Capto Blue affinity chromatography resin

Product Information

Cat#No#	Ca-353C

Product Overview

Capto Blue affinity resin with Cibacron Blue ligand has outstanding pressure and flow properties, allowing rapid processing of large sample volumes of albumin and other proteins.
Excellent chemical stability ensures tolerance of harsh solvents used in repeated cleaning-in-place and sanitization procedures.
Capto Blue can be autoclaved repeatedly.
Highly rigid agarose base matrix allows high flow rates and processing of large sample volumes with no reduction in binding capacity. Ligand functionality can be modified through the use of appropriate buffer salts and buffer conductivity to increase selectivity for desired targets.
Excellent choice for the removal or purification of proteins at both laboratory and process scales.
Resin fulfills industrial demands for security of supply, robust performance, and regulatory support.

Description

Capto Blue is affinity chromatography media (resins) for the capture of human serum albumin (HSA), as well as purification of HSA fusion proteins, blood coagulation factors, enzymes, and recombinant proteins in laboratory and process scales. Developed from Blue Sepharose 6 Fast Flow, Capto Blue products are more chemically stable and have a more rigid agarose base matrix than their predecessor. These improvements allow the use of higher flow rates and larger sample volumes, enabling increased throughput and improved process economy.

Characteristic

Excellent chemical stability for tolerance to the harsh solvents used in repeated cleaning-in-place (CIP) and sanitization procedures. Highly rigid agarose base matrix allows high flow rates and processing of large sample volumes.
Ligand functionality may be modified through the choice of buffer salt and conductivity to increase selectivity for desired targets.
Excellent choice for the removal or purification of proteins in both laboratory and process scales.

Applications

Tel: 1-631-559-9269 1-516-512-3133
Email:info@creative-biomart.com
Fax:1-631-938-8127
45-1 Ramsey Road, Shirley, NY 11967, USA

CREATIVE BIOMART®
Chromatography

Capto Blue affinity chromatography resin

Capture of human serum albumin (HSA).
Purification of HSA fusion proteins.
Purification of blood coagulation factors, enzymes, and recombinant proteins.

Maximum operating pressure
300 kPa at 600 cm/h, 1 m diameter column, 20 cm bed height
Sample preparation
HSA, 1 mg/mL
Medium Preparation
Capto Blue media are based on a highly rigid agarose base matrix that offers outstanding pressure and flow properties, allowing rapid processing of large sample volumes. The Cibacron Blue ligand is attached to the base matrix via a hydrophilic spacer and is immobilized with a stable amine bond.
Ligand Coupling Method
Ether linkages and hydrophilic spacer arm
Packing Column
All large-scale columns can be supplied as variable bed height columns. Do not choose large diameter columns if the bed height is low.
Column
Tricorn 5/100 (10 cm bed height)
Matrix
Highly cross-linked agarose
Average particle size
~75 µm
Ligand
Cibacron blue
Ligand density

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Approx. 13 μmol/ml
Dynamic binding capacity
> 24 mg HSA/mL resin (Qb10 at 4 min residence time)
Recommended flow rate
300 kPa at 600 cm/h, 1 m diameter column, 20 cm bed height
Recommended column height
20 cm
Chemical stability
Stable to commonly used aqueous buffers:0,5 M NaOH, 8 M urea, 6 M guanidine hydrochloride.
pH working range
2–13
CIP stability
2–13
Storage
2 to 8°C, Solution of 0.1 M KH2PO4 and 20% Ethanol; 0.1 M Potassium Phosphate containing 20% Ethanol
Binding buffer
20 mM citric acid, pH 5.5
Elution buffer
50 mM sodium phosphate, 2 M NaCl, pH 7
Evaluation of Packing
Test the column efficiency to check the quality of packing. Testing should be done after packing, at regular intervals during the working life of the column or 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% acetone solution to the column. Sodium chloride can also be used as a test substance.

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Use a concentration of 0.8 M NaCl in water with 0.4 M NaCl in water as eluent.

Binding

1.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.
2.After the sample has been loaded, wash the medium with binding buffer until the base line is stable.

Equilibration

After packing, and before a chromatographic run, equilibrate with start buffer by washing with at least 5 bed volumes, or until the column effluent shows stable conductivity and pH values.

Elution

1.Elution conditions vary with the sample. Elution may be accomplished by a change in pH, polarity (e.g., ethylene glycol) 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 may be used.

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 TrisHCl, 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.

Cleaning-in-place

Remove precipitated proteins by:1.washing the column with 4 column volumes (CV) of 0.5 M NaOH at 40 cm/h, followed by washing the column with 3–4 CV of 70% ethanol or 2 M potassium thiocyanate.
2.Alternatively, wash the column with 2 CV of 6 M guanidine hydrochloride. 3.In both cases wash immediately with at least 5 CV filtered binding buffer, pH 8.0.
Remove strongly bound hydrophobic proteins, lipoproteins and lipids by:1.washing the column with 3–4 CV of up to 70% ethanol or 30% isopropanol. 2.Alternatively, wash the column with 2 CV detergent in a basic or acidic solution, e.g., 0.1% non-ionic detergent in 1 M acetic acid. Wash at a flow rate of 40 cm/h. Remove residual detergent by washing with 5 CV of 70% ethanol. 3.In both cases wash immediately with at least 5 CV

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filtered binding buffer, pH 8.0.

Sanitization

Capto Blue (high sub) can also be sanitized by autoclaving, which is particularly appropriate if microbial contamination is suspected. When Capto Blue (high sub) was autoclaved 10 times (121°C, 2.5 to 2.7 bar), no decrease in ligand density was observed. Capto Blue has not been exposed to autoclaving but is expected to exhibit the same tolerance as Capto Blue (high sub).

Scaling up

Scale-up is typically performed by keeping bed height and linear liquid velocity constant while increasing bed diameter and volumetric flow rate. However, since optimization is preferentially performed with small column volumes, in order to save sample and buffer, some parameters such as the dynamic binding capacity may be optimized using shorter bed heights than those being used in the final scale. As long as the residence time is kept constant, the binding capacity for the target molecule remains the same.

Other factors, such as clearance of critical impurities, may change when column bed height is modified and should be validated using the final bed height.

The residence time is approximated as the bed height (cm) divided by the linear liquid velocity (cm/h) applied during sample loading. Select the bed volume according to required binding capacity. Keep sample concentration and gradient slope constant.

The larger equipment used when scaling up may cause some deviations from the method optimized at small scale. In such cases, check the buffer delivery system and monitoring system for time delays or volume changes. Different lengths and diameters of outlet tubing can cause zone spreading on larger systems.

Purification procedures

1.Pack a column with Capto Blue (or use the prepacked HiScreen Capto Blue) or Capto Blue (high sub).
2.Wash the medium bed with binding buffer.
3.Adjust the sample pH to that of the binding buffer (e.g., binding of HSA occurs at pH 5.5).
4.Filter the sample through a 0.22 to 0.45 µm filter.
5.Load the sample.
6.Wash the medium bed with binding buffer to remove weakly bound proteins.
7.Optimize elution conditions to attain maximum purity and throughput of the captured proteins. Bound proteins can be eluted, for example, by the addition of NaCl.

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Pack size
25 mL
BioProcess resin
Yes
Dimensions
1 m

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