SARTORIUS

Product Datasheet

Blue Trisacryl® M

Affinity Chromatography Resin

Product Information

Blue Trisacryl® M is an affinity chromatographic resin used for the purification of a wide variety of enzymes and proteins such as kinases, albumin, interferons, and some coagulation factors.

The basic matrix is Trisacryl®, a macroporous non ionic resin on which Blue dye is covalently immobilized.

The initial matrix is a copolymer of N-acryloyl-2-amino-2 hydroxymethyl-1,3-propane-diol in bead form. Its macroporous structure allows a good diffusion and improves exchange kinetics.

Blue dye is strongly bound to the matrix through a six carbon spacer arm (Figure 1). The reaction is performed with the EEDQ coupling agent. This type of coupling prevents any leakage of the dye in normal working conditions.

Chemical and mechanical stability

The excellent chemical stability of Blue Trisacryl® M is a function of the synthetic nature of Trisacryl® resin and the enhanced stability of the ligand coupling mechanism.

The resin has an excellent mechanical stability. High flow rates with working pressures up to 3 bar can be used.

Experimental results have shown that the flow rate increases linearly with pressure up to about 40 cm per hour. However, the absolute linear flow rate and its relation to working pressure will vary according to the column height.

Due to their extended lifetime, Blue Trisacryl® M columns do not require frequent repacking, which is cost- and labour-saving.

Experimental results showed no modification of the chromatographic properties of the resin after more than 100 cycles of albumin separation from whole plasma.

On an industrial scale, Blue Trisacryl® M was used over 700 cycles in the production of human albumin from IgG depleted plasma.

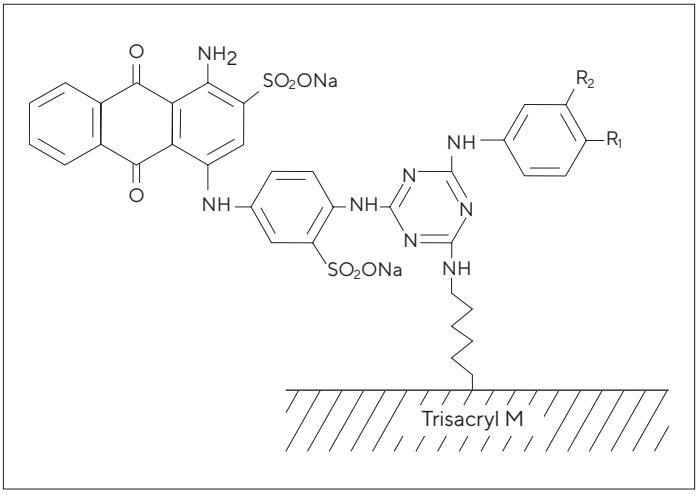


Figure 1: Structure of Blue Trisacryl® M.

Table 1: Main Properties of Blue Trisacryl® M.

Particle size	40 – 80 µm
Exclusion limit	10^7 dt
Capacity for human albumin*	10 – 15 mg/ml
Capacity tor bovine albumin*	5 – 7 mg/ml
Working pH stability	1 – 10
Cleaning pH stability	1 – 12
Heat stabibity	–20°C to +121°C
Pressure stability	Up to 3 bar (45 psi)
Stability to detergents and denaturing agents	Excellent

* Capacity determined in PBS buffer using 5 mg/ml.

Capacity

The binding capacity of Blue Trisacryl® M depends on the protein involved. Additionally, the capacity for a given protein may differ according to the animal species. For example, the binding capacity for bovine albumin is lower than for human albumin. Binding capacity can also vary according to the working conditions, especially pH (see Figure 2).

Due to its binding capacity and its outstanding flow rate, Blue Trisacryl® M is more efficient than any commonly used resin. Its long lifetime and the absence of non-specific adsorption contribute to improve yields.

