Blue Sepharose 6 Fast Flow is Cibacron Blue 3G covalently attached to Sepharose 6 Fast Flow. A member of the BioProcess Media family, this medium is the ideal adsorbent for albumin and interferon at both laboratory and process scale. Blue Sepharose 6 Fast Flow has been developed and tested in co-operation with leading large scale manufacturers and is used in routine commercial production. A detailed study of this separation medium has been presented (1).

Blue Sepharose 6 Fast Flow was originally developed by Amersham Biosciences Custom Designed Media Group. This is a specialist group that focuses on the design, production and delivery of media to customers’ specific requirements.

Features
- High dynamic capacity.
- Fast Flow matrix gives high flow rates.
- Ideal for separation of albumin and interferon.
- Specially developed in co-operation 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 medium with minimal non-specific adsorption. The molecular weight for the dye as a sodium salt is 726.1 (2).

The swollen medium has a Cibacron Blue content of 7.3 ± 0.6 µmol dye per ml drained medium (3). The total binding capacity for human serum albumin is greater than 18 mg per ml drained medium. Figure 2 shows the partial structure of Blue Sepharose 6 Fast Flow. Table 1 summarizes its characteristics.
Sepharose 6 Fast Flow is a highly cross-linked 6% agarose with impressive kinetics, leading to excellent chromatographic qualities in the affinity adsorbent. Its rigidity also makes it ideal for use at process scale. Blue Sepharose 6 Fast Flow is suitable for recovery and purification of albumin and interferon. The high degree of substitution, together with the rigidity of the Sepharose 6 Fast Flow matrix, enables the rapid processing of large volumes. Purification and concentration are achieved in a single step.

**Stability**

Blue Sepharose 6 Fast flow has high chemical and mechanical stability. It withstands high concentrations of denaturing agents such as urea and guanidine hydrochloride. It has high thermal stability and is autoclavable at 125°C for 20 minutes.

**Process scale use**

**Columns and flow rates**

Table 2 lists columns recommended for Blue Sepharose 6 Fast Flow. When packing medium with a 5 cm bed height, the recommended packing flow rate is at least 700 cm/h, with a 15 cm bed height, at least 300 cm/h, and with a 30 cm bed height, at least 150 cm/h. The working flow rate should not exceed 80% of the packing flow rate.

![Flow rate curve](image)

**Fig. 3.** Pressure / flow rate curves for Blue Sepharose 6 Fast Flow packed in BP113 column, bed height 10 cm; mobile phases H2O and 50% ethylene glycol (Work by Amersham Biosciences AB).

Figure 3 shows pressure/flow rate curves for Blue Sepharose 6 Fast Flow packed in a BioProcess Column BP 113. Flow properties of the medium are better than its predecessor, Blue Sepharose CL-6B, which is important when elution buffers contain viscous liquids like ethylene glycol (see Fig. 4).

Detailed instructions for packing BPG Columns can be found in the relevant Instruction Manual.
**Dynamic capacity**

The dynamic capacity of chromatographic adsorbents, a function of the low rate used for loading samples, increases with decreasing flow rate. In addition, individual samples differ in their affinity to Blue Sepharose 6 Fast Flow. To obtain an optimal 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 during the loading phase can be determined to achieve maximum binding of the sample in minimum time.

**Process hygiene**

Good process hygiene ensures the safety and integrity of the final product by removing or controlling any unwanted substances which 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 protocols are:

1. a) Equilibrate the packed column with a buffer consisting of 2 % hibitane digluconate and 20 % ethanol.
   b) Allow to stand for 6 hours.
   c) Wash with at least 5 bed volumes of sterile buffer.
2. a) Equilibrate the packed column with sterile filtered 70 % ethanol.
    b) Allow to stand for 12 hours.
    c) 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:

a) Wash the packed column with 0.1 M NaOH.

b) Wash with 3–4 bed volumes of 2 M potassium thiocyanate.

c) Wash immediately with at least 5 column volumes of sterile filtered binding buffer at pH 8.

The medium can also be washed with 0.5 M NaOH at 4°C. For a further discussion of process hygiene see Reference 4.

**Operation**

Blue Sepharose 6 Fast Flow is supplied pre-swollen in 20 % ethanol, 0.1 M KH₂PO₄, pH 8.0.

1. After packing in the column, the medium bed should be washed with at least 3 column volumes of starting buffer to remove preservative.

2. When loading the sample, bear in mind:
   - We recommend that the sample pH be the same as that of the starting buffer. See Figure 5 which shows the influence of pH on the adsorption of the sample to the medium.
   - We recommend that the sample be filtered through a 0.22–0.45 µm filter to prolong the working life of the medium.

3. After loading the sample, wash the medium with starting buffer until the base line is stable. The medium can be washed at high flow rates. (See the pressure/flow rate curves of Blue Sepharose 6 Fast Flow in Fig. 3.)

4. Elution conditions then have to be optimized for different samples in order to obtain maximum purity and throughput.

**Storage**

For longer periods of storage, e.g. several weeks, we recommend that the medium be stored at +4 to +8 °C in 20 % ethanol, 0.1 M KH₂PO₄, pH 8.0.
Applications
The most important application areas for Blue Sepharose 6 Fast Flow are the purification of interferon and albumin (3), and albumin removal. The high capacity and high flow rates make the medium ideal for both laboratory and process scale separations (1).

Interferon

Figure 4 shows that albumin and interferon ß are both adsorbed to the medium, but eluted under different conditions. In the first step, albumin is desorbed when salt is added in the buffer, while interferon ß is desorbed in the second step when ethylene glycol is present in the buffer.

Human serum albumin

Figure 5 shows that the adsorption of human serum albumin to the medium is dependent on 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 medium and less elutes from the column (see how the shaded albumin peak decreases in Fig. 5). Controlling binding in this way can help you raise the efficiency of albumin purification.

Ordering information

Blue Sepharose 6 Fast Flow is supplied pre-swollen in 20 % ethanol, 0.1 M KH₂PO₄, pH 8.0.

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References


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