0 - 8.0), but can vary over the range 5.5 - 8.5. To avoid ionic interchange, add 0.15 - 0.5 M of NaCl.

Please note: In some cases addition of imidazole (10 - 40 mM) to the binding buffer will increase the selectivity of protein binding. Usage of high purity imidazole will avoid affecting OD_{280} measurements. Avoid the presence of chelating agents such as EDTA or citrate during the complete purification process.

3. Application of the sample

Add the sample containing the His-tagged protein while keeping the lower cap in its place. Manually shake the spin column to maximize contact between the resin and the target-protein. Remove the bottom cap, introduce the spin column in a microcentrifuge tube and centrifuge (thus eliminating proteins not retained in the column).

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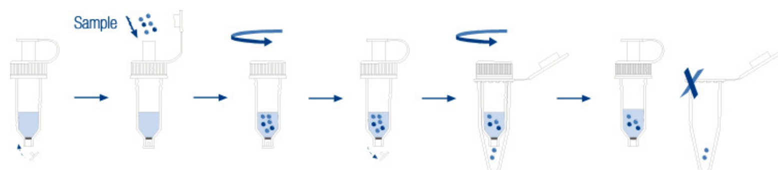
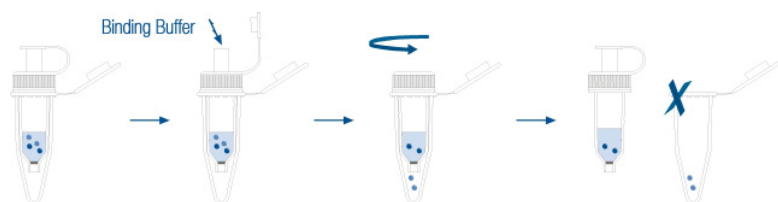


or with chelating agents such as EDTA or EGTA (0.05 M). However, these will also cause desorption of the metal from the resin.

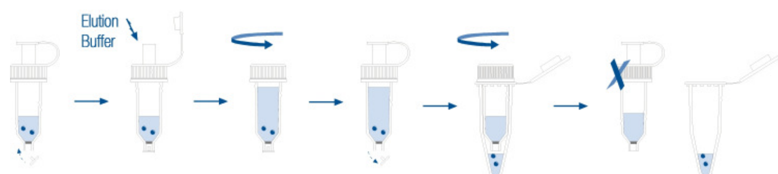
4. Washing of the spin column

Introduce the spin column in a microcentrifuge tube and add the binding buffer through the top. Centrifuge and discard the residue collected in the tube.



5. Elution of the pure protein

Add the elution buffer through the top of the column while keeping the bottom cap of the spin column in place. Manually shake to drive the elution of the target protein. Remove the bottom cap, introduce the spin column in a microcentrifuge tube and centrifuge, finally collecting the pure protein in the tube.



Please note:

- Elution buffer is usually 20 mM disodium phosphate, 500 mM NaCl, 500 mM imidazole at pH 7.5. This imidazole concentration is generally sufficient for elution of the target protein. If the desired result is not achieved, the concentration of imidazole can be increased up to 2.0 M.
- Other reagents that can be used to elute the protein are histidines and ammonium chloride. Elution may also be performed by decreasing the pH to 4.0 or 3.0, or with chelating agents such as EDTA or EGTA (0.05 M). However, these will also cause desorption of the metal from the resin.

Recombinant proteins often form insoluble inclusion bodies which have to be rendered soluble by a purification under denaturing conditions, e.g. by using 7 M urea or 6 M guanidine chloride during the purification process.

Selected References

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