cytiva

Blue **Sepharose** 6 Fast Flow

Affinity chromatography

Instructions for Use

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71705500 AL

Abstract

Blue Sepharose™ 6 Fast Flow is Cibacron Blue 3G covalently attached to the Sepharose 6 Fast Flow matrix by the triazine coupling method. The blue dye binds many proteins, such as albumin, interferon, lipoproteins, and blood coagulation factors.
It also binds several enzymes including kinases, dehydrogenases, and most enzymes requiring adenyl-containing cofactors, for example NAD+.
The cross-linked matrix provides a stable, rigid resin.
Blue Sepharose Fast Flow belongs to the BioProcess™ resin range. BioProcess chromatography resins are developed and supported for production-scale chromatography. BioProcess resins are produced with validated methods and are tested to meet manufacturing requirements. Secure ordering and delivery routines give a reliable supply of resins for production-scale. Regulatory Support Files (RSF) are available to assist process validation and submissions to regulatory authorities. BioProcess resins cover all purification steps from capture to polishing.

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Important

Read these instructions carefully before using the product.

Safety

For use and handling of the product in a safe way, refer to the Safety Data Sheet.

1 Product description

Table 1. Resin characteristics

Matrix	Cross-linked agarose, 6% spherical
Particle size, d_{50v}[1]	~ 90 µm
Ligand concentration	6.7 to 7.9 µmol Cibacron blue 3G/mL resin
Total binding capacity[2]	≥ 18 mg human serum albumin/mL resin
Pressure/flow characteristics	≥ 400 cm/h at 0.1 MPa in an XK 50/30 column with 5 cm diameter and 15 cm bed height (at 25°C using 0.1 M NaCl solution)[3]
pH stability, operational[4]	4 to 12
pH stability, CIP[5]	3 to 13
Chemical stability	Stable to commonly used aqueous buffers, 70% ethanol, 6 M guanidine hydrochloride, 8 M urea
Operating temperature	4°C to 40°C
Autoclavability	20 min at 121°C in distilled water
Storage	0.1 M KH_2PO_4, pH 8.0 and 20% ethanol, 2°C to 8°C

[1] Median particle size of the cumulative volume distribution

[2] Protein in excess is loaded in 0.050 M potassium dihydrogen phoshate, 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 phoshate, 1.5 M potassiuim chloride, pH 7.0.

[3] The pressure/flow characteristics describes the relationship between pressure and flow under the set circumstances. The pressure is not the maximum pressure of the resin.

[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

2 Column packing

Blue Sepharose 6 Fast Flow is supplied preswollen in 20% ethanol, 0.1 M KH_2PO_4, pH 8.0. Decant the solution and replace it with binding buffer. The binding buffer must not contain agents which significantly increase the viscosity, but the column can be equilibrated with viscous buffers at reduced flow rates after packing is completed.

Packing lab-scale columns

1. Assemble the column (and packing reservoir if necessary).
2. Remove air from the end-piece and adapter by flushing with water. Make sure no air has been trapped under the column bed support. Close the column outlet leaving the bed support covered with water.
3. Resuspend the resin and pour the slurry into the column in a single continuous motion. Pouring the slurry down a glass rod held against the column wall will minimize the introduction of air bubbles.
4. If using a packing reservoir, immediately fill the remainder of the column and reservoir with water. Attach the adapter or lid of the packing reservoir.
5. Connect the column to a pump. Avoid trapping air bubbles under the adapter or in the inlet tubing.
6. Open the bottom outlet of the column and set the pump to run at the desired flow rate.
7. Ideally, Sepharose 6 Fast Flow resins are packed in XK, HiScale, or Tricorn columns in a two-step procedure:

   1. Use maximum 0.05 MPa (0.5 bar, 7.25 psi).

   2. Use maximum 0.15 MPa (1.5 bar, 21.75 psi).

   If the packing equipment does not include a pressure gauge, use the packing flow rates given in Table 2.

   If the recommended pressure or flow rate cannot be obtained, use the maximum flow rate your pump can deliver. This usually also gives a well packed bed.

