Affinity chromatography

Blue Sepharose™ 6 Fast Flow

Blue Sepharose 6 Fast Flow is Cibacron Blue 3G coupled to Sepharose 6 Fast Flow (Fig 1). As a member of the BioProcess™ family of resins, this product is a well-suited adsorbent for albumin and interferon at both laboratory and process scale. Blue Sepharose 6 Fast Flow has been developed and tested in cooperation with leading large-scale manufacturers and is widely used in routine commercial production. A detailed study of this separation resin has been presented (1).

Features

- High binding capacity
- Sepharose Fast Flow matrix enables high flow rates
- Suitable for separation of albumin and interferon
- Specially developed in cooperation with commercial manufacturers

Blue Sepharose 6 Fast Flow

Blue Sepharose 6 Fast Flow is Cibacron Blue 3G covalently attached to the matrix by the triazine coupling method, giving a highly stable resin with minimal nonspecific adsorption. The molecular weight of the dye is 774.16 g/mole.

The swollen resin has a Cibacron Blue content of 6.7 to 7.9 µmol Cibacron Blue 3G/mL resin. The total binding capacity for human serum albumin is greater than 18 mg per mL resin. Figure 2 shows the partial structure of Blue Sepharose 6 Fast Flow. Table 1 summarizes the resin characteristics.

Sepharose 6 Fast Flow is base on a highly cross-linked agarose base matrix. The rigidity of the matrix, together with a high degree of substitution, enables rapid processing of large volumes at production scale. With excellent binding kinetics, Blue Sepharose 6 Fast Flow is suitable for recovery and purification of albumin and interferon. Purification and concentration are achieved in one single step.

Fig 1. Blue Sepharose 6 Fast Flow for rapid, process-scale purification of albumin and interferon.

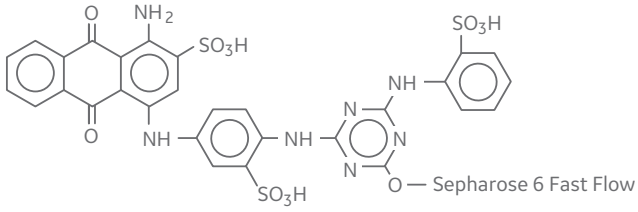

Fig 2. Partial structure of Blue Sepharose 6 Fast Flow.

For information on additional GE affinity chromatography resins suitable for large-scale manufacturing, please visit gelifesciences.com/chromatography.

Stability

Blue Sepharose 6 Fast Flow has high chemical and mechanical stability. The resin withstands high concentrations of denaturing agents such as urea and guanidine hydrochloride. Blue Sepharose 6 Fast Flow has high thermal stability and is autoclavable at 121°C for 20 min.

Table 1. Medium characteristics

Pressure/flow characteristics	≥400 cm/h at 0.1 MPa in a XK 50/30 column with 5 cm diameter and 15 cm bed height (at 25°C using 0.1 M NaCl solution)[1]
Total binding capacity[2]	≥ 18 mg human serum albumin/mL resin
Ligand density	6.7 to 7.9 µmol Cibacron Blue 3G/mL resin
Particle size, d_{50V}[3]	~90 µm.
Matrix	cross-linked agarose, 6%, spherical
pH stability Operational[4] CIP[5]	 4 to 12 3 to 13
Chemical stability	Stable to commonly used aqueous buffers, 70% ethanol 6 M guanidine hydrochloride 8 M urea
Working temperature	4°C to 40°C

[1] 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.
[2] Protein in excess is loaded in 0.050 M Potassium dihydrogen phosphate, pH 7.0 on a PEEK 7.5/50 column. The binding capacity is obtained by measuring the amount of bound and eluted protein in 0.050 M Potassium dihydrogen phosphate, 1.5 M Potassium chloride, pH 7.0.
[3] Median particle size of the cumulative volume distribution.
[4] pH range where resin can be operated without significant change in function.
[5] pH range where resin can be subjected to cleaning- or sanitization-in-place without significant change in function.

Process scale use

Columns and flow velocities

Table 2 lists columns recommended for Blue Sepharose 6 Fast Flow. The working flow velocity should not exceed 80% of the packing flow velocity. Detailed instructions for packing BPG columns can be found in the relevant instruction manual.backpressure below 3 bar.

