

nProtein A Sepharose 4 Fast Flow

nProtein A Sepharose™ 4 Fast Flow is native protein A coupled to Sepharose 4 Fast Flow. It has nearly twice the total IgG binding capacity of Protein A Sepharose CL-4B, and is the ideal adsorbent for recovery and purification of monoclonal antibodies from cell culture at both laboratory and process scale.

nProtein A Sepharose 4 Fast Flow (Fig 1) has been developed and tested in cooperation with leading manufacturers of purified monoclonal antibody products, and is used in routine commercial production.

nProtein A Sepharose 4 Fast Flow features:

- Low leakage of protein A
- Used in large-scale FDA approved processes
- Manufactured without using animal-derived components

Medium characteristics

Native protein A has a molecular weight of 42 000 daltons and a structure consisting of several regions (Fig 2). Five of these (E, D, A, B, and C) show strong specific affinity for the Fc part of IgG, leaving the antigen combining sites within the regions free. One molecule of immobilized protein A binds at least two molecules of IgG.

Staphylococcal Protein A

S	E	D	A	B	C	Xr	Xc
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Fig 2. Schematic drawing of regions encoded by the gene for Staphylococcal protein A. S is the signal sequence. E, D, A, B, and C are the homologous repetitive immunoglobulin binding regions. Xr and Xc are C-terminal located, non-immunoglobulin binding regions thought to be responsible for the binding of Protein A to the bacterial cell.



Fig 1. nProtein A Sepharose 4 Fast Flow is available in a range of pack sizes.

GE Healthcare's native protein A is produced by fermenting a selected strain of *Staphylococcus aureus*. The purified protein is coupled to Sepharose 4 Fast Flow by the cyanogen bromide technique, giving a highly stable medium with minimal non-specific adsorption. nProtein A Sepharose 4 Fast Flow is manufactured without using animal-derived components.

The swollen medium has a protein A content of approximately 6 mg/ml drained medium. The total binding capacity for human IgG is approximately 35 mg/ml drained medium.

Sepharose 4 Fast Flow is a highly cross-linked, 4% agarose derivative with impressive kinetics, leading to excellent chromatographic qualities in the immobilized affinity adsorbent. Its rigidity also makes it well-suited for process scale applications. nProtein A Sepharose 4 Fast Flow is particularly suitable for recovery and purification of monoclonal antibodies from cell culture supernatants. The rigidity and high degree of substitution of the Sepharose 4 Fast Flow matrix enables the rapid processing of large volumes of dilute cell culture fluid.



Stability

nProtein A Sepharose 4 Fast Flow has high chemical and mechanical stability. It withstands high concentrations of hydrogen bond disrupting agents such as urea, guanidine hydrochloride, and sodium thiocyanate. It has high thermal stability, but is not autoclavable. The characteristics of the product are summarized in Table 1.

Table 1. Characteristics of nProtein A Sepharose 4 Fast Flow

Ligand	native Staphylococcal protein A
Ligand density	approx. 6 mg native protein A/ml drained medium
Ligand coupling method	cyanogen bromide activation
Dynamic binding capacity ¹	min 20 mg human IgG/ml drained medium
Matrix	highly cross-linked 4% agarose
Average particle size	90 µm (45–165 µm)
Chemical stability	no significant change in chromatographic performance after 1 week storage using 8 M urea, 6 M guanidine-HCl, 2% benzyl alcohol or 20% ethanol
pH stability ²	
Long term	3–9
Short term	2–10
Recommended flow rate	50–300 cm/h
Max operating back pressure	0.1 MPa (1 bar, 14 psi)
Sanitization	wash the packed column with 2% hibitane/20% ethanol or 70% ethanol
Storage	20% ethanol at +4°C to +8°C

¹ The binding capacity was determined at a linear flow rate of 100 cm/h, column: 7.5 × 50 mm, sample volume: 250 ml, sample concentration: 1 mg human IgG/ml. Please note that there might be considerable deviations in binding capacity for different immunoglobulins derived from the same species even if they are of the same subclass.