Note: *For subsequent chromatography procedures, do not exceed 75% of the packing flow rate. See Table 2 for flow rates for chromatography.*

Table 2. Recommended lab-scale columns and flow rates

Empty Column[1]	Packing flow rate (mL/min)		Max. recommended flow rate for chromatography (mL/min)
	First step	Second step	
HiScale™ 16/20	2.5	8.7	5
HiScale 16/40	2.5	8.7	5
HiScale 26/20	6.6	23	13
HiScale 26/40	6.6	23	13
HiScale 50/20	24.5	85	49
HiScale 50/40	24.5	85	49
Tricorn 10/20	0.9	4.7	2
Tricorn 10/50	0.9	4.7	2
Tricorn 10/100	0.9	4.7	2
XK 16/20	2.5	8.7	5
XK 26/20	6.6	23	13
XK 50/20	24.5	85	49
XK 50/30	24.5	85	49

[1] For inner diameter, maximum bed volumes, and bed heights, see *Ordering information*.

8 Maintain packing flow rate for at least 3 bed volumes after a constant bed height is reached. Mark the bed height on the column.

9 Stop the pump and close the column outlet.

10 If using a packing reservoir, disconnect the reservoir and fit the adapter to the column.

11 With the adapter inlet disconnected, push the adapter down into the column until it reaches the mark. Allow the packing solution to flush the adapter inlet. Lock the adapter in position.

12 Connect the column to a pump or a chromatography system and start equilibration. Re-adjust the adapter if necessary.

Packing process-scale columns

For general process-scale column packing instructions, visit the support section at *cytiva.com/protein-purification*.

Table 3. Recommended process-scale columns

Column	Inner diam (mm)	Bed volume (L)	Bed height max (cm)
AxiChrom™ 50-200	50 to 200	up to 16.7 L	50
AxiChrom 300-1600	300 to 1600	1005 L	50
BPG 100/500	100	up to 2.0 L	26
BPG 140/500	140	up to 4.0 L	26
BPG 200/500	200	up to 8.2 L	26
BPG 300/500	300	up to 18.0 L	26
BPG 450/500	450	up to 36.0 L	23
Chromaflow™ 400/100-300	400	13 to 37 L	30
Chromaflow 600/100-300	600	28 to 85 L	30

3 Evaluation of packing

Intervals

Test the column efficiency to check the quality of packing. Testing must be done after packing, at regular intervals during the working life of the column or when separation performance is seen to deteriorate.

Column efficiency testing

The best method of expressing the efficiency of a packed column is in terms of the height equivalent to a theoretical plate (HETP) and the asymmetry factor (A_s). These values are easily determined by applying a sample such as 1% acetone solution to the column. Sodium chloride can also be used as a test substance. Use a concentration of 0.8 M NaCl in water with 0.4 M NaCl in water as eluent.

For more information about column efficiency testing, consult the application note *Column efficiency testing* (28937207).

Note: *The calculated number of plates will vary according to the test conditions and must only be used as a reference value. It is important that test conditions and equipment are kept constant so that results are comparable. Changes of solute, solvent, eluent, sample volume, flow velocity, liquid pathway, temperature, etc. will influence the results.*

Sample volume and flow velocity

For optimal results, the sample volume must be at maximum 2.5% of the column volume and the flow velocity between 15 and 30 cm/h. If an acceptance limit is defined in relation to column performance, the column plate number can be used as one of the acceptance criteria for column use.

Method for measuring HETP and A_S

Calculate HETP and A_S from the UV curve (or conductivity curve) as follows:

$$HETP = \frac{L}{N}$$

$$N = 5.54 \times \left(\frac{V_R}{W_h}\right)^2$$

L = bed height (cm)

N = number of theoretical plates

V_R = volume eluted from the start of sample application to the peak maximum

W_h = peak width measured as the width of the recorded peak at half of the peak height

V_R and W_h are in the same units

The concept of reduced plate height is often used for comparing column performance.

The reduced plate height, h, is calculated as follows:

$$h = \frac{HETP}{d_{50v}}$$

d_{50v} = Median particle size of the cumulative volume distribution (cm)

As a guideline, a value of < 3 is very good.

The peak must be symmetrical, and the asymmetry factor as close to 1 as possible
(A typical acceptable range could be $0.7 < A_S < 1.3$).

A change in the shape of the peak is usually the first indication of bed deterioration due to excessive use.

Peak asymmetry factor calculation:

$$A_s = \frac{b}{a}$$

a = ascending part of the peak width at 10% of peak height

b = descending part of the peak width at 10% of peak height

Figure 1 shows a UV trace for acetone in a typical test chromatogram from which the HETP and A_s values are calculated.

