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HiTrap rProtein A FF column, 1 mL and 5 mL

Catalog Number: HP001, HP002, HP003, HP004

Table 1. Package and Storage

Cat No.	Material	Unit Size	Storage	Stability
HP001	HiTrap rProtein A FF column	1 × 1 mL	2-8 °C 20% ethanol	The product is stable for at least 24 months when stored as directed.
HP002	HiTrap rProtein A FF column	1 × 5 mL		
HP003	HiTrap rProtein A FF column	5 × 1 mL		
HP004	HiTrap rProtein A FF column	5 × 5 mL		

Product Description

HiTrap rProtein A FF columns are prepacked with cytiva rProtein A Sepharose™ Fast Flow resin for purification and fractionation of IgG subclasses and IgG from different sources, including cell supernatants and ascites.

The recombinant protein A ligand (produced in E. coli) is coupled to Sepharose™ Fast Flow resin by a technique which generates a stable thioether linkage between rProtein A and the base matrix. The coupling technique is optimized to give high binding capacity for IgG.

The special design of the column, together with the matrix, provide fast, simple and easy separations in a convenient format. The column can be operated with a syringe, peristaltic pump or liquid chromatography system such as ÄKTA™.

HiTrap Column Characteristics

The columns are made of biocompatible polypropylene that does not interact with biomolecules.

The columns are delivered with a stopper at the inlet and a snap-off end at the outlet. Table 1 lists the characteristics of HiTrap columns.

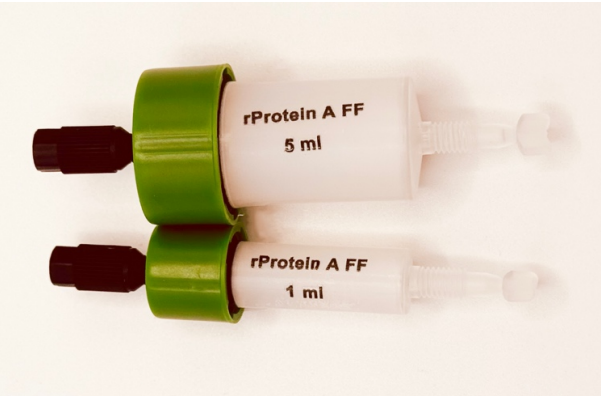

Table 1. Characteristics of HiTrap columns.

Column volume (CV)	1 mL	5 mL
Column dimensions	0.7 × 2.5 cm	1.6 × 2.5 cm
Column pressure limit	5 bar (0.5 MPa)	5 bar (0.5 MPa)

Resin Properties

Cytiva rProtein A Sepharose™ Fast Flow resin is designed for purification and isolation of monoclonal antibodies from ascites and cell culture supernatants. The ligand has been specially engineered to give very high binding capacities.

The characteristics of the products are summarized in Table 2.

Table 2. HiTrap rProtein A FF characteristics

Matrix	cross-linked agarose, 4%, spherical
Particle size	~ 90 µm
Ligand	Recombinant protein A, (E. coli)
Dynamic binding capacity	~ 35 mg human IgG/mL resin
Dynamic binding capacity	~ 23 mg mouse monoclonal IgG_{2a}/mL resin ~ 12 mg mouse monoclonal IgG_1/mL resin ~ 11 mg monoclonal humanized IgG_4/mL resin
Maximum flow rate	1 mL column: 4 mL/min 5 mL column: 20 mL/min
Recommended flow rate	1 mL column: 0.5 mL/min 5 mL column: 2.5 mL/min
Chemical stability	Stable to commonly used aqueous buffers, 6 M guanidine hydrochloride, 2% benzyl alcohol, 1 mM NaOH (pH 11), 0.1 M sodium citrate/HCl (pH 3), 20% ethanol
pH stability, operational pH stability, CIP	3 to 10 3 to 12
Temperature stability	2°C to 40°C
Storage	20% ethanol, 2°C to 8°C

The degree to which protein A binds to IgG varies with respect to both origin and antibody subclass and may even vary substantially within a single subclass, see Tables 3. The binding capacity of protein A for IgG depends on the source species of the particular immunoglobulin. The total capacity depends also upon several other factors such as the flow rate during sample application, and the sample concentration. This resin has a binding capacity for human IgG of approximately 35 mg IgG/mL resin.

