instructions

Blue Sepharose CL-6B

Blue Sepharose™ CL-6B is Cibacron™ Blue F3G-A covalently attached to Sepharose CL-6B by the triazine coupling method. The structure of the blue dye makes it a very versatile tool for separating many proteins, e.g. albumin, interferon, lipoproteins and blood coagulation factors. It also binds several enzymes including kinases, dehydrogenases and most enzymes requiring adenylcontaining cofactors (including NAD^+ and $NADP^+$). The cross-linked matrix provides a stable, rigid medium even in the presence of dissociating agents.

71-7082-00 Edition AE

Amersham Biosciences

Table 1. Medium characteristics

Ligand density:	~2 µmole Cibacron Blue F3G-A/ml drained medium
Available capacity*:	5 mg human serum albumin/ml drained medium
Bead structure:	6% cross-linked agarose
Bead size range:	45-165 µm
Mean bead size:	90 µm
Max linear flow rate**:	150 cm/h at 25°C, HR16/10 column, 5 cm bed height
pH stability***.	
Long term:	4-12
Short term:	3-13
Chemical stability:	Stable to all commonly used aqueous buffers and additives such as 8 M urea and 6 M guanidine hydrochloride.
Physical stability:	Negligible volume variation due to changes in pH or ionic strength.
Autoclavable:	121 °C for 30 min.

* The binding capacity was estimated in 0.1 M phospate buffer pH 7.0

** Linear flow rate = $\frac{\text{volumetric flow rate (cm}^3\text{/h)}}{\text{column cross-sectional area (cm}^2\text{)}}$

*** 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 gel 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.

Preparing the medium

Blue Sepharose CL-6B is supplied freeze-dried in the presence of additives. These additives must be washed away at neutral pH. Weigh out the required amount of freeze dried powder (1 g freeze-dried powder gives about 4 ml final medium volume) and suspend it in distilled water. The medium swells immediately and should now be washed for 15 minutes with

distilled water on a sintered glass filter. Use approximately 200 ml distilled water per gram freeze-dried powder, added in several aliquots.

Prepare a slurry with binding buffer in a ratio of 75% settled medium to 25% buffer. The binding buffer should not contain agents which significantly increase the viscosity. The column may be equilibrated with viscous buffers at reduced flow rates after packing is completed.

Packing Sepharose CL-6B

1. Equilibrate all material to the temperature at which the chromatography will be performed.
2. De-gas the medium slurry.
3. Eliminate air from the column dead spaces by flushing the end pieces with buffer. Make sure no air has been trapped under the column net. Close the column outlet with a few centimeters of buffer remaining in the column.
4. Pour the slurry into the column in one continuous motion. Pouring the slurry down a glass rod held against the wall of the column will minimize the introduction of air bubbles.
5. Immediately fill the remainder of the column with buffer, mount the column top piece onto the column and connect the column to a pump.
6. Open the bottom outlet of the column and set the pump to run at the desired flow rate. This should be at least 133%

of the flow rate to be used during subsequent chromatographic procedures. However, the maximum flow rate, see Table 1, is typically employed during packing.

Note: If you have packed at the maximum linear flow rate, do not exceed 75% of this in subsequent chromatographic procedures.

7. Maintain the packing flow rate for 3 bed volumes after a constant bed height is reached.

Using an adaptor

Adaptors should be fitted as follows:

1. After the medium has been packed as described above, close the column outlet and remove the top piece from the column. Carefully fill the rest of the column with buffer to form an upward meniscus at the top.
2. Insert the adaptor at an angle into the column, ensuring that no air is trapped under the net.
3. Make all tubing connections at this stage. There must be a bubble-free liquid connection between the column and the pump.
4. Slide the plunger slowly down the column so that the air above the net and in the capillary tubings is displaced by eluent. Valves on the inlet side of the column should be turned in all directions during this procedure to ensure that air is removed.
5. Lock the adaptor in position on the medium surface. Open the column outlet and start the eluent flow. Pass eluent

through the column at the packing flow rate until the bed is stable. Re-position the adaptor on the medium surface as necessary.

The column is now packed and equilibrated and ready for use.

Binding

Different substances differ in their affinity for Blue Sepharose CL-6B. The available capacity will depend upon parameters such as sample concentration, flow rate, pH, buffer composition and temperature. To obtain an optimal purification with respect to capacity, the capacity must first be determined over a range of different pH and flow rates.

- Sample pH should be the same as that of the binding buffer. Filter the sample through a 0.22 µm or 0.45 µm filter to prolong the working life of the medium.
- After the sample has been loaded, wash the medium with binding buffer until the baseline is stable.

