Capto Blue

Capto™ Blue is an affinity medium for the capture of albumin, as well as purification of enzymes and recombinant proteins at laboratory and process scale. Developed from Blue Sepharose™ 6 Fast Flow, Capto Blue is more chemically stable and has a more rigid agarose base matrix than its predecessor. These improvements allow the use of faster flow rates and larger sample volumes, leading to higher throughput with no significant reduction in binding capacity.

Capto Blue offers the following benefits:

- Excellent chemical stability ensures tolerance of harsh solvents used in repeated cleaning-in-place and sanitization procedures
- Capto Blue can be repeatedly autoclaved
- Highly rigid agarose base matrix allows high flow rates and processing of large sample volumes with no reduction in binding capacity
- Ligand functionality may 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

Principles

Capto Blue is a medium for affinity chromatography that can be used in the same way as Blue Sepharose 6 Fast Flow. Binding of albumin occurs at neutral pH and elution is performed by increasing the conductivity using sodium chloride. The Cibacron™ Blue ligand contains sulfonic groups that can take part in ion exchange interactions and also groups that can bind to the target molecule by hydrophobic interactions.

Depending on the target molecule, the effect of these groups can be enhanced or weakened by the choice of buffer salt and conductivity. To increase yield or to regenerate the chromatography medium, elution with salt can be complemented by adding an organic solvent such as ethanol or by changing pH.

Fig 1. Capto Blue is an affinity medium for the capture of albumin, as well as purification of enzymes and recombinant proteins at laboratory and process scale.

A typical protocol for purification using Capto Blue is described as follows:

1. Pack a column with Capto Blue.
2. Wash the medium bed with starting buffer.
3. Adjust the sample pH to that of the starting buffer, for example, optimal binding of human serum albumin (HSA) occurs at neutral pH.
4. Filter the sample through a 0.22 to 0.45 µm filter.
5. Load the sample.
6. Wash the medium bed with starting buffer to remove weakly bound proteins.
7. Optimize elution conditions to attain maximum purity and throughput of the captured proteins. Bound proteins may be eluted, for example, by the addition of NaCl.

Characteristics of the medium

Capto Blue medium is based on a highly rigid agarose base matrix which 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 (Fig 2). The main characteristics of Capto Blue are shown in Table 1.

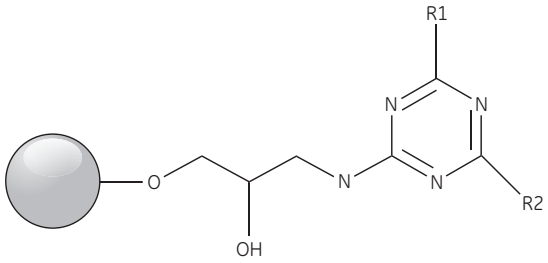


Fig 2. Capto Blue medium showing the base matrix, spacer, and ligand.

Table 1. Physical and performance characteristics of Capto Blue

Matrix	Highly cross-linked agarose
Average particle size	75 µm
Ligand	Cibacron Blue
Ligand density	11 to 16 µmol/ml
Dynamic binding capacity of HSA at 10% breakthrough	12 mg/ml at 4.0, 2.0, and 1.3 min residence times
Total binding capacity (HSA)	~25 mg/ml
Maximum flow velocity	At least 600 cm/h in a 1 m column with 20 cm bed height at 20°C using process buffers with the same viscosity as water; corresponds to a residence time of 2 min
pH stability (long and short term)	2 to 13

Cibacron Blue binds targets through a combination of aromatic and electrostatic interactions, which makes the medium suitable for many different applications. The ligand has structural similarities to naturally occurring molecules such as the cofactor NAD^+, which enables it to bind a wide range of proteins.

Binding capacity

The binding capacity of Capto Blue for HSA was evaluated in terms of the 10% breakthrough (Q_{B10}, the point that 10% of unbound HSA is detected in collected fractions). The binding capacity of Capto Blue was found to be 12 mg/ml when the residence time (the time taken for a nonbinding molecule to transit the medium) was 2 min (data not shown). Shorter residence times (i.e., the use of faster flow rates) were not shown to result in any significant loss in binding capacity.

The conditions used in the evaluation of binding capacity at 10% breakthrough are shown in Table 2.

Table 2. Conditions used in the evaluation of binding capacity of Capto Blue for HSA at 10% breakthrough

Column	Tricorn™ 5/100 (10 cm bed height)
Sample	HSA, 4 mg/ml
Starting buffer	50 mM sodium phosphate, pH 7
Elution buffer	50 mM sodium phosphate, 1.5 M KCl, pH 7
Flow rate	300 cm/h
Residence time	2 min
Dynamic binding capacity	~12 mg/ml

Purification of HSA from plasma

The selectivity of Capto Blue was verified in a one-step purification of HSA from serum (Fig 3). The loaded, washed and eluted samples were analyzed by SDS-PAGE (Fig 4).