The ligand rProtein A is coupled to cross-linked 4% agarose beads by a technique which generates a stable thioether linkage between rProtein A and the base matrix. The coupling technique is optimized to give a high binding capacity for IgG.

Table 3. Affinity of protein A for selected classes of monoclonal antibodies.

Antibody	Affinity	Binding pH	Elution pH
Human			
IgG_1	very high	6.0-7.0	3.5-4.5
IgG_2	very high	6.0-7.0	3.5-4.5
IgG_3	low-none	8.0-9.0	<7.0
IgG_4	low-high	7.0-8.0	3.0-6.0
Mouse			
IgG_1	low	8.0-9.0	4.5-6.0
IgG_{2a}	moderate	7.0-8.0	3.5-5.5
IgG_{2b}	high	~7.0	3.0-4.0
IgG_3	low-high	~7.0	3.5-5.5

Operation

Protein A binds IgG over a wide pH range, and thus permits the use of a wide variety of buffers, depending on the applications. Elution is often achieved by a decrease in pH. Different subclasses of IgG elute at different pH values depending on the species from which they originate.

Buffer preparation

Water and chemicals used for buffer preparation must be of high purity. It is recommended to filter the buffers by passing them through a 0.45 µm filter before use.

Recommended buffers

Binding buffer: 20 mM sodium phosphate, pH 7.0

Elution buffer: 0.1 M sodium citrate, pH 3 to 6

With some antibodies, e.g., mouse IgG_1, it might be necessary to add sodium chloride up to 4 M in the binding buffer, to achieve efficient binding.

High salt binding buffer: 1.5M glycine, 3M NaCl, pH 8.9 or 20mM sodium phosphate, 3 M NaCl, pH 7.0

Elution buffer: 0.1 M sodium citrate, pH 3 to 6

As a safety measure to preserve the activity of acid labile IgG when using very acidic elution conditions, we recommend adding 60 to 200 µL of 1 M Tris-HCl, pH 9.0 per mL of eluted fraction to becollected, so that the final pH of the sample will be approximately neutral.

Sample preparation

The sample should be adjusted to the composition of the binding buffer. This can be done by either diluting the sample with binding buffer or by buffer exchange using Desalting columns. The sample should be filtered through a 0.45 µm filter or centrifuged immediately before it is applied to the column. Never apply a turbid solution to the column. (This is especially important to prevent clogging of column when loading large volumes of serum or plasma).

Purification

We recommend to use a flow rate of 0.5 mL/min for HiTrap rProtein A FF 1 mL column and 2.5 mL/min for HiTrap rProtein A FF 5 mL column.

1. Prepare collection tubes by adding 60 to 200 µL of 1 M Tris-HCl, pH 9.0 per mL of fraction to be collected.
2. Remove the stopper from the inlet and the snap-off end at the column outlet.
3. Connect the column to the system with 1/16" male connectors.

   Note: Make a drop-to-drop connection to prevent air from entering the column. Make sure that the connections are tight to prevent leakage.
4. Wash out the ethanol preservative with at least 5 column volumes of distilled water or binding buffer.
5. Regenerate the column with 5 column volumes of elution buffer.
6. Equilibrate the column with 5 to 10 column volumes of binding buffer.
7. Apply the sample, using a syringe fitted to the luer connector or by pumping it onto the column.
8. Wash with 5 to 10 column volumes of binding buffer or until no material appears in the effluent. Excessive washing should be avoided if the interaction between the protein of interest and the ligand is weak, since this might decrease the yield.
9. Elute with elution buffer 2 to 5 column volumes is usually sufficient, but other volumes (or different elution buffer) will be required if the interaction is difficult to break.
10. The purified IgG fractions can be buffer exchanged using Desalting columns if necessary.

    Note: The reuse of HiTrap rProtein A FF depends on the nature of the sample and should only be performed with identical monoclonals to prevent cross-contamination.

Storage

Before storage, we recommend to wash the column with 5 column volumes of 20% ethanol to prevent microbial growth. Seal the column with the supplied stoppers. Store the HiTrap rProtein A FF column at 2°C to 8°C.