Capto™ Blue and Capto Blue (high sub)

Capto Blue and Capto Blue (high sub)† are 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 (Fig 1). 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.

Capto Blue and Capto Blue (high sub) offer the following benefits:

- 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

Principles

Capto Blue and Capto Blue (high sub) affinity chromatography media can be used in the same way as Blue Sepharose 6 Fast Flow. Binding of albumin occurs near pH 5.5 and elution is performed by increasing pH and the conductivity by using sodium chloride. The Cibacron™ Blue ligand contains sulfonic groups that can take part in ion exchange interactions and other groups that can bind to the target molecule by hydrophobic interactions.

Depending on the target molecule, the effects 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 with addition of an organic solvent such as ethylene glycol.

Fig 1. Capto Blue and Capto Blue (high sub) are affinity media for the capture of albumin, as well as purification of HSA fusion proteins, enzymes, and recombinant proteins in laboratory and process scales.

A typical protocol for purification is as follows:

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.

† Capto Blue (high sub) is part of the Custom Designed Media line and available by request.

Characteristics of the chromatography medium

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 (Fig 2). The main characteristics of Capto Blue and Capto Blue (high sub) are listed in Table 1.

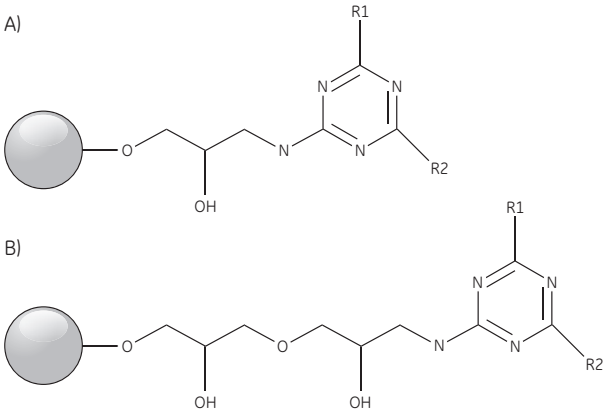


Fig 2. Image of (A) Capto Blue and (B) Capto Blue (high sub) media, showing the base matrix, spacer, and ligand.

Table 1. Physical and performance characteristics of Capto Blue and Capto Blue (high sub)

Matrix	Highly cross-linked agarose
Average particle size	75 µm
Ligand	Cibacron Blue
Ligand density	~ 18 µmol/mL, Capto Blue (high sub) ~ 13 µmol/mL, Capto Blue
Dynamic binding capacity of HSA at 10% breakthrough	30 mg/mL at 4 min residence time for Capto Blue (high sub) 24 mg/mL at 4 min residence time for Capto Blue
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 operational during CIP	 3 to 13 2 to 13.5

Cibacron Blue binds the target molecule 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 and recovery

The binding capacity for HSA was evaluated at 10% breakthrough (Q_{B10}, the point where 10% of unbound HSA is detected in collected fractions).

The conditions used in the evaluation of binding capacity are listed in Table 2.

Table 2. Conditions used in the evaluation of binding capacity of Capto Blue and Capto Blue (high sub) for HSA at 10% breakthrough in comparison with Blue Sepharose 6 Fast Flow

Column	Tricorn™ 5/100 (10 cm bed height)
Sample	HSA, 1 mg/mL
Binding buffer	20 mM citric acid, pH 5.5
Elution buffer	50 mM sodium phosphate, 2 M NaCl, pH 7
Flow velocity	150 cm/h
Retention time	4 min

Figure 3 shows the breakthrough curves for the three products. The results show that the dynamic binding capacity for Capto Blue (high sub) is 25% higher than that of Capto Blue. Capto Blue and Blue Sepharose 6 Fast Flow dynamic binding capacities are comparable. The Capto base matrix tolerates a much higher flow rate and bed height compared with the Sepharose base matrix. Hence, there is much to gain in terms of productivity, especially in equilibration, wash, elution and CIP with the use of Capto Blue products.