² Complete data on the stability of protein A as a function of pH are not available. The ranges given are estimates based on our knowledge and experience. Please note the following:

pH stability, long term refers to the pH interval where the medium is stable over a long period of time without adverse effects on its subsequent chromatographic performance.

pH stability, short term refers to the pH interval for regeneration, cleaning-in-place, and sanitization procedures.

pH below 3 is sometimes required to elute strongly bound Ig's. However, protein ligands may hydrolyze at very low pH.

Process-scale use

Columns

Columns recommended for nProtein A Sepharose 4 Fast Flow are shown in Table 2.

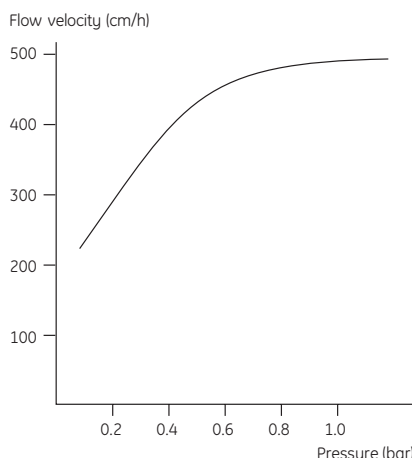
Table 2. Recommended GE Healthcare columns for nProtein A Sepharose 4 Fast Flow

Column	Bed height	Medium volume
XK Column 50/30 Fast Flow ¹	5–15 cm	100–300 ml
BPG™ 100/500		Up to 2.4 l

¹ These are columns fitted with a special Fast Flow adaptor to increase throughput.

When packing the medium at a 5-cm bed height, the recommended packing flow velocity is at least 700 cm/h, and at a 15-cm bed height at least 300 cm/h. The working flow velocity should not exceed 80% of the packing flow velocity. As a guide, pressure/flow velocity curves for the Sepharose 4 Fast Flow base matrix packed in XK 50/30 Fast Flow and BP 113 columns are shown in Figure 3.

(a)



(b)

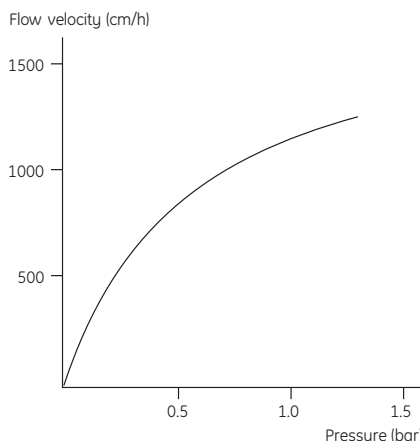


Fig 3. Pressure/flow velocity curve for Sepharose 4 Fast Flow in XK 50/30, bed height 15 cm (a) and BP 113, bed height 5 cm (b); mobile phase H₂O.

Dynamic capacity

The dynamic capacity of chromatographic adsorbents is a function of the flow velocity used for loading samples, and increases with decreasing flow velocity. Furthermore, individual antibodies differ in their affinity to protein A. To obtain an optimal purification scheme with respect to capacity and time, it is necessary to first determine the capacity for the specific antibody to be purified over a range of different flow velocities (see the example in Fig 4). Once this is known it is then possible to control the flow velocity during the loading phase to achieve maximum binding of the antibody in minimum time. In practice, this means initially loading the sample at a high flow velocity (e.g., 300 cm/h) and reducing the flow velocity successively with increasing sample load.