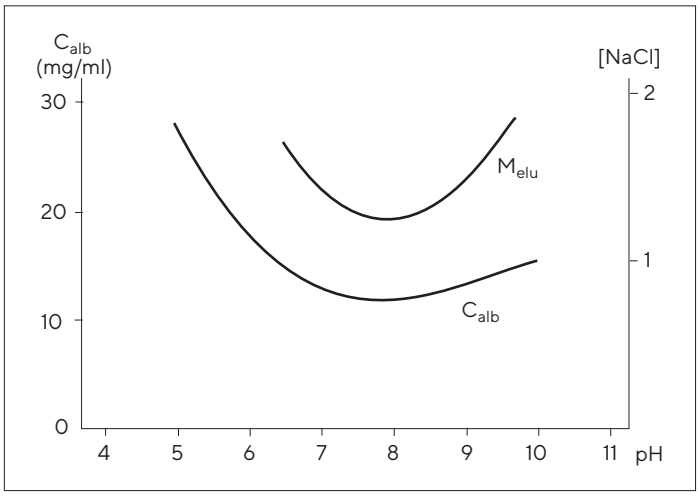


Figure 2. Binding capacity (C_{alb}) and sodium chloride elution molarity (M_{elu}) of human albumin at different pHs.

Delivery and storage

Blue Trisacryl® M is supplied as ready-to-use labpacks suspended in 1 M sodium chloride and 20% ethanol as bacteriostatic.

Before the first use, the resin can be stored at room temperature.

Once opened, Blue Trisacryl® resin or columns must be stored at +4°C in a neutral buffer containing a bacteriostatic agent. They must never be frozen.

Applications

Blue Trisacryl® M can be used for the purification of many proteins. Generally speaking, the Blue dye chromophore interacts with most enzymes which need NAD as cofactor (kinases, dehydrogenases, phosphatases). Other proteins with a non-enzymatic activity can also be separated: albumins, lipoproteins, growth factors such as maturation promoting factor and heparin-binding growth factors, clotting factors, interferons (Example I) and plasma proteins.

The interaction mechanism between Blue dye and proteins involves one or more of the following:

- Stereospecific recognition of NAD analogs,
- Electrostatic and hydrophobic interaction,
- Electron exchange.

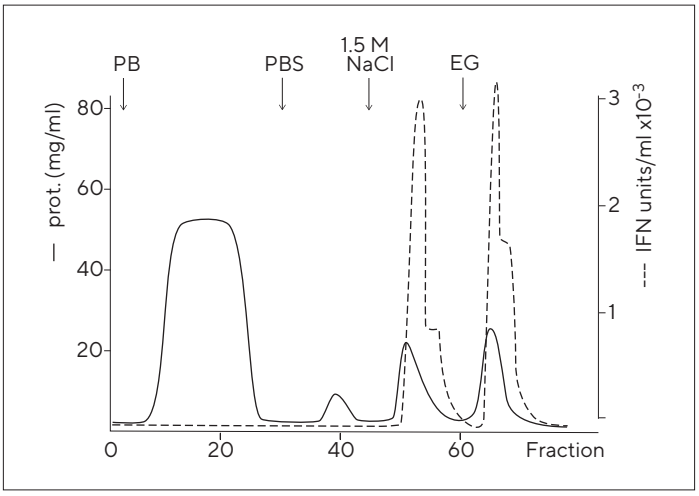
Due to its high selectivity and resolution power, Blue Trisacryl® M may be used on the laboratory level for rapid analytical and preparative separations of numerous proteins (Example II). The separation speed allows several chromatographic cycles to be performed in a single day.

On the industrial level, Blue Trisacryl® M is the ideal resin for human albumin separation in a single step, for interferon purification and for TPA isolation.

Blue Trisacryl® M may be used in any other purification of Blue dye-affine proteins. Many other enzymes may be purified on Blue Trisacryl® M, such as sulfatases, phosphatases, RNA polymerases, mono-oxygenases, and oxydoreductases.

