rmp Protein A **Sepharose** Fast Flow

AFFINITY CHROMATOGRAPHY

rmp Protein A Sepharose™ Fast Flow is a low-leakage, animal component-free affinity chromatography resin designed for high-purity separation of monoclonal and polyclonal antibodies in laboratory and process scale (Fig 1).

Key features of rmp Protein A Sepharose Fast Flow:

- Very low ligand leakage for high purity of the target antibody
- Animal component-free production facilitates regulatory filing
- Increased chemical stability due to multipoint ligand attachment
- High throughput due to high capacity and good pressure/flow characteristics

Parallel screening in a miniaturized format allows for higher throughput, better process understanding, and low sample consumption. The availability of PreDictor™ 96-well filter plates, prefilled with BioProcess™ chromatography resins, and Assist software for experimental setup and data evaluation enables high-throughput screening of downstream purification process conditions, while keeping sample consumption at a minimum.

Fig 1. rmp Protein A Sepharose Fast Flow is chemically stable and exhibits high capacity and good pressure/flow characteristics.

Characteristics

The recombinant rmp Protein A is designed for therapeutic applications that require extremely pure eluate fractions of antibodies, and where it is vital that the antibodies are not exposed to human or animal derivatives during the purification process. rmp Protein A has a molecular weight of 44 600 and contains five antigen binding domains (E, D, A, B, C), which allow multiple attachment points to the Sepharose Fast Flow base matrix. Sepharose 4 Fast Flow base matrix is a highly cross-linked, 4% agarose derivative with high chemical and physical stability, which makes it well-suited for process-scale applications.

A single rmp Protein A molecule can bind in average two antibody molecules, for example IgG. The dynamic binding capacity of chromatographic adsorbents is a function of the flow rate used and increases with decreasing flow rate. The basic characteristics are summarized in Table 1.

Table 1. Characteristics of rmp Protein A Sepharose Fast Flow

Matrix	Cross-linked agarose, 4%, spherical
Particle size, d_{50v}[1]	~ 90 µm
Ligand	Recombinant protein A (*E. coli*)
Coupling chemistry	Reductive amination
Dynamic bindng capacity Q_{B10}[2]	≥ 25 mg hIgG/mL resin
Pressure/flow characteristics	150–250 cm/h at < 0.1 MPa in a XK 50/60 column with 5 cm diameter and 25 cm bed height (at 20°C using buffers with the same viscosity as water)[3,4]
Chemical stability	Stable to commonly used aqueous buffers, 20% ethanol, 20 mM Sodium phosphate, 0.1 M Glycine/HCl pH 3.5, 0.1 M Glycine/HCl pH 2.5
pH stability, operational[5] pH stability, CIP[7]	3 to 10[6] 3 to 12[6]
Temperature stability	2°C to 40°C
Delivery conditions	20% ethanol
Storage	20% ethanol, 2°C to 8°C

[1] Median particle size of the cumulative volume distribution.
[2] Dynamic binding capacity at 10% breakthrough by frontal analysis at a mobile phase velocity of 100 cm/h in a PEEK 7.5/50 column at 5 cm bed height (3 min residence time) for human IgG in 0.020 M NaH_2PO_4, pH 7.
[3] The pressure/flow characteristics describes the relationship between pressure and flow under the set circumstances. The pressure given shall not be taken as the maximum pressure of the resin.
[4] Pressure/flow test performed on the base matrix.
[5] pH range where resin can be operated without significant change in function.
[6] pH below 3 is sometimes required to elute strongly bound IgG species. However, protein ligands may hydrolyze at pH below 2.
[7] pH range where resin can be subjected to cleaning- or sanitization-in-place without significant change in function.

Animal component-free production for facilitated regulatory filing

Recombinant Protein A is expressed in *E. coli*, fermented in animal component-free soy medium and highly purified through multiple steps of ion exchange. This manufacturing process is entirely free from contact with human and animal products, minimizing the risk of impurities of mammalian origin, such as viruses and prions, in the eluate.

Low ligand leakage for high antibody purity

rmp Protein A is coupled to Sepharose Fast Flow resin at several attachment points through reductive amination. Reductive amination creates chemically stable amide bonds, resulting in little detachment of the ligand from the support during cleaning and product elution (Table 2). In addition, reductive amination improves flow properties. Low ligand leakage increases purity of the eluate fraction, which improves the efficiency of subsequent downstream purification steps.