Column: Tricorn 10/100 (10 cm bed height)
Equilibration: 10 column volumes (CV) of 50 mM Tris, 500 mM NaCl, pH 8, 535 cm/h
Load: 2.5 ml of filtered human serum, 300 cm/h
Wash: 10 CV of 50 mM Tris, 500 mM NaCl, pH 8, 535 cm/h
Elution: 7.5 CV of 50 mM Tris, 200 mM sodium thiocyanate (NaSCN), 300 cm/h

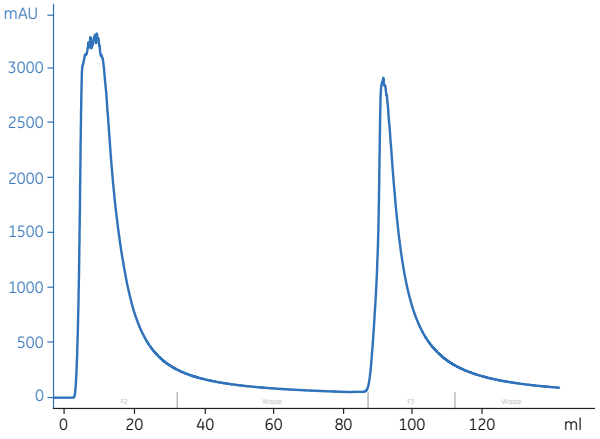
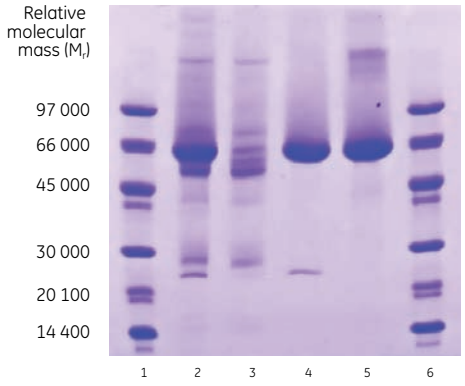


Fig 3. Chromatogram of the purification of HSA from serum using Capto Blue. The total load of HSA corresponds to 50% of the maximum capacity.



Lane
1. Low Molecular Weight Marker Kit
2. Serum
3. Flowthrough
4. Eluate
5. HSA reference sample
6. Low Molecular Weight Marker Kit

Fig 4. SDS-PAGE using PhastGel™ Gradient 10–15 and PhastSystem™ of whole human serum before application to and elution from Capto Blue under reducing conditions. Note that most HSA binds to the Capto Blue chromatography medium (lane 3) and that the purity (lane 4) is similar to the reference sample (lane 5).

Cleaning-in-place and sanitization

A cleaning or sanitization protocol should be designed for each application in order to prevent the accumulation of chemical or microbial contaminants over time.

The tolerance of Capto Blue to cleaning with NaOH was evaluated by studying binding capacity for HSA after repeated exposure to NaOH. Each purification cycle consisted of equilibration, loading of plasma, elution, and cleaning-in-place (CIP) with 0.5 M NaOH for 30 min. After every tenth cycle, the total binding capacity of the medium for HSA was determined. Figure 5 shows that the binding capacity for HSA over 50 cycles of CIP remained similar to the initial measurement and that 0.5 M NaOH can therefore be used for routine CIP.

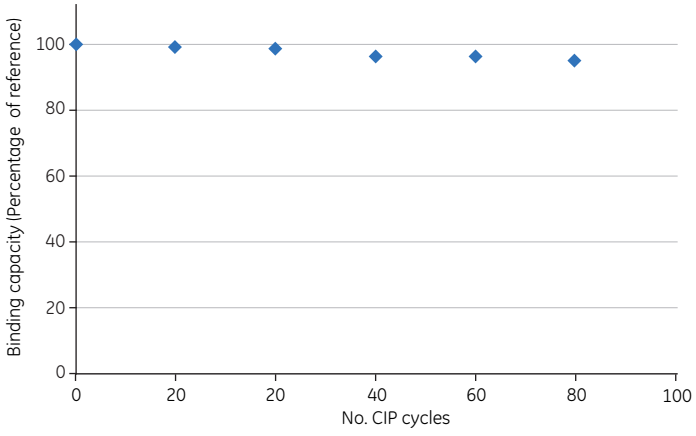


Fig 5. Cleaning-in-place with 0.5 M NaOH.

Capto Blue can also be sanitized by autoclaving, which is particularly appropriate if microbial contamination is suspected. When Capto Blue was autoclaved 10 times (121.1°C, 2.5 to 2.7 bar), no decrease in ligand density was recorded.

Chemical stability and recommended storage

To evaluate chemical stability, Capto Blue was stored at room temperature for three weeks in the storage solutions listed in Table 3. No change in ligand density was observed following storage in any of the storage solutions. Although exposure to 0.1 M HCl did not decrease ligand density, this solution may hydrolyze the base matrix over extended periods of contact and is thus not recommended for prolonged storage.

Capto Blue is supplied, pre-swollen, in a solution of 0.1 M KH_2PO_4 and 20% ethanol, pH 8. The medium should be stored in this solution at 4°C to 8°C.

Table 3. Storage solutions used in the study of chemical stability of Capto Blue

0.1 M KH_2PO_4 + 20% ethanol (pH 8)
8 M urea
0.1 M NaOH + 20% ethanol
6 M guanidine-HCl
0.01 M HCl
0.1 M HCl
0.1 M NaOH
0.5 M NaOH

Ordering information

Product[1]	Quantity[2]	Code no.
Capto Blue	25 ml	17-5448-01
Capto Blue	500 ml	17-5448-02

[1] Part of the Custom Designed Media program from GE Healthcare
[2] Larger quantities are available, please contact your local GE Healthcare representative

Capto Blue is made available through Custom Designed Media. The product is not a stock item, but usually only produced on order. Thus delivery time is typically longer than for standard products and may vary depending on quantity and availability of raw materials. The product is in late development phase, but not yet with a validated production process. For Custom Designed Media products, scale up and validation is performed when sales orders for a suitable batch size are received.

For contact information for your local office, please visit, www.gelifesciences.com/contact

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