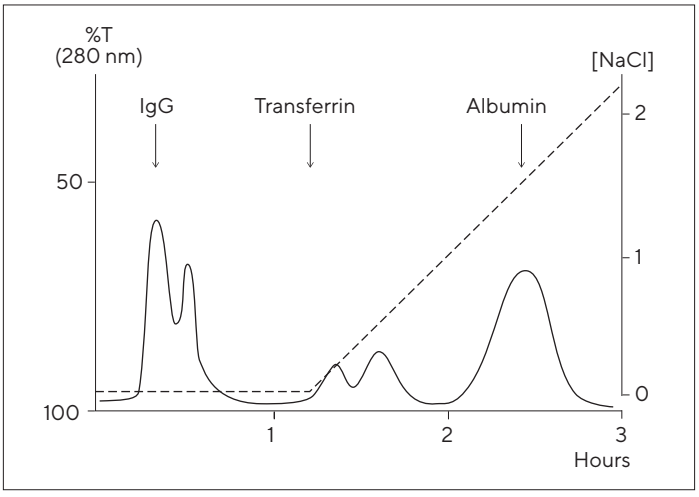
Application examples

Example I: Separation of phytohemagglutinininduced interferon (-IFN) on Blue Trisacryl® M.
Courtesy of S. Stefanos, J. Wietzerbin, published by Mary Ann Liebert Inc. Publications, NY.



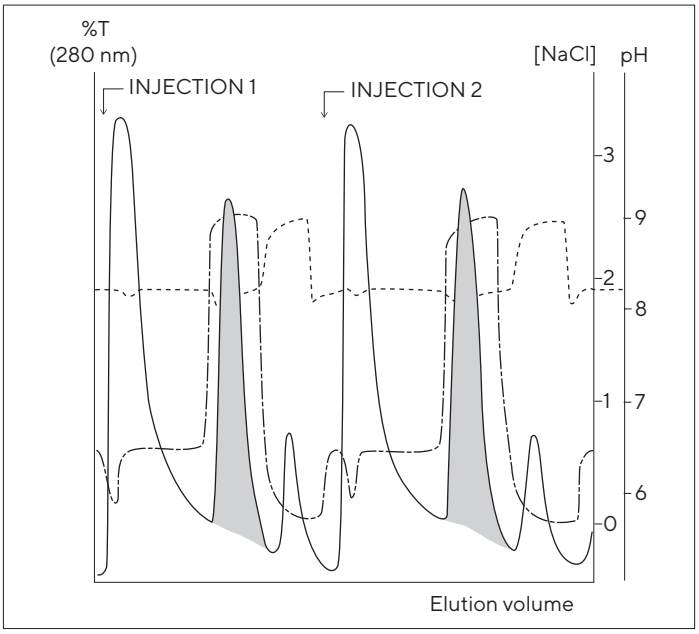
— Protein concentration ---- Interferon activity
PB = 0.02 M phosphate buffer, pH 7.2
PBS = phosphate buffer saline
EG = ethylene-glycol mixed with PBS (50:50)

Example II: Analytical separation of human plasma proteins on Blue Trisacryl® M.



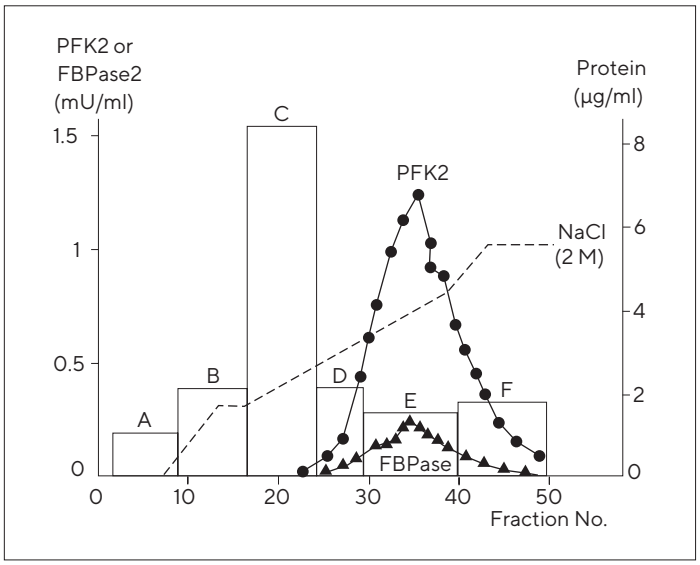
Column: 1.6 cm I.D. x 10 cm; Buffer: 0 05 M Tris-HCl, pH 8 8; Elution performed by a continuous sodium chloride gradient from 0 to 3 M; Flow rate: 100 cm/h; Separation time: 180 min; Temperature: 20°C.

