Untagged package

With our prepacked columns and verified protocols using ÄKTA™ chromatography systems, you can purify almost any untagged or native protein with ease.

Contents of Untagged package:

- HiTrap™ Capto™ Q ImpRes (1 × 1 mL)
- HiTrap Capto SP ImpRes (1 × 1 mL)
- HiPrep™ 16/60 Sephacryl™ S-200 HR (1 × 120 mL)

HiTrap Capto Q ImpRes 1 mL, HiTrap Capto SP ImpRes 1 mL, and HiPrep 16/60 Sephacryl S-200 HR, 120 mL columns.

Introduction

Untagged recombinant and native proteins usually require a multistep purification protocol to obtain sufficient purity. These proteins may come from natural sources or have been over-expressed without a tag because the presence of a tag would interfere with the use of the protein.

Untagged proteins can usually be sufficiently purified by combining purification methods that separate on the basis of the different physicochemical characteristics of the proteins (orthogonal methods).

This purification protocol is a good starting point for most applications. It utilizes a first ion exchange chromatography (IEX) step, which is followed by a size exclusion chromatography (SEC)[1] step. The end purity will be > 80%, and the yield will be high.

[1] *Also called gel filtration*

How to use the kit

The following workflow describes the steps involved in purifying untagged proteins.

1. Sample preparation

   First, you need to prepare your sample for the first capture step. For optimal growth, induction, and cell lysis conditions for your untagged or native clones, please refer to established protocols.

   Note that it is important to have the same conditions (salt concentration, pH etc.) in the sample as well as in the binding buffer for the capture step.

2. Capture

   The capture step is intended to isolate, concentrate, and stabilize your target protein. HiTrap Capto Q ImpRes (anion exchanger) or HiTrap Capto SP ImpRes (cation exchanger) may be used for this. Capto Q ImpRes and Capto SP ImpRes ion exchangers are based on a high-flow agarose base matrix with good pressure/flow properties and a small bead size (approx. 40 µm), which gives high resolution.

   The pH of the binding buffer should be at least 0.5–1 pH unit above the pI of the target molecule when using an anion exchanger and at least 0.5–1 pH unit below the pI when using a cation exchanger.

   Recommended buffers to start with:

   Anion exchange (HiTrap Capto Q ImpRes)

   Binding buffer: 20 mM Tris-HCl, pH 8.0
   Elution buffer: 20 mM Tris-HCl, 1 M NaCl, pH 8.0

   Cation exchange (HiTrap Capto SP ImpRes)

   Binding buffer: 50 mM sodium acetate, pH 5.0
   Elution buffer: 50 mM sodium acetate, 1 M NaCl, pH 5.0

3. Intermediate purification (optional)

   The intermediate purification step can be added for removal of potential bulk impurities such as other proteins, endotoxins, and viruses. Use HiTrap Capto Q ImpRes or HiTrap Capto SP ImpRes if they were not used in the capture step. . Use the recommended buffers listed under Capture.

4. Polishing

   To achieve final purity, HiPrep 16/60 Sephacryl S-200 HR SEC column will help you remove any remaining trace impurities or closely related substances. The column is packed with Sephacryl S-200 HR chromatography resin, which has a broad fractionation range of M_r 5000 to ~ 250 000 for globular proteins and allows loading of sample volumes of up to 5 mL.

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 structure 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
Untagged package	1	29497631
HiTrap Capto Q ImpRes	1 × 1 mL	29400460
HiTrap Capto SP ImpRes	1 × 1 mL	29400462
HiPrep 16/60 Sephacryl S-200 HR	1 × 120 mL	17116601

Related literature

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

Ion Exchange Chromatography - Principles and Methods
Size Exclusion Chromatography - Principles and Methods

cytiva.com/purify

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