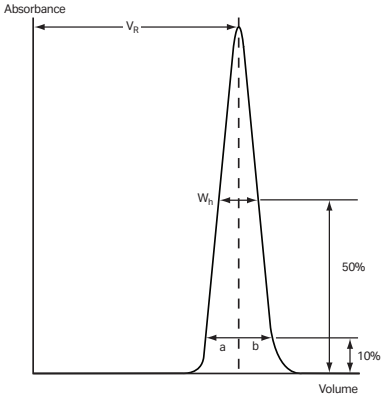

Fig 1. A typical test chromatogram showing the parameters used for HETP and A_s calculations.

4 Operation

Binding

Different substances differ in their affinity for Blue Sepharose 6 Fast Flow. The available capacity will depend upon parameters such as flow rate, pH, buffer composition, and temperature.

1. Sample pH must 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 resin.
2. After the sample has been loaded, wash the resin with binding buffer until the base line is stable.

Elution

1. Elution conditions vary with the sample. Elution can be accomplished by a change in pH, polarity or ionic strength of the buffer. Enzymes can often be eluted at less than 1 M NaCl.
2. Competitive elution with low concentrations of the cofactor is required for very specifically bound proteins. Either step or continuous gradients can be used.

5 Regeneration, Cleaning, Sanitization, and Storage

Regeneration

Depending on the nature of the sample, reversibly bound material can be eluted with 4 to 5 washing cycles of alternate 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, followed by re-equilibration in binding buffer.

In some applications, substances like denaturated proteins or lipids do not elute in the regeneration procedure. These can be removed by Cleaning-In-Place (CIP).

Cleaning-In-Place

Remove precipitated proteins by washing the column with 4 bed volumes of 0.1 M NaOH solution at a low flow velocity (40 cm/h), followed by washing the column with 3 to 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 sterile filtered binding buffer at pH 8.0.

Remove strongly bound hydrophobic proteins, lipoproteins, and lipids by washing the column with 3 to 4 bed volumes of up to 70% ethanol. (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. Wash at a flow velocity of 40 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 sterile filtered binding buffer at pH 8.0.

Sanitization

Sanitize the column by equilibrating it with 70% ethanol and allow to stand for 12 hours.

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

Column performance is not significantly changed by the CIP and sanitization procedures described above.

All of the above recommended procedures can be performed directly on the packed column.

Storage

For longer periods of storage, for example weeks, we recommend that the resin be stored at 2°C to 8°C in 20% ethanol, 0.1 M KH_2PO_4, pH 8.0.

The resin must not be frozen.

6 Ordering information

Product	Pack size	Product code
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 product	Pack size	Product code
HiScale 16/20 column, 16 mm i.d., max 40 mL bed volume, max bed height 20 cm	1	28966441
HiScale 16/40 column, 16 mm i.d., 16-80 mL bed flow, 8-40 cm bed height	1	28964424
HiScale 26/20 column, 26 mm i.d., max 106 mL bed volume, 8-40 cm bed height	1	28964514
HiScale 26/40 column, 26 mm i.d., 69-212 mL bed volume, max 20 cm bed height	1	28964513
HiScale 50/20 column, 50 mm i.d., max 393 mL bed volume, max 20 cm bed height	1	28964445
HiScale 50/40 column, 50 mm i.d., 274-785 mL bed volume, 8-40 cm bed height	1	28964444
Tricorn 10/20 column, 10 mm i.d., max 2.2 mL bed volume or 2.8 cm bed height	1	28406413
Tricorn 10/50 column, 10 mm i.d., max 4.5 mL bed volume or 5.8 cm bed height	1	28406414
Tricorn 10/100 column, 10 mm i.d., max 8.5 mL bed volume or 10.8 cm bed height	1	28406415
XK 16/20 column, 16 mm i.d., max 30 mL bed volume or 15 cm bed height	1	18877301
XK 26/20 column, 26 mm i.d., max 65 mL bed volume or 12.5 cm bed height	1	18100072
XK 50/20 column, 50 mm i.d., max 270 mL bed volume or 14 cm bed height	1	18100071
XK 50/30 column, 50 mm i.d., max 550 mL bed volume or 28.5 cm bed height	1	18875101

Data files	Product code
AxiChrom	28929041
Blue Sepharose 6 Fast Flow	18106075
BPG columns	18111523
CHROMAFLOW columns	18113892

Literature	Product code
Affinity Chromatography Handbook	18102229
Handbook of Process Chromatography: A Guide to Optimization, Scale-Up, and Validation (Academic Press, 1997, Sofer, G. and Hagel, L.)	18112156

7 Further information

For the latest news, more product information and our handbooks, visit: cytiva.com/protein-purification

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