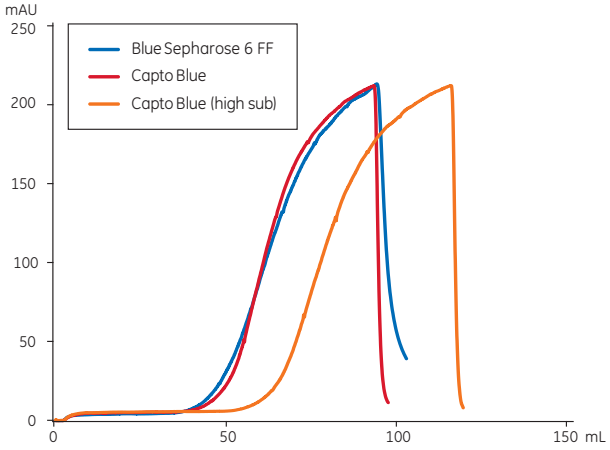


Fig 3. Breakthrough (Q_{B10}) curves for Blue Sepharose 6 Fast Flow (FF), Capto Blue, and Capto Blue (high sub).

Figure 4 shows how the dynamic binding capacity increases with an increased residence time until a plateau is reached. A more concentrated feed also resulted in a higher binding capacity (data not shown).

In a recovery study, 70% of Q_{B10} for HSA was loaded. The study shows similar results for Capto Blue and Blue Sepharose 6 Fast Flow, whereas Capto Blue (high sub) binds more strongly to HSA. To improve recovery for Capto Blue (high sub), 20% ethylene glycol was added to the elution buffer (Table 3). With 50% ethylene glycol, recovery in the elution peak was 95%.

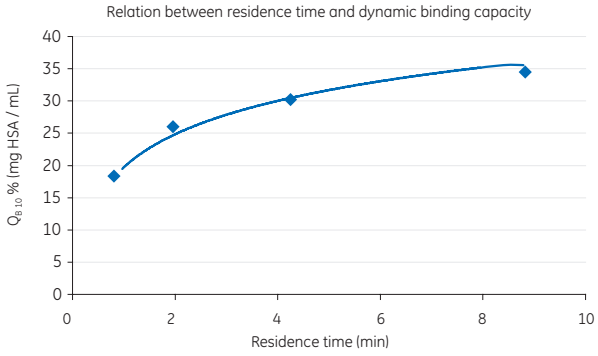


Fig 4. Dynamic binding capacity relative to residence time for Capto Blue (high sub). The dynamic binding capacity increases with increased residence time until a plateau is reached.

Table 3. Dynamic binding capacity at Q_{B10} and recovery of HSA for Blue Separose 6 FF, Capto Blue, and Capto Blue (high sub)

	Blue Sepharose 6 FF[1]	Capto Blue[1]	Capto Blue (high sub)[1]	Capto Blue (high sub)[2]
Q_{B10} (mg/mL)	22	24	30	
Load (mg)	30.9	32.5	41.7	41.7
Eluate (mg)	27.7	29.1	26.6	34.5
Recovery (%)	90	90	64	83
CIP (mg)	1.6	7.,4	19.7	5.6
Recovery (%)	95	113	111	96

[1] Elution buffer: 50 mM NaH_2PO_4 + 2 M NaCl, pH 7
[2] Elution buffer: 50 mM NaH_2PO_4 + 2 M NaCl, pH 7 + 20% (w/w) ethylene glycol

Binding buffer: 20 mM citric acid, pH 5.5
CIP: 10 mM NaOH (Blue Sepharose 6 FF), 0.5 M NaOH (Capto Blue products)
Sample: HSA in binding buffer ~ 1 mg/mL

Figure 5 shows the chromatograms from the recovery study. Flowthrough and wash were equal with baseline and samples were only analyzed sporadically to verify that they contained very little protein.

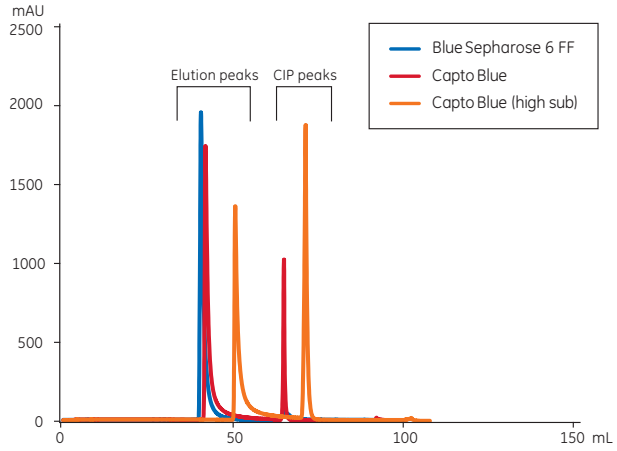


Fig 5. Comparative chromatograms from the HSA recovery study including Blue Sepharose 6 FF, Capto Blue, and Capto Blue (high sub). HSA was eluted with 50 mM NaH2PO4 + 2 M NaCl, pH 7.