Elution

Blue Sepharose CL-6B is a group specific adsorbent with affinity for a wide variety of biomolecules. Some proteins interact biospecifically with the dye due to its structural similarity to nucleotide cofactors, while others, such as albumin and interferon, bind in a less specific manner by electrostatic and/or hydrophobic interactions with the aromatic anionic ligand.

- Specifically bound enzymes may be eluted by competitive elution with low concentrations of free cofactor, NAD^+ or $NADP^+$, in the buffer. Specifically bound biomolecules

normally elute in the range 1-20 mM. Either step or continuous gradients may be used.

- Less specifically bound biomolecules can be eluted with increased ionic strength. Elution is normally complete at salt concentrations of 2 M or less of NaCl or KCl. Either step or continuous gradients may be used. For elution of serum albumin, KCl is most commonly used. Elution can also be achieved by a change in pH or polarity (e.g. ethylene glycol).

Regeneration

Depending of the nature of the sample, Blue Sepharose CL-6B may be regenerated for re-use by washing the medium with at least 5 column volumes of alternating high pH (0.1 M Tris-HCl, 0.5 M NaCl, pH 8.5) and low pH (0.1 M sodium acetate, 0.5 M NaCl, pH 4.5) buffers. This cycle should be repeated 4-5 times followed by re-equilibration in binding buffer.

In some applications, substances like denatured proteins or lipids do not elute in the regeneration procedure. These can be removed by cleaning-in-place procedures.

Cleaning-in-place (CIP)

Remove precipitated proteins by washing the column with 4 bed volumes of 0.1 M NaOH solution at a low linear flow rate (20 cm/h), followed by washing with 3-4 bed volumes of 70% ethanol or 2 M potassium thiocyanate. Alternatively, wash the column with 2 bed volumes of 6 M guanidine hydrochloride. In both cases, wash immediately with at least 5 bed volumes of binding buffer.

Remove strongly bound hydrophobic proteins, lipoproteins and lipids by washing the column with 3-4 bed volumes of up to 70% ethanol or 30% isopropanol. (Apply increasing gradients to avoid air bubble formation when using high concentrations of organic solvents.)

Alternatively, wash the column with 2 bed volumes of detergent in a basic or acidic solution, e.g. 0.1% non-ionic detergent in 1 M acetic acid at a flow rate of 20 cm/h. Remove residual detergent by washing with 5 bed volumes of 70% ethanol.

In both cases, wash immediately with at least 5 bed volumes of binding buffer.

Sanitization

Sanitize the column by equilibrating it with a buffer consisting of 2% hibitane digluconate and 20% ethanol and allow it to stand for 6 hours.
Alternatively, equilibrate the column with 70% ethanol and allow it to stand for 12 hours.

In both cases, wash with at least 5 bed volumes of sterile filtered binding buffer at pH 8.0.

Storage

Store freeze-dried Blue Sepharose CL-6B below 8°C. Store swollen medium at 4-8°C in 20% ethanol, 0.1 M KH_2PO_4, pH 8.0.
Swollen Blue Sepharose CL-6B must not be frozen.

Further information

Check www.chromatography.amershambiosciences.com for

more information. Useful information is also available in the Affinity Chromatography Handbook, see ordering information.

Ordering information

Product	Pack size	Code No.
Blue Sepharose CL-6B	25 g	17-0830-01
Literature		
Affinity Chromatography Handbook, Principles and Methods	1	18-1022-29

Trademarks

Sepharose and Drop Design are trademarks of Amersham Biosciences Limited. Amersham and Amersham Biosciences are trademarks of Amersham plc. Cibacron is a trademark of Ciba-Geigy Corp.

© Amersham Biosciences AB 2002 – All rights reserved

All goods and services are sold subject to the terms and conditions of sale of the company within the Amersham Biosciences group which supplies them. A copy of these terms and conditions is available on request.

Amersham Biosciences AB
SE-751 84 Uppsala Sweden

Amersham Biosciences UK Limited
Amersham Place, Little Chalfont
Buckinghamshire, England HP7 9NA

Amersham Biosciences Corporation
800 Centennial Ave.
Piscataway, NJ 08855 USA

Amersham Biosciences Europe GmbH
Postfach 5480
D-79021 Freiburg Germany

Amersham Biosciences K.K.
Sanken Building, 3-25-1
Shinjuku-ku, Tokyo 169-0073 Japan

Printed in Sweden by TK i Uppsala AB, 2003

Amersham Biosciences