Table 2. Protein A leakage to the eluate fraction measured after each of five chromatographic cycles*

Cycle	ng released protein A/mL eluate	ng released protein A/mg purified IgG (ppm)
1	6.8	2.6
2	6.2	2.6
3	5.9	2.5
4	5.6	2.5
5	5.4	2.3

* Column size: HR 5/5; Sample: human IgG, 1.0 mg/mL; equilibration buffer: PBS, pH 7.5; elution buffer: 0.1 M glycin/HCl, pH 3.5; regeneration buffer: 0.1 M glycin/HCl, pH 2.5; flow velocity = 150 cm/h; eluate volume = 4 mL/cycle; recovered IgG = 10 mg/cycle

Multipoint ligand attachment to the support allows extremely stringent cleaning procedures

Tightly bound impurities from the crude sample feed can only be effectively removed by a stringent cleaning protocol. The multiple attachment points and strong reductive amide bonds between the ligand and the support in rmp Protein A Sepharose Fast Flow create a high chemical stability that can withstand very stringent washing conditions. Even with repeated antibody purification cycles containing cleaning and sanitization protocols, the resin retains a high breakthrough capacity, leading to a long lifetime of the resin (Fig 2).

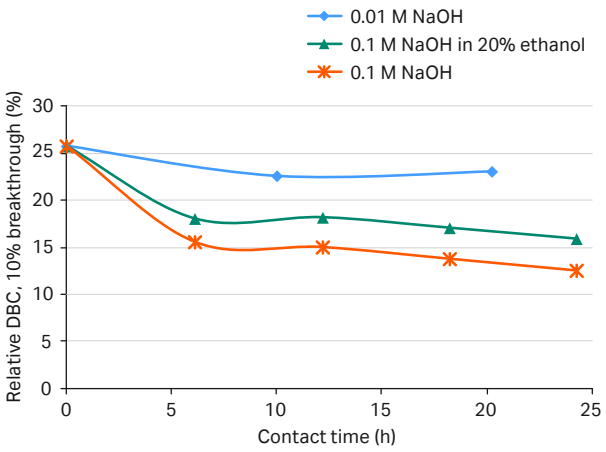

Fig 2. Cleaning and sanitization in place study using different concentrations of NaOH. rmp Protein A Sepharose Fast Flow was treated with the respective cleaning agent for two hours, and then washed and equilibrated before the CIP procedure was repeated. The dynamic binding capacity (DBC) for hIgG at 10% breakthrough (QB10) was checked every 6 hours after treatment with 0.1 M NaOH in 20% ethanol or 0.1 M NaOH and every 10 hours when treated with 0.01 M NaOH. Column: HR 10/10, bed height 7 cm, flow velocity equilibration = 140 cm/h, flow velocity CIP = 50 cm/h.

High binding capacity and flow properties enables high productivity

There is an increasing demand for cost-effective purification steps within biopharmaceutical manufacturing processes, which requires the development of high productivity chromatography adsorbents. rmp Protein A Sepharose Fast Flow offers high dynamic binding capacity and high flow properties, contributing to an enhanced total productivity (Fig 3).

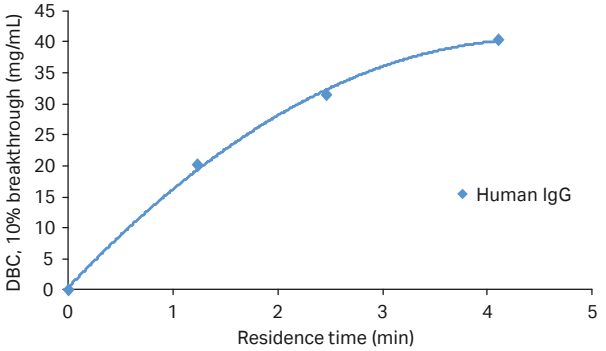

Fig 3. Example of flow velocity/dynamic binding capacity (DBC) dependence for rmp Protein A Sepharose Fast Flow. Dynamic binding capacity at 10% breakthrough for hIgG was determined at three different flow velocities. Breakthrough capacity is defined as mg hIgG applied per mL resin at the point where the concentration of hIgG in the column effluent reaches a certain % of the concentration in the sample. The IgG sample was loaded at 5 mg/mL in PBS (pH 7.4). Column i.d. 5 mm, bed height 4.1 cm.

