# Data Sheet

IFTA AG
Certified QMS according to
DIN EN ISO 9001:2000-12
Reg. No. IC 03214 034

Jena Bioscience

# Low Density Nickel Agarose
## His-Columns

| Cat. No. | Amount |
|---|---|
| AC-304C | 8 columns (1 ml each) |
| AC-304C-5 | 5 XL columns (5 ml each) |

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

### Description

Pre-packed columns are ready-to-use for the purification of His-tagged proteins by gravity flow. By this method, target proteins can be purified rapidly and obtained in good yield. All our chelating resins are available in this format. The columns are suitable for use in both native or denaturing conditions.

### Form

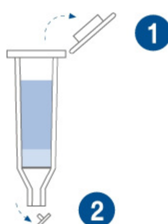
This product is supplied as a suspension in 20 % ethanol.

### General Procedure for the Purification of His-tagged Proteins

The following procedure describes the purification of His-tagged protein under native conditions.

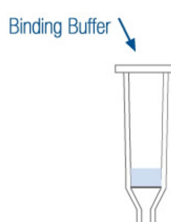
*1. Removal of the storage solution*

First, remove the upper and then the lower cap of the column, to allow removal of the storage solution by gravity flow.




*2. Equilibration of the pre-packed column*

Equilibrate the column with 5 - 10 column bed volumes of binding buffer. Add the binding buffer at the upper part of the column and make sure no air has been trapped. Mix manually inverting the pre-packed column.



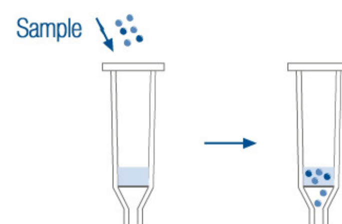

The typical binding buffer is 20 mM disodium phosphate, 500 mM NaCl, 10 mM imidazole at pH 7.5.

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*

Bring the bottom cap of the column back in place. Add the sample containing the His-tagged protein to be purified through the top of the column, keeping sample and resin in contact at least 15 minutes before removing the bottom cap.

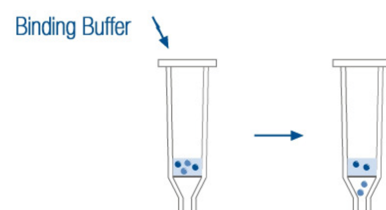



*Please note:*

- In some cases, a slight increase of contact time may facilitate binding.
- Pouring the sample down a glass rod held against the wall of the column will minimize the introduction of air bubbles.
- Binding capacity can be affected by several factors such as sample concentration, binding buffer and the flow rate during sample application.

*4. Washing of the pre-packed column*

Add the binding buffer through the top to eliminate all



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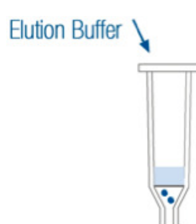
the proteins that have not been retained in the column. Mix manually inverting the pre-packed column. Wash the column with binding buffer until $OD_{280}$ of the eluent reaches baseline level.

*5. Elution of the pure protein*

Add the elution buffer to the column. Mix manually inverting the pre-packed column. It is recommended to keep elution buffer and resin in contact for at least 10 minutes before removing the bottom cap.




*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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