rmp Protein A Sepharose Fast Flow

rmp Protein A Sepharose™ Fast Flow is a low leakage, non-mammalian based affinity medium designed for high purity separation of monoclonal and polyclonal antibodies at the laboratory and process scales. It has the following characteristics:

- Few regulatory concerns as a result of the very low ligand leakage and the absence of mammalian culture in ligand production and purification
- Increased chemical stability due to multipoint ligand attachment
- High throughput due to high capacity and good pressure/flow characteristics

Characteristics

The recombinant protein rmp Protein A has been 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. The 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 support. The base matrix, Sepharose 4 Fast Flow, is a highly cross-linked, 4 % agarose derivative with high chemical and physical stability, which makes it ideal for process scale applications. A single rmp Protein A molecule can bind on average two antibody molecules, for example IgG. The dynamic capacity of chromatographic adsorbents is a function of the flow rate used and it increases with decreasing flow rate. The basic characteristics are summarised in Table 1.

Non-mammalian based medium production eliminates mammalian cell culture related risks

Recombinant Protein A is expressed in *E. coli*, fermented in animal-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. This removes the risk of the eluate being contaminated by undesirable products of mammalian cell origin such as viruses and prions.

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Composition	highly cross-linked 4% agarose
Particle size	60–165 µm
Ligand	recombinant protein A (*E. Coli*)
Coupling chemistry	reductive amination
Dynamic binding capacity	minimum 22 mg hIgG/ml drained medium
Chemical stability	No significant change in chromatographic performance after 1 week storage using 8 M urea, 6 M gua-HCl, 2% benzyl alcohol or 20% ethanol
Cleaning-In-Place stability	No significant change in chromatographic performance after 100 cycles normal use at room temperature, which corresponds to a total contact time of 16.7 hours using 50 mM NaOH+1 M NaCl or 50 mM NaOH + 1 M Na_2SO_4 or 6 M Gua-HCl
Recommended pH	
working range	3–10
cleaning-in-place	2–11
Recommended flow velocity	30–300 cm/h
Temperature stability[1]	4–40 °C
Delivery conditions	20% ethanol

[1] Recommended long term storage conditions: +4 to +8 °C, 20 % ethanol

Table 1. Characteristics of rmp Protein A Sepharose Fast Flow.

Low ligand leakage leads to a purer eluate fraction

The rmp Protein A is coupled to Sepharose Fast Flow medium 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 (see Table 2). In addition, reductive amination improves flow properties. Low ligand leakage produces a purer eluate fraction, which improves the efficiency of subsequent downstream clean-up procedures.

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

Table 2. Representation of the protein A leakage in the eluate measured after each of 5 chromatographic cycles. 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 steps

Tightly bound impurities from the crude feed sample 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 purification cycles containing cleaning and sanitization protocols, the medium retains a high breakthrough capacity. This leads to an increased life-length of the medium (see Figure 1).

High binding capacity and flow properties gives high productivity

There is an increasing demand for cost effective purification steps within drug 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, which boost total productivity. An example of the flow rate/capacity dependence for three different flow rates is shown in Figure 2.

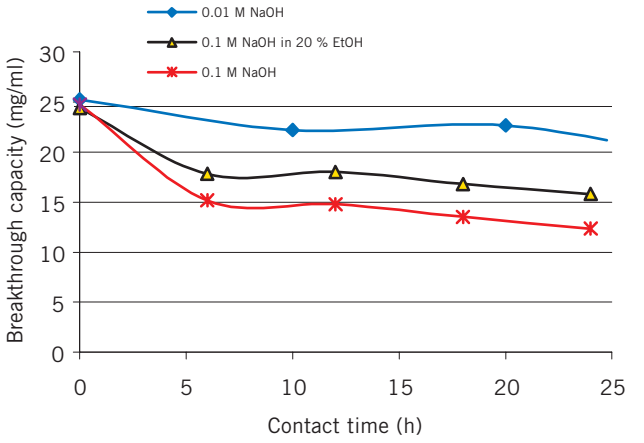

Fig. 1. Cleaning/Sanitization in Place study using different concentrations of NaOH. The adsorbent was treated with a specific cleaning agent for 2 hours. Then it was washed and equilibrated and the CIP procedure was repeated. The hIgG breakthrough capacity at 10% breakthrough was checked every 6th hour after treatment with 0.1 M NaOH in 20% ethanol or 0.1 M NaOH and every 10th hour when treated with 0.01 M NaOH. Column:HR 10/10, Bed height 7 cm, Flow velocity equil. = 140 cm/h, Flow rate CIP = 50 cm/h