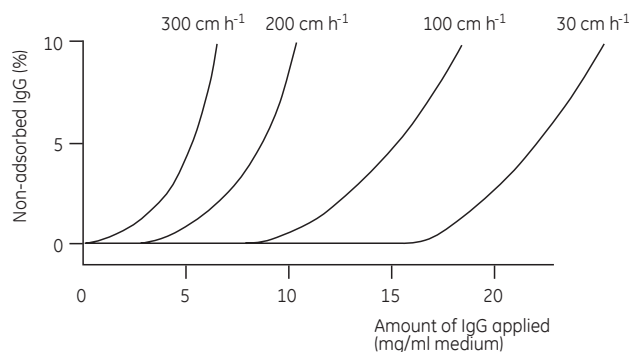


Fig 4. One example of how the capacity for human IgG depends on the flow velocity with nProtein A Sepharose 4 Fast Flow. The non-adsorbed IgG (%) was measured as a function of the amount applied to the column at 4 different flow velocities, 300, 200, 100, and 30 cm/h. Concentration of the applied sample: 0.33 mg IgG/ml. Column: 0.5 × 5 cm (i.d. × h). Buffer system: 0.1 M Na₂HPO₄, pH 7.0.

Operation

nProtein A Sepharose 4 Fast Flow is supplied in suspension in 20% ethanol.

1. After packing, wash the medium bed with at least three column volumes of starting buffer to remove preservative.
2. Note the following points when loading the sample:
 - The sample pH should be the same as the starting buffer pH.
 - The sample should be filtered through a 0.22–0.45 μm filter (This prolongs the working life of the medium).
3. After loading the sample, wash the medium with starting buffer until the baseline is stable.
4. When eluting the sample, reverse the direction of flow.

Process Hygiene

Good process hygiene ensures the safety and integrity of the final product by removing or controlling any unwanted substances that might be present or generated in the raw material, or derived from the purification system itself. In practice, process hygiene of most affinity media usually means reduction of product contamination by sanitization, followed by a cleaning step.

Sanitization

Sanitization is the reduction of microbial populations on the medium. Two suggested alternative protocols are:

- i) Equilibrate with a buffer consisting of 2% hibitane digluconate and 20% ethanol.
 - ii) Allow to stand for 6 hours.
 - iii) Wash with sterile buffer.
- or
- i) Equilibrate with 70% ethanol.
 - ii) Allow to stand for 12 hours.
 - iii) Wash with sterile buffer.

Cleaning

The general recommendation for cleaning nProtein A Sepharose 4 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.

Regeneration

After each separation cycle, regenerate the medium bed by washing with approximately three column volumes of 0.1 M citrate buffer, pH 3 until the baseline is stable.

Storage

For longer periods of storage, keep nProtein A Sepharose 4 Fast Flow in a suitable bacteriostat (e.g., 20% ethanol), at 4°C to 8°C. The medium must not be frozen.

Applications

The most important application area for nProtein A Sepharose 4 Fast Flow is the purification of monoclonal antibodies from cell culture. High IgG capacity and high flow velocities make the medium ideal for both laboratory- and process-scale separations.

There is a natural diversity between the different subclasses of IgG and even within subclasses. Therefore the binding and elution system must be optimized for every monoclonal antibody to be purified.

Ordering information

Product	Pack size	Code No.
nProtein A Sepharose 4 Fast Flow	5 ml	17-5280-01
nProtein A Sepharose 4 Fast Flow	25 ml	17-5280-04
nProtein A Sepharose 4 Fast Flow	200 ml	17-5280-02
nProtein A Sepharose 4 Fast Flow	1 liter	17-5280-03
nProtein A Sepharose 4 Fast Flow	5 liter	17-5280-05

Related products

Product	Pack size	Code No.
HiTrap™ Protein A HP	2 × 1 ml	17-0402-03
HiTrap Protein A HP	5 × 1 ml	17-0402-01
HiTrap Protein A HP	1 × 5 ml	17-0403-01
HiTrap Protein A HP	5 × 5 ml	17-0403-03

Literature

Product	Code No.
Antibody Purification Handbook	18-1037-46
Affinity Chromatography Handbook	18-1022-29
Affinity Columns and Media, Selection Guide	18-1121-86
Convenient Protein Purification, HiTrap Column Selection Guide	18-1129-81

www.gelifesciences.com/protein-purification

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