- **Chemical treatments**

The following procedures are recommended:

Method	Procedure
Diethylpyro-carbonate-ethanol washing	- Wash 100 mL of Blue **Trisacryl** M with 1 M sodium chloride. Mix the sorbent with 50 mL of 1 M sodium chloride. -Prepare a 2-5% solution of diethylpyrocarbonate in pure ethanol. Add 5 mL of this solution to the sorbent suspension. -Stir for 2-5 hours at 20-25°C (68-77°F) in sterile conditions (do not use magnetic stirrer). -Wash with a sterile buffer.
Ethanol - acetic acid washing	Wash with at least 3 cv of a solution of 20% ethanol containing 1 M acetic acid. One hour contact time.

After sanitization, the column must be reequilibrated in the normal sterile pyrogen-free buffer.
For more information, please contact our technical service.

Thermal Stability and Storage

Temperature of use	2-30°C (36-86°F)
Shipping temperature	Ambient
Storage temperature	2-8°C (36-46°F)
Recommended storage solution (between runs)	Neutral buffer containing bacteriostatic agents such as 1 M NaCl and 20% (v/v) ethanol.

Ordering information

Pack size	Part Number
5 mL	25896-051
25 mL	25896-045
100 mL	25896-010
1 L	25896-028
10 L	25896-036

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BioSepra™ Blue Trisacryl® M

Blue Trisacryl® M

Affinity Chromatography Sorbent

Product Description

Blue **Trisacryl** M is an affinity chromatographic sorbent 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** GF2000 macroporous non ionic support on which Cibacron Blue has been covalently immobilized.

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

Properties

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
pH stability	1-10
Heat stability	-20°C to 121°C (-4°F to 250°F)
Pressure stability	Up to 3 bar (44 psi)
Stability to detergents and denaturing agents	Excellent

* Capacity determined in PBS using 5 mg/mL.

Column preparation

- **Before a first use**

- Wash the sorbent (on frit or in batch) with 5 volumes of 1 M NaCl. The sorbent does not leak any blue dye. A washing solution with 6 M urea can be used to elute any possible non-covalently bound dye.

- Equilibrate the sorbent in the buffer chosen for the chromatography until the required conditions are reached (pH, ionic strength, ...).

- Add to the sorbent an equivalent amount of buffer and stir the solution to obtain a homogeneous suspension. Transfer it to a vacuum flask. The vacuum flask should be 4-5 times bigger than the sorbent volume.

• Elimination of dissolved gas

Carefully degas the suspension under vacuum while gently shaking (do not use magnetic stirrers).

• Column packing

Packing a Blue **Trisacryl** M column may be done under normal or medium pressure.

- Set up the column and insert the lower adapter.

- Connect a tubing of about 1 m (39.4 in.) long to the adapter outlet.

- Add a syringe to the outlet tubing, then add degassed buffer up to a 1-2 cm (0.4-0.8 in.) height. Check that all air bubbles are thoroughly eliminated.

- Clamp the column outlet tubing and remove the syringe.

- If necessary, put a filling reservoir on the top of the column. Pour the degassed suspension into the column.

- Allow it to settle for a few seconds. Open the column outlet and set the hydrostatic pressure at a height of at least 80 cm (31 in.).

The column packing is considered terminated when the support is totally sedimented. Never let the upper column packing dry (add buffer it necessary).

- Close the column outlet and dip the upper adapter a few millimeters into the sorbent.

- After checking air bubble elimination, clamp the outlet of the upper tubing.

- Connect a pump to the column inlet.

Column washing and equilibration

- After packing the column, run through the support 3 volumes of buffer (use the same buffer as previously) but at a concentration of 0.5 or 1 M.

- At the end of equilibration, make sure that the ionic strength and pH are identical at both the outlet and inlet.

Sample application

- Check for the absence of bubbles.

- Inject the sample into the column through the pump tubing, then connect the tubing to a buffer reservoir or a stepwise system.

- Start simultaneously the pump, the recorder and the elution gradient maker.

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Working flow rate

Blue **Trisacryl** M is a semi-rigid sorbent. It will withstand high flow rate without undergoing any shrinking phenomenon, provided the column has been prepared according to our recommendations.

When high flow rate are to be used, it is advisable to pack the column at an operating pressure of 0.5 bar (7 psi) higher than the required pressure during the normal run.

Elution

When using Blue **Trisacryl** M for the first time for a particular sample, the following procedure is recommended:

- Make an ionic strength linear gradient using sodium chloride up to 3 M, pH 6.9.

- After running, optimize the elution conditions, particularly the final NaCl concentration. The elution can also be performed more specifically by cofactor gradients (NAD, NADP) or with more energic eluents such as ethylene-glycol, urea, potassium isothiocyanate.

Regeneration and Maintenance

In order to avoid frequent regeneration, it is advised to introduce into the column only samples and buffers which are perfectly clear and previously filtered through a 0.22 µm membrane. Verify that the changes in pH and ionic strength which occurred in elution will not cause precipitation of certain components of the samples.

After repeated uses and if necessary, Blue **Trisacryl** M may be regenerated in column or in batch. The regeneration procedure must be adapted to the type of adsorbed material to be eliminated. The following suggestions must be checked first for their degree of efficiency:

Situation	Recommendation
General Cleaning-In-Place (CIP)	Wash with 3 M sodium chloride and demineralized water. Make repeated and alternated washings with buffer solutions at acidic pH (2-3) and basic pH (9-10).
Adsorbed Contaminants	Wash with chaotropic agents such as 6-8 M urea, 6 M guanidine hypochloride or 50% ethylene-glycol (v/v).

After regeneration treatment, reequilibrate Blue **Trisacryl** M in the starting buffer.

Sanitization

• Autoclaving

Equilibrate the gel in a neutral buffer The buffer should not contain phosphate and other products unstable at high temperature. Autoclave the sorbent at 121°C (250°F) for 20 min.