Table 2. Recommended columns for Blue Sepharose 6 Fast Flow

Lab-scale columns			
Column	**i.d. (mm)**	**Bed volume (mL)**	**Bed height max (cm)**
Tricorn™	10	up to 8.4	10
HiScale™	16 to 50	up to 785	40
XK	16 to 50	up to 559	28
Production-scale columns			
AxiChrom™ 50–200	50 to 200	up to 16.7	50
AxiChrom 300–1600	300 to 1600	1005 L	50
BPG 100–450	100 to 450	up to 36	23
Chromaflow™ 400/100–300	400	13 to 37	30
Chromaflow 600/100–300	600	28 to 85	30

Figure 3 shows pressure/flow curves for Blue Sepharose 6 Fast Flow packed in a process-scale column with i.d. 113 mm (BP 113). Flow properties of the resin is better than its predecessor, Blue Sepharose CL-6B, which become important when elution buffers contain viscous liquids like ethylene glycol (Fig 4).

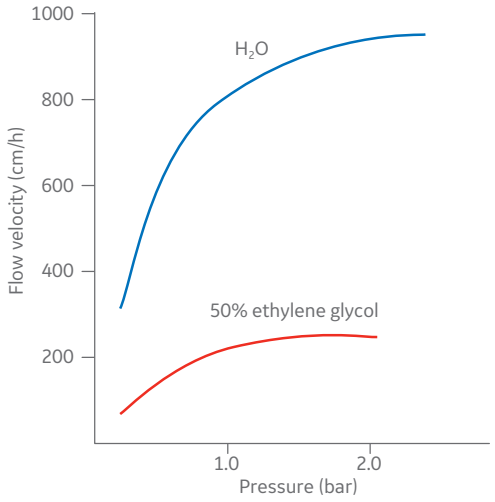

Fig 3. Pressure/flow curves for Blue Sepharose 6 Fast Flow packed in a BP 113 column, bed height 10 cm; mobile phases H_2O and 50% ethylene glycol, respectively.

Binding capacity

The binding capacity of a chromatography resin is a function of the flow rate used for loading of the sample. The binding capacity increases with decreasing flow rate. In addition, individual samples differ in their affinity for Blue Sepharose 6 Fast Flow. To obtain an optimized purification scheme with respect to capacity and time, the capacity for the specific sample to be purified must first be determined over a range of different flow rates. Once this is known, the flow rate for loading can be determined to maximize binding of the sample in minimum time.

Process hygiene

Good process hygiene enables 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 contamination of the resin.

One suggested protocols is:

1. Equilibrate the packed column with sterile filtered 70% ethanol.
2. Allow to stand for 12 h.
3. Wash with at least 5 bed volumes of sterile filtered buffer.

Cleaning-in-place (CIP)

Cleaning-in-place is the removal from the purification system of precipitated or denatured substances generated in previous production runs.

A suggested protocol is:

1. Wash the packed column with 0.1 M NaOH.
2. Wash with 3 to 4 bed volumes of 2 M potassium thiocyanate.
3. Wash immediately with at least 5 column volumes of sterile filtered binding buffer at pH 8.

The resin can also be washed with 0.5 M NaOH at 4°C. Further information on process hygiene can be found from Process chromatography—a practical guide (3).

Operation

Blue Sepharose 6 Fast Flow is supplied preswollen in 20% ethanol, 0.1 M KH_2PO_4, pH 8.0.

1. After column packing, the resin bed should be washed with at least 3 column volumes of starting buffer to remove preservative.
2. When loading the sample, bear in mind the following:
   - We recommend the sample pH to be the same as that of the starting buffer (see Human serum albumin under Applications for more details).
   - We recommend that the sample is filtered through a 0.22 to 0.45 µm filter to prolong the working life of the resin.
3. After loading the sample, wash the resin with starting buffer until the base line is stable. The resin can be washed at high flow rates.
4. Elution conditions need to be specifically optimized for each sample to obtain high purity and throughput.

Storage

For long-time storage (e.g. several weeks), we recommend the resin to be stored at +2°C to +8°C in 20% ethanol, 0.1 M KH_2PO_4, pH 8.0.

Applications

The most important application areas for Blue Sepharose 6 Fast Flow are the purification of interferon and albumin (2), as well as albumin removal. The high capacity and high flow rates make the resin suitable for both laboratory and process scale separations (1).

Interferon

In Figure 4, it is shown that albumin and interferon β both are adsorbed to the resin, but eluted under different conditions. In the first step, albumin is eluted using a salt-containing buffer, while interferon β is eluted in the second step using a buffer containing both salt and ethylene glycol.

Column:	NAP™-5 with an inner diameter of 10 mm containing 0.5 mL Blue Sepharose 6 Fast flow.
Sample:	0.5 mL interferon β (1.000.000 U/mL) in 0.1 M phosphate pH 7.4 with 1 mg/mL of human serum albumin.
Flow rate:	Gravity.
Start buffer (1):	0.02 M phosphate, 0.15 M NaCl, pH 7.2.
Elution buffer A (2):	0.02 M phosphate, 2 M NaCl, pH 7.2.
Elution buffer B (3):	0.02 M phosphate, 2 M NaCl, 50% ethylene glycol, pH 7.2.