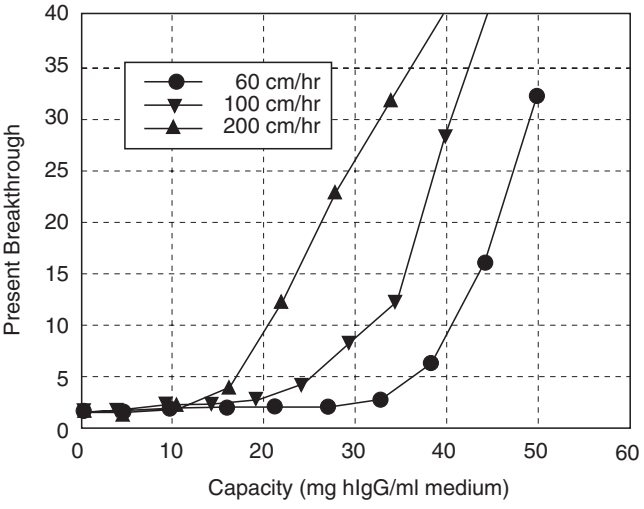

Fig. 2. Example of flow velocity/capacity dependence for rmp Protein A Sepharose Fast Flow. Breakthrough capacity for hIgG was determined at three different flow velocities. Breakthrough capacity is defined as mg hIgG applied per ml medium at the point where the concentration of hIgG in the column effluent reaches a value of 10% 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 media rmp Protein A Sepharose Fast Flow offers high selectivity, which renders 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 protein A binds to IgG varies with respect to both origin and antibody subclass, and even within the same subclass. This is an important consideration when developing the purification protocol. Typical binding conditions are low salt concentration buffers at 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 species and subclass.

Cleaning and Sanitization

The general recommendation for cleaning rmp Protein A Sepharose Fast Flow is to use a mixture of 50 mM NaOH and 1 M NaCl. As an alternative cleaning protocol 6 M guanidine hydrochloride can be used. Phosphoric acid (100 mM) has also been used for cleaning. To remove hydrophobically bound substances a solution of non-ionic 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 optimisation, cleaning-sanitization and column packing of rmp Protein A Sepharose Fast Flow can be found in the instructions enclosed with each pack of medium.

Scale up

After optimizing the antibody purification at 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. We recommend a bed height of 5-15 cm, allowing high flow rates to be used. Pressure/flow velocity curve for 5 cm i.d column is shown in Figure 3.

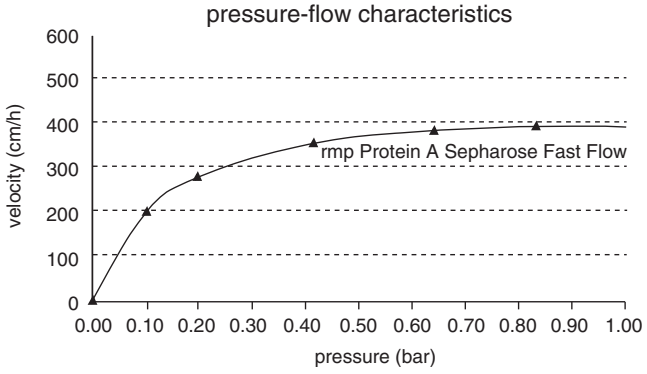

Fig. 3. Pressure and flow velocity dependence performed on rmp Protein A Sepharose Fast. The data were determined in a XK 50/30 (50 mm i.d) column packed to a bed height of 15 cm using 0.1 M NaCl as the mobile phase at 20 ˚C.

Equipment

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

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			
BPG™ variable bed, glass column	100–450	2.4–131 liters	max. 83
BioProcess™ Stainless Steel (BPSS) fixed bed columns	400–1400	12–1500 liters	10–100
INdEX™ variable bed columns	70–200	Up to 24.8 liters	max. 79
Chromaflow™ variable bed columns	280–2000		

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

Ordering information

Product	Pack size	Code No.
rmp Protein A Sepharose Fast Flow	5 ml	17-5138-01
	25 ml	17-5138-02
	200 ml	17-5138-03
	1 l	17-5138-04
	5 l	17-5138-05

All bulk media products are supplied in suspension in 20 % ethanol

to order:

Asia Pacific Tel: +852 2811 8693 Fax: +852 2811 5251 **Australasia** Tel: +61 2 9899 0999 Fax: +61 2 9899 7511 **Austria** Tel: 01 576 0616 20 Fax: 01 576 0616 27 **Belgium** Tel: 0800 73 888 Fax: 03 272 1637 **Canada** Tel: 1 800 463 5800 Fax: 1 800 567 1008 **Central, East, South East Europe** Tel: +43 1 982 3826 Fax: +43 1 985 8327 **Denmark** Tel: 45 16 2400 Fax: 45 16 2424 **Finland & Baltics** Tel: +358 (0)9 512 3940 Fax: +358 (0)9 512 1710 **France** Tel: 0169 35 67 00 Fax: 0169 41 9677 **Germany** Tel: 0761 4903 401 Fax: 0761 4903 405 **Italy** Tel: 02 27322 1 Fax: 02 27302 212 **Japan** Tel: 81 3 5331 9336 Fax: 81 3 5331 9370 **Latin America** Tel: +55 11 3933 7300 Fax: +55 11 3933 7315 **Middle East and Africa** Tel: +30 2 10 96 00 687 Fax: +30 2 10 96 00 693 **Netherlands** Tel: 0165 580 410 Fax: 0165 580 401 **Norway** Tel: 2318 5800 Fax: 2318 6800 **Portugal** Tel: 21 417 7035 Fax: 21 417 3184 **Russia & other C.I.S. & N.I.S.** Tel: +7 (095) 232 0250,956 1137 Fax: +7 (095) 230 6377 **South East Asia** Tel: 60 3 8024 2080 Fax: 60 3 8024 2090 **Spain** Tel: 93 594 49 50 Fax: 93 594 49 55 **Sweden** Tel: +46 18 612 19 00 Fax: +46 18 612 19 10 **Switzerland** Tel: 01 802 81 50 Fax: 01 802 81 51 **UK** Tel: 0800 616 928 Fax: 0800 616 927 **USA** Tel: +1 800 526 3593 Fax: +1 877 295 8102

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