Example III: Automatic separation of human albumin from plasma on Blue Trisacryl® M by step elution chromatography.



Column: 2.5 cm I.D. x 6 cm; Adsorption buffer: 0.05 M Tris-HCl, 0 5 M NaCl, pH 8; Albumin elution buffer: 2.5 M NaCl in the same buffer; Regeneration solution: water-ethylene glycol mixture (50:50).
—— UV absorbance at 280 nm
--- Ionic strength ---- pH

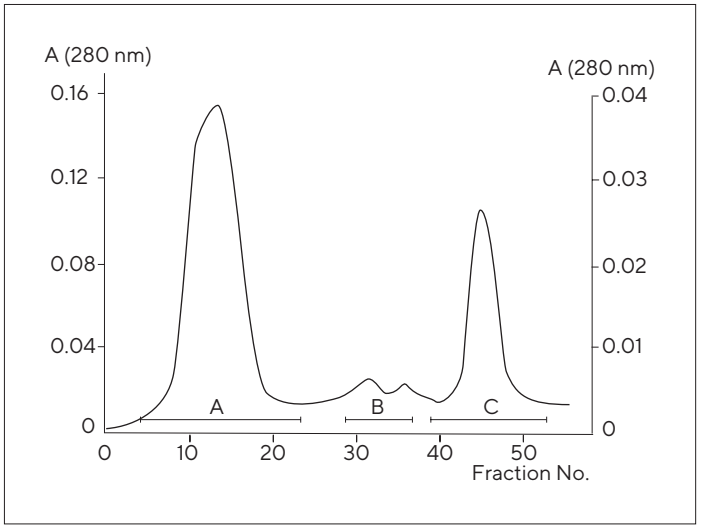
Example IV. Purification of phosphofructokinase-2/fructose-2,6-bisphosphatase (PFK 2/FBP) on a Blue Trisacryl® M column from an anion exchange chromatography of a spinach extract.
(Courtesy of Y. Larondelle, E. Mertens, E. Van Schaftingen & H.G. Hers, Université Catholique de Louvain, Eur. J. of Biochem.).



21 mU ot PFK 2/FBP were injected into a 2 cm I.D. x 0.9 cm Blue Trisacryl® M column pre-equilibrated with 25 mM Tris-acetate buffer, pH 7.8 containing 5 mM magnesium acetate, 5 mM dithiothreitol and 0.2% Triton X-100. Elution was performed by a sodium chloride gradient up to 2 M. For the determination of proteins (histograms), six pools (A to F) were prepared and concentrated. The purification factor from the previous column was 27.6; The recovery was approximately 44%.

Example V: Purification of MPF (Maturation-Promoting Factors) on a Blue Trisacryl® M column from a hydroxyapatite fraction obtained using a x.laevis unfertilized egg extract.

Courtesy of P. Nguyen-Gia, M. Bomsel, J.P. Labrouase, C.L. Gallien & H. Weintraub, CNRS, Unité d'Enseignement et de Recherche Biomédicale des Saints-Pères, Paris, Eur. J. of Biochem.



7 ml (12 mg/ml) of the MPF extract were applied to a 10 ml Blue Trisacryl® M column pre-equilibrated with 80 mM sodium glycerol-2-phosphate, 20 mM EGTA, 15 m M MgCl2, 1 mM dithiothreitol, ATP and phenyl-methylsulfonylfluoride. The MPF activity was retained and eluted with the same buffer containing 500 mM NaCl.
The horizontal lines show fractions pooled to give three sets: Inactive peaks A and B (emerging with the flowthrough and eluted with extraction buffer containing 100 mM NaCl), and the active MPF (peak C) eluted with extraction buffer and 500 mM NaCl.
Left absorbance axis relates to peak A, right axis to peaks B and C.
Purification factor from the previous column was 12. Recovery was 51%.

Ordering Information

Product	Cat. No.	Size
Blue Trisacryl® M	25896-045	25 mL
	25896-010	100 mL
	25896-028	1 L
	25896-044	5 L
	25896-036	10 L

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