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# Antibody package, modern Protein A (2-step protocol)

With our prepacked columns and verified protocols using ÄKTA™ chromatography systems, you can purify almost any human monoclonal and polyclonal antibodies with ease.

Contents of Antibody package, modern Protein A (2-step protocol):

- HiTrap™ MabSelect™ PrismA (1 × 1 mL)
- HiPrep™ 16/60 Sephacryl™ S-300 HR (1 × 120 mL)



HiTrap MabSelect PrismA 1 mL and HiPrep 16/60 Sephacryl S-300 HR 120 mL columns.

## Introduction

The basis for antibody affinity chromatography is the high affinity and specificity of protein A for the Fc-region of IgG of humans. Protein A immobilized on chromatography resins is highly effective in isolating subclasses of IgG from cell culture supernatants and serum. Protein A is widely used for the routine purification of human antibodies. Antibodies from other species, for example rabbit, can also be purified with Protein A.

All antibody purification protocols typically start with an affinity chromatography step (AC). After the AC step, the purity level is usually high (> 90%). However, if antibody aggregates and/or fragments need to be removed to obtain monomeric antibodies, then a size exclusion chromatography (SEC)<sup>1</sup> step is recommended. The purity level after a 2-step purification will be very high (95% to 99%).

This kit contains columns suitable for a successful 2-step protocol of human antibodies. The 1- and 2-step protocols are recommended for research use.

## How to use the kit

The following workflow describes the steps involved in purifying human antibodies.

### 1. Sample preparation

First, you need to prepare your sample for the affinity purification step. Depending on the source of your human antibody (e.g., serum or cell culture supernatant), different preparation steps can be used, please refer to established protocols.

### 2. Affinity purification

Your kit includes a HiTrap MabSelect Prisma column prepacked with MabSelect Prisma resin for preparative purification of monoclonal and polyclonal antibodies from serum and cell culture supernatant. The protein A-derived ligand on MabSelect Prisma is produced in *Escherichia coli*. Fermentation and subsequent purification are performed in the absence of animal products. The ligand has been specially engineered to create an affinity resin with enhanced alkali and protease stability. The specificity of binding to the Fc region of IgG is similar to that of conventional Protein A and provides excellent purification in one step.

The following buffers are recommended:

- Binding buffer: 20 mM sodium phosphate, 150 mM NaCl, pH 7.2
- Elution buffer: 100 mM sodium citrate, pH 3.0 to 3.63

### 3. Polishing

To achieve final purity, the HiPrep 16/60 Sephacryl S-300 HR size exclusion column included in the kit will help you remove antibody aggregates and/or fragments. The column is packed with Sephacryl S-300 HR chromatography resin, which has a broad fractionation range of  $M_r$  10 000 to 1 500 000 for globular proteins, is well-suited for antibody purification and allows loading of sample volumes of up to 5 mL.

<sup>1</sup> 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](https://www.cytiva.com/instructions)

#### 4. Analysis

Depending on your purpose, 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 produce 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
Antibody package, modern Protein A (2-step protocol)	1	29497629
HiTrap MabSelect Prisma	1 × 1 mL	17549851
HiPrep 16/60 Sephacryl S-300 HR	1 × 120 mL	17116701

## Related literature

The following handbooks can be downloaded at [cytiva.com/handbooks](https://www.cytiva.com/handbooks)

Affinity Chromatography Vol. 1: Antibodies

Size Exclusion Chromatography - Principles and Methods

## **cytiva.com/purify**

For local office contact information, visit [cytiva.com/contact](https://cytiva.com/contact)

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