Operation

Method development

As for most affinity chromatography resins, rmp Protein A Sepharose Fast Flow offers high selectivity, which makes efficiency-related parameters such as sample load, flow rate, bead size, and bed height less important for resolution. The primary aim of method optimization is to establish the conditions that will bind the highest amount of target molecule, in the shortest time, and with the highest product recovery. The degree to which IgG binds to protein A varies with respect to both origin and antibody subclass, and even within the same subclass, which is an important consideration when developing the purification protocol. Typical binding conditions are low salt concentration and neutral pH. However, to achieve efficient capture of weakly bound antibodies, it is often necessary to increase the pH and/or salt concentration in the binding buffer. Elution is normally achieved at reduced pH, down to pH 3.5 depending on antibody subclass.

Cleaning and sanitization

The general recommendation for cleaning rmp Protein A Sepharose Fast Flow is to use sodium hydroxide at a concentration of 10–50 mM. As an alternative cleaning protocol, 6 M guanidine hydrochloride can be used. To remove hydrophobically bound substances, a solution of nonionic detergent or ethanol is recommended. For sanitization of rmp Protein A Sepharose Fast Flow, we recommend treatment with a solution containing 0.1 M acetic acid/20% ethanol or 2% hibitane digluconate/20% ethanol. Detailed recommendations for method design and optimization, cleaning-sanitization, and column packing for rmp Protein A Sepharose Fast Flow can be found in the instructions enclosed with each pack of resin.

Scaling up

After optimizing the antibody purification in laboratory scale, the process can be scaled up by keeping the linear flow rate and sample-to-bed volume ratio constant, and by increasing the column diameter. Typical bed height range between 5 to 15 cm, allowing high flow rates to be used. Pressure/flow velocity curve for a 50 mm i.d. column is shown in Figure 4.

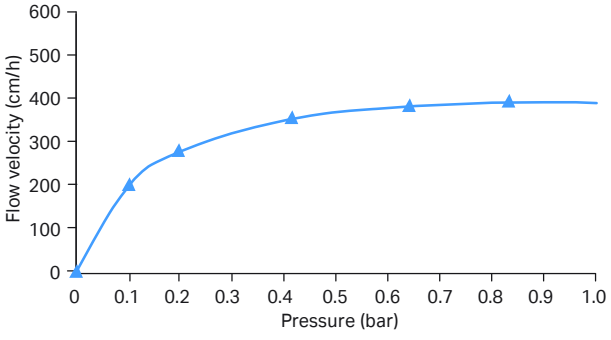

Fig 4. Pressure/flow characteristics for rmp Protein A Sepharose Fast Flow. The results were obtained in a XK 50/30 (50 mm i.d.) column packed to a bed height of 15 cm using 0.1 M NaCl as the mobile phase at 20°C.

Equipment

rmp Protein A Sepharose Fast Flow can be used together with most equipment available for chromatography, from laboratory to process scale. Recommended columns from Cytiva are listed in Table 3.

Table 3. Recommended columns for rmp Protein A Sepharose Fast Flow

Columns	Inner diameter (mm)	Bed volume	Bed height (cm)
Lab scale			
XK 16/40	16	8–74 mL	max. 35
XK 26/40	26	32–196 mL	max. 35
Production scale			
AxiChrom™ column, variable bed height	50–1000	Up to 393 L	max. 50
BPG glass column, variable bed height	100–446	2.4–131 L	max. 83

Ordering information

Product	Pack size	Code number
rmp Protein A Sepharose Fast Flow	5 mL	17-5138-01
rmp Protein A Sepharose Fast Flow	25 mL	17-5138-02
rmp Protein A Sepharose Fast Flow	200 mL	17-5138-03
rmp Protein A Sepharose Fast Flow	1 L	17-5138-04
rmp Protein A Sepharose Fast Flow	5 L	17-5138-05

Bulk resins products are supplied in suspension in 20% ethanol.

cytiva.com/bioprocess

For local office contact information, visit cytiva.com/contact

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