Blue Sefinose™ 6 Fast Flow

Product information for SF018-BL:

Introductions:

Blue Sefinose™ 6 Fast Flow is Cibacron™ Blue 3G covalently attached to the Sefinose 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 e.g. NAD+.The highly cross-linked matrix provides a stable, rigid medium.

Medium characteristics

Total binding capacity	>18 mg human serum albumin/ml drained medium
Ligand density	approx 7 µmol Cibacron Blue 3G/ml drained medium
Average particle size	90 µm (45–165 µm)
Bead structure	6% highly cross-linked agarose
Linear flow velocity	About 200-400cm/h 300 cm/h (25 °C, XK 50/30 column,15 cm bed height)
pH stability	Long term 4–12,Short term 3–13
Chemical stability	7 days at 40 °C in: 70% ethanol 6M guanidine hydrochloride 8 M Urea
Temperature stability	4–40 °C
Autoclavable	121 °C for 15 min in distilled water
Storage	+4–8 °C in 0.1 M KH2PO4, pH 8.0 and 20% ethanol

Column packing

Blue Sefinose™ 6 Fast Flow is supplied preswollen in 20% ethanol, 0.1 M KH2PO4, pH 8.0. Decant the 20% ethanol solution and replace it with binding buffer. The binding buffer should not contain agents which significantly increase the viscosity, but the column may be equilibrated with viscous buffers at reduced flow rates after packing is completed.some column ,such as Tricorn™ 10/100, XK 16/20 can be used for lab-scale for Blue Sefinose 6 Fast Flow. BPG 140/500, chromaflow™ 400/100-300,BPG™ 100/500 column can be uesd for process-scale for Blue Sefinose™ 6 Fast Flow.

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 medium 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. Mount the adapter or lid of the packing reservoir and connect the column to a pump. Avoid trapping air bubbles under the adapter or in the inlet tubing.
5. Open the bottom outlet of the column and set the pump to run at the desired flow rate. Ideally, Sefinose 6 Fast Flow media are packed in XK or Tricorn columns in a two-step procedure: Do not exceed 0.5 bar (0.05 MPa) in the first step and 1.5 bar (0.15 MPa) in the second step.If the

packing equipment does not include a pressure gauge, use a packing flow rate of 2.5 ml/min (XK 16/20 column) or 0.9 ml/min (Tricorn 10/100 column) in the first step, and 8.7 ml/min (XK 16/20 column) or 4.7 ml/min (Tricorn 10/100 column) in the second step. for packing flow rates of other columns,please refer to the individual manuals. If the recommended pressure or flow rate cannot be obtained, use the maximum flow rate your pump can deliver. This should also give a wellpacked bed.

Note: For subsequent chromatography procedures, do not exceed 75% of the packing flow rate.

6. Maintain packing flow rate for at least 3 bed volumes after a constant bed height is reached. Mark the bed height on the column.
7. Stop the pump and close the column outlet.
8. If using a packing reservoir, disconnect the reservoir and fit the adapter to the column.
9. 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.
10. Connect the column to a pump or a chromatography system and start equilibration. Re-adjust the adapter if necessary.

Evaluation of packing

To check the quality of the packing and to monitor this during the working life of the column, column efficiency should be tested directly after packing, prior to re-use, and when separation performance is seen to deteriorate.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, As. These values are easily determined by applying a sample such as 1% (v/v) acetone solution to the column. Sodium chloride can also be used as a test substance. Use a concentration of 2.0 M NaCl in water with 0.5 M NaCl in water as eluent.

The calculated plate number will vary depending on the test conditions and it should therefore be used as a reference value only.

It is also important that conditions and equipment are kept constant so that results are comparable. Changes in solute, solvent, eluent, sample volume, flow rate, liquid pathway, temperature, etc., will influence the results. For optimal results, the sample volume should be at max. 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 part of the acceptance criteria for column use

Restore the binding property of old Blue Sefinose™ 6 fast flow:

The procdure 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.
d) For longer periods of storage, e.g. several weeks,we recommend that the resin be stored at +4 to +8 °C in 20 % ethanol, 0.1 M KH2PO4, pH 8.0.