nProtein A **Sepharose** 4 Fast Flow

AFFINITY CHROMATOGRAPHY

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 an excellent 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

Resin characteristics

Native protein A has a molecular weight of 42 000 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

Fig 1. 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.

Cytiva'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 resin with minimal non-specific adsorption. nProtein A Sepharose 4 Fast Flow is manufactured without using animal-derived components.

The total binding capacity for human IgG is ≥ 30 mg/mL resin.

Sepharose 4 Fast Flow is a 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 ligand concentration 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 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

Matrix	Cross-linked agarose, 4%, spherical
Ligand	Native Staphylococcal protein A
Ligand coupling method	Cyanogen bromide activation
Particle size, d_{50v} [1]	~ 90 µm
Total binding capacity[2]	≥ 30 mg hIgG/mL resin
Recommended operating flow velocity[3]	30 to 200 cm/h
Pressure/flow characteristics[4,5]	150 to 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)
pH stability, operational[6]	3 to 9[7]
pH stability, CIP[8]	3 to 10[7]
Chemical stability	Stable to commonly used aqueous buffers, 6 M guanidine-HCl, 70% ethanol, 3 M NaSCN, 0.1 M glycine (pH 3.0), 2% benzyl alcohol and 20% ethanol
Sanitization	Wash the packed column with 2% hibitane/20% ethanol or 70% ethanol
Storage	20% ethanol, 2°C to 8°C

[1] Median particle size of the cumulative volume distribution.
[2] Protein in excess is loaded in 0.020 M NaH_2PO_4 at pH 7 on a 7.5/50 PEEK-column. The binding capacity is obtained by measuring the amount of eluted protein in 0.1 M Glycine at pH 3.
[3] 60 cm diameter, 20 cm bed height, at 20°C using buffer with the same viscosity as water.
[4] 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.
[5] Pressure/flow test performed on the base matrix.
[6] pH range where resin can be operated without significant change in function.
[7] pH below 3 is sometimes required to elute strongly bound IgG species. However, protein ligands may hydrolyze at pH below 2.
[8] pH range where resin can be subjected to cleaning-or sanitization-in-place without significant change in function.

Process-scale use

Columns

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

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

Column	Bed height	Resin volume
XK Column 50/30 Fast Flow[1]	5 to 15 cm	100 to 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 resin 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.

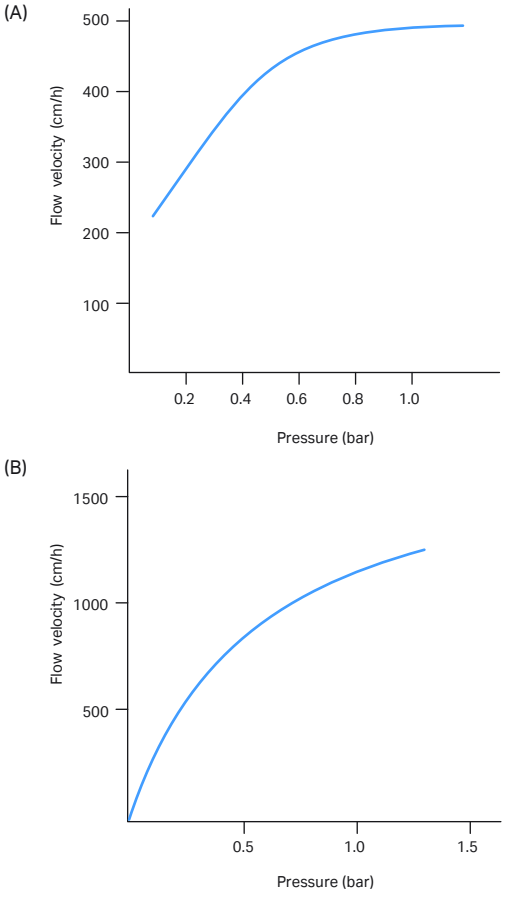

Fig 3. Pressure/flow velocity curve for Sepharose 4 Fast Flow in XK 50/30, bed height 15 cm (A) and BP 113, bed height 5 cm (B); mobile phase (water).

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 (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.

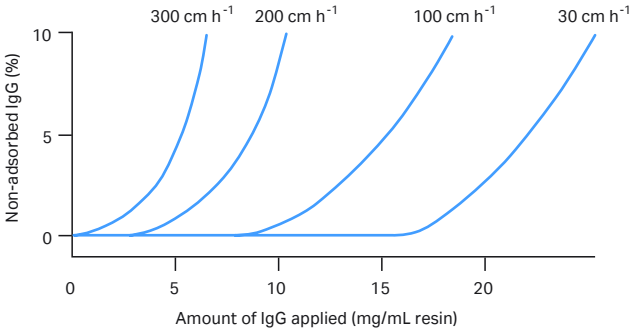

Fig 4. One example of how the capacity for human IgG depends on the flow velocity with nProtein A Sepharose 4 Fast Flow. The non-adsorbed IgG (%) was measured as a function of the amount applied to the column at four different flow velocities, 300, 200, 100, and 30 cm/h. Concentration of the applied sample: 0.33 mg IgG/mL. Column: 0.5 × 5 cm (i.d. × h). Buffer system: 0.1 M Na_2HPO_4, pH 7.0.

Operation

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

1. After packing, wash the resin 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 resin)
3. After loading the sample, wash the resin 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 resins usually means reduction of product contamination by sanitization, followed by a cleaning step.

Sanitization

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

i) Equilibrate with a buffer consisting of 2% hibitane digluconate and 20% ethanol
ii) Allow to stand for 6 h
iii) Wash with sterile buffer

or

i) Equilibrate with 70% ethanol
ii) Allow to stand for 12 h
iii) Wash with sterile buffer

Cleaning

As 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 resin 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 2°C to 8°C. The resin 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 resin 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 number
nProtein A Sepharose 4 Fast Flow	5 mL	17528001
nProtein A Sepharose 4 Fast Flow	25 mL	17528004
nProtein A Sepharose 4 Fast Flow	200 mL	17528002
nProtein A Sepharose 4 Fast Flow	1 L	17528003
nProtein A Sepharose 4 Fast Flow	5 L	17528005
Related products		
HiTrap™ Protein A HP	2 × 1 mL	17040203
HiTrap Protein A HP	5 × 1 mL	17040201
HiTrap Protein A HP	1 × 5 mL	17040301
HiTrap Protein A HP	5 × 5 mL	17040303
Literature		
Antibody Purification Handbook		18103746
Affinity Chromatography Handbook		18102229
Affinity Columns and Media, Selection guide		18112186
Convenient Protein Purification, HiTrap Column Selection guide		18112981

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