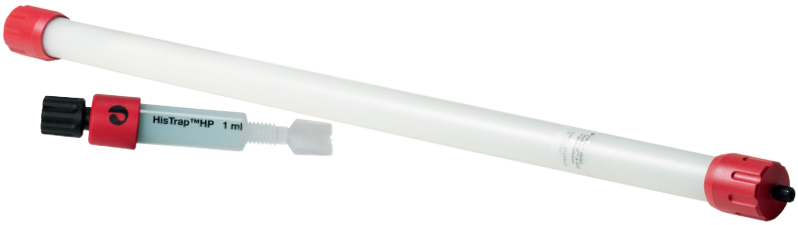
Tagged package, His (2-step protocol)

With our prepacked columns and verified protocols using ÄKTA™ chromatography systems, you can purify almost any his-tagged protein with ease.

Content of Tagged package, His (2-step protocol):

- HisTrap™ HP (1 × 1 mL)
- HiPrep™ 16/600 Sephacryl™ S-200 HR (1 × 120 mL)



HisTrap HP 1 mL and HiPrep 16/600 Sephacryl S-200 HR 120 mL columns.

Introduction

His-tags are widely used because they are small and rarely interfere with the function, activity, or structure of target proteins.

All his-tagged protein purification protocols typically start with an affinity chromatography (IMAC = immobilized metal ion affinity chromatography) step. It enables the isolation of the target his-tagged protein from initial sample (e.g., lysate, cell culture).

After the first affinity chromatography step is run on an IMAC column, the purity level is usually moderate (> 80%).

For applications such as functional studies, a moderate purity level is not sufficient. This is why a size exclusion chromatography (SEC)[1] step should be added to remove remaining impurities. The purity level after a 2-step purification protocol will be very high (95% to 99%).

This kit contains suitable columns to perform a successful 2-step purification of his-tagged proteins.

How to use the kit

The following workflow describes the steps involved in purifying his-tagged proteins.

1. Sample preparation

   First, you need to prepare your sample for the affinity purification step. For optimal growth, induction, and cell lysis conditions for your his-tagged clones, please refer to established protocols. Note that it is important to include the same concentration of imidazole in your sample as well as in the binding buffer for affinity purification.

2. Affinity purification

   Your kit includes a HisTrap HP column prepacked with Ni Sepharose™ High Performance (HP) resin, which is an affinity chromatography resin for preparative purification of his-tagged proteins. Ni Sepharose HP affinity resin consists of highly cross-linked agarose beads to which a chelating group has been coupled. This chelating group is precharged with nickel, which selectively retains proteins with exposed histidine groups.

   This IMAC resin has a binding capacity that exceeds 40 mg/mL, resulting in high protein recovery.

   Recommended buffers:

- Binding buffer: 20 mM sodium phosphate, 500 mM NaCl, **20 to 40 mM imidazole,** pH 7.4 (the optimal imidazole concentration is protein-dependent; 20 to 40 mM is suitable for many proteins).
- Elution buffer: 20 mM sodium phosphate, 500 mM NaCl, 500 mM imidazole, pH 7.4.

3. Polishing

   To achieve final purity, the HiPrep 16/60 Sephacryl S-200 HR size exclusion chromatography column included in this kit will help you remove any remaining trace impurities or closely related substances. Imidazole, which might affect subsequent analysis, will be removed at the same time.

   The column is packed with Sephacryl S-200 HR chromatography resin, which has a broad fractionation range of M_r 5 000 to ~ 250 000 for globular proteins and allows loading of sample volumes of up to 5 mL.

[1] *Also called gel filtration*

Recommended buffer:

50 mM sodium phosphate, 150 mM NaCl, pH 7.2 or select the buffer in which the sample should be solubilized for the next step. To avoid pH-dependent, nonionic interactions with the matrix, include at least 150 mM NaCl in the buffer (or use buffer with equivalent ionic strength).

Detailed information on how to use the columns and purification protocols can be found in the instructions for the respective columns. Download the instructions at cytiva.com/instructions

4. Analysis

   Depending on your goal, several analytical methods can be used. SDS-PAGE may be used for purity analysis, Western blotting for detection of target molecules, X-ray crystallography for structural determination, and mass spectrometry for identification.

About the columns

To ensure that the columns do not interact with biomolecules, we manufacture them from biocompatible polypropylene. HiTrap columns can be used individually or connected in series for easy scale-up, together with a syringe, peristaltic pump, or chromatography system. HiTrap and HiPrep columns are well-suited for use with ÄKTA chromatography systems.

Ordering information

Product	Quantity	Product code
Tagged package, His (2-step protocol)	1	29497630
HisTrap HP	1 × 1 mL	29051021
HiPrep 16/60 Sephacryl S-200 HR	1 × 120 mL	17116601

Related literature

The following handbooks can be downloaded at cytiva.com/handbooks

Affinity Chromatography Vol. 2: Tagged Proteins
Size Exclusion Chromatography - Principles and Methods

cytiva.com/purify

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