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HiTrap rProtein G FF column, 1 mL and 5 mL

Catalog Number: HP005, HP006, HP007, HP008

Table 1. Package and Storage

Cat No.	Material	Unit Size	Storage	Stability
HP005	HiTrap rProtein G FF column	1 × 1 mL	2-8 °C 20% ethanol	The product is stable for at least 24 months when stored as directed.
HP006	HiTrap rProtein G FF column	1 × 5 mL		
HP007	HiTrap rProtein G FF column	5 × 1 mL		
HP008	HiTrap rProtein G FF column	5 × 5 mL		

Product Description

HiTrap rProtein G FF columns are prepacked with cytiva rProtein G Sepharose™ Fast Flow resin for purification of monoclonal and polyclonal antibodies from most species including rat.

The recombinant protein G ligand (produced in E. coli) is coupled to Sepharose™ Fast Flow resin by the CNBr method. 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.

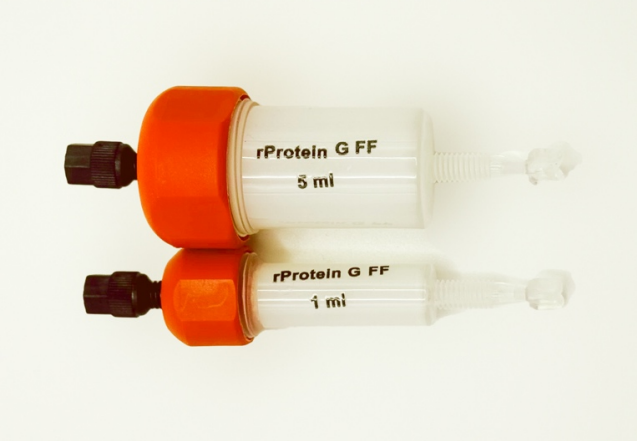

Table 1. Characteristics of HiTrap columns.

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 G Sepharose™ Fast Flow resin is designed for purification and isolation of monoclonal and polyclonal IgG from ascites, serum 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 G FF characteristics

Matrix	cross-linked agarose, 4%, spherical
Particle size	~ 90 µm
Ligand	Recombinant protein G, (E. coli), Mr ~17000
Degree of substitution	~ 2 mg Protein G/mL resin
Binding capacity	≥20 mg Human IgG/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(pH 4.7), 6 M urea, 20 mM sodium phosphate with 1% SDS (pH 7), 70% ethanol, 100 mM glycine-phosphoric acid
pH stability, operational pH stability, CIP	3 to 9 2 to 10
Temperature stability	2°C to 40°C
Storage	20% ethanol, 2°C to 8°C

Protein G, a cell surface protein of Group G streptococci, is a Type III Fc receptor that binds to the Fc region of IgG by a non-immune mechanism similar to that of protein A of Staphylococcus aureus. Protein G and protein A, however, have different IgG binding specificities, dependent on the origin of the IgG. Compared with protein A, protein G binds more strongly to polyclonal IgG, for example, from cow, sheep and horse. Furthermore, unlike protein A, protein G binds polyclonal rat IgG, human IgG3 and mouse IgG1 (Table 3).

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Table 3. Relative binding strengths for protein A and protein G.

Species	Subclass	Protein A Binding	Protein G Binding
Human	IgA	variable	-
	IgD	-	-
	IgE	-	-
	IgG_1	++++	++++
	IgG_2	++++	++++
	IgG_3	-	++++
	IgG_4	++++	++++
Cow		++	++++
Dog		++	+
Goat		-	++
Guinea pig	IgG_1	++++	++
	IgG_2	++++	++
Horse		++	++++
Monkey		++++	++++
Mouse	IgG_1	+	++++
	IgG_{2a}	++++	++++
	IgG_{2b}	+++	+++
	IgG_3	++	+++
	IgM	variable	-
Pig		+++	+++
Rabbit		++++	+++
Rat	IgG_1	-	+
	IgG_{2a}	-	++++
	IgG_{2b}	-	++
	IgG_3	+	++
Sheep		+/-	++

++++ = strong binding, ++ = medium binding, - = weak or no binding

Operation

Protein G 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 glycine-HCl, pH 2.7

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 be collected, 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 G FF 1 mL column and 2.5 mL/min for HiTrap rProtein G 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 10 column volumes of binding buffer.
5. Apply the sample, using a syringe fitted to the luer connector or by pumping it onto the column.
6. Wash with 5 to 10 column volumes of binding buffer or until no material appears in the effluent.
7. 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.
8. The purified IgG fractions can be buffer exchanged using Desalting columns if necessary.

   Note: The reuse of HiTrap rProtein G 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 G FF column at 2°C to 8°C.