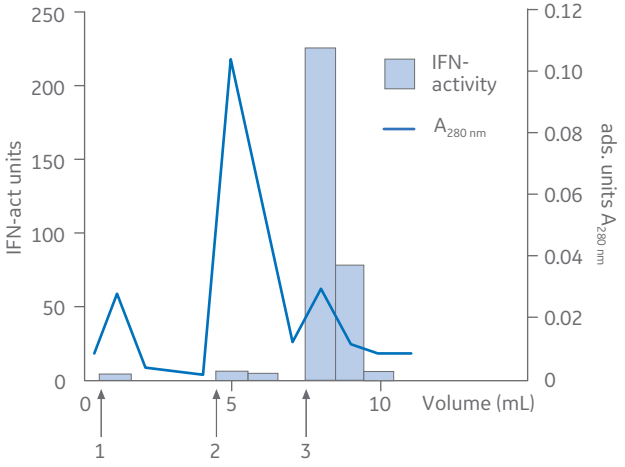

Fig 4. Purification of human serum albumin and interferon β on Blue Sepharose 6 Fast Flow.

Human serum albumin

In Figure 5, it is shown that the adsorption of human serum albumin to the resin is dependent of the pH in the starting buffer. As the pH of the starting buffer decreases from 7.0 to 4.0, more albumin binds to the resin and less elutes from the column.

Controlling binding in this way can help you increase the efficiency of albumin purification.

Column:	HR 10/2 containing 2 mL Blue Sepharose 6 Fast Flow.
Sample:	1.5 mL human serum albumin (20 mg/mL) in starting buffer.
Flow rate:	2.0 mL/min (153 cm/h)
Start buffer:	0.05 M citric acid, 0.1 M Na_2HPO_4, pH from 7.0 to 4.0.
Elution buffer:	0.05 M KH_2PO_4, 1.5 M KCl, pH 7.0.

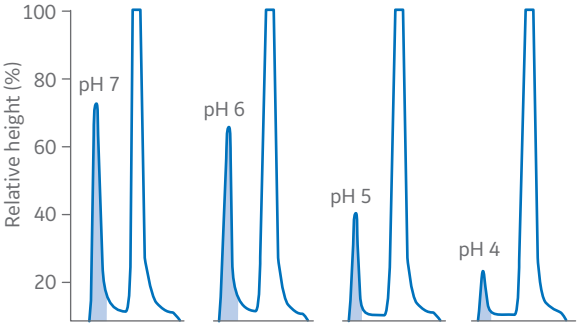

Fig 5. Adsorption of human serum albumin to Blue Sepharose 6 Fast Flow. The binding is dependent on the pH of the starting buffer used. Shaded peak = albumin in eluate.

References

1. Larsson, A. *et al.* Blue Sepharose 6 Fast Flow – a new process dye affinity chromatography gel. Poster presented at the *23rd IUPAC Congress*, 2–7 Aug., Stockholm, Sweden (1989).
2. Knight. E. Jr, and Fahey, D. Human fibroblast interferon, an improved purification. *J. Biol. Chem.* **256**, 3609–3611 (1981).
3. Sofer G.K.; Nyström L-E., eds. *Process chromatography – a practical guide*, Academic Press, London (1989).

Ordering information

Product	Pack size	Code number
Blue Sepharose 6 Fast Flow	50 mL	17094801
Blue Sepharose 6 Fast Flow	500 mL	17094802
Blue Sepharose 6 Fast Flow	1 L	17094803
Blue Sepharose 6 Fast Flow	5 L	17094804

Related literature	Code number
Application note: Use of sodium hydroxide for cleaning and sanitizing chromatography media and systems	18112457
Handbook: Affinity Chromatography, Principles and Methods	18102229
Handbook: Antibody Purification	18103746
Instruction: Blue Sepharose™ 6 Fast Flow	71705500
Instruction: Constant flow packing in BPG columns, brief overview	29001796
Instruction: Constant flow packing in BPG columns, method description and practical example	29001795
Instruction: Determining the compression factor and slurry concentration, brief overview	29001794
Instruction: Determining the compression factor and slurry concentration, method description and practical example	29001351
Instruction: Pack-in-place Chromaflow columns, brief overview,	29001798
Instruction: Pack-in-place Chromaflow columns, method description and practical example	29001797
Selection guide: Affinity chromatography columns and media	18112186

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