Small-scale column formats for fast screening and method development

Using a small-scale column format in screening for the most suitable chromatography process conditions at early stages of process development saves both time and sample. Capto Blue is available in the small, prepacked HiScreen column format (4.7 mL). Together with a chromatography system, such as ÄKTA™ avant, prepacked HiScreen columns are convenient to use when developing an efficient and robust separation method. Further development and optimization using HiScale™ columns permit straightforward scale-up. Basic characteristics of HiScreen prepacked columns are summarized in Table 4.

Table 4. Main characteristics of prepacked HiScreen Capto Blue columns

Column volume	4.7 mL
Column dimensions	0.77 × 10 cm
Maximum flow velocity	600 cm/h
Recommended flow velocities	150–300 cm/h
Maximum back pressure over the packed bed	3 bar (0.3 MPa)
Maximum pressure over the hardware	8 bar (0.8 MPa)

Purification of HSA from plasma

The selectivity of Capto Blue and Capto Blue (high sub) for HSA was verified in a one-step purification of HSA from serum. The result from HSA purification using Capto Blue (high sub) is shown in Figure 6. Similar result was obtained when using Capto Blue. Samples and fractions were analyzed by SDS-PAGE (Fig 7). The selectivity of the media for HSA was high and the eluted fraction had purity similar to the reference material.

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), pH 8, 300 cm/h

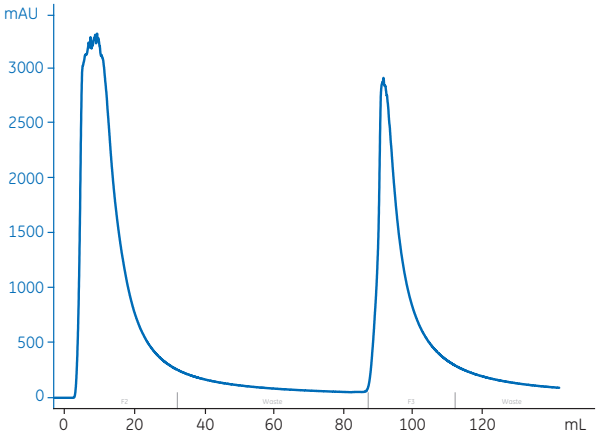
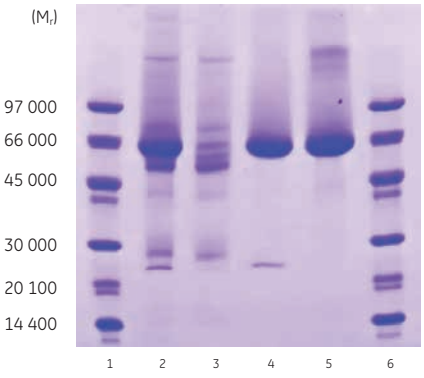


Fig 6. Purification of HSA from serum using Capto Blue (high sub). The total load of HSA corresponds to 50% of the maximum binding capacity.



Lane
1. Low molecular weight marker
2. Serum
3. Flowthrough
4. Eluate
5. HSA reference sample
6. Low molecular weight marker

Fig 7. SDS-PAGE using PhastGel* Gradient 10–15 and PhastSystem* analysis of whole human serum before application and eluate from Capto Blue (high sub) under reducing conditions (similar results were obtained when using Capto Blue) using PhastGel™ Gradient 10–15 and PhastSystemTM electrophoresis unit. Note that most HSA binds to the Capto Blue (high sub) (lane 3) and that the purity (lane 4) is similar to the reference sample (lane 5).

Cleaning-in-place and sanitization

A lifetime study was performed on Capto Blue media to determine the stability towards repeated CIP cycles using 1.5 M sodium hydroxide (NaOH). Capto Blue and Capto Blue (high sub) were exposed to 200 CIP cycles each, using 1.5 M NaOH. A 5 mL HSA sample was applied to the columns between each cycle. Binding capacity and chromatographic performance were determined by UV measurements (280 nm). The results show that Capto Blue and Capto Blue High Sub tolerate repeated CIP with up to 1.5 M NaOH without loss of capacity or chromatographic performance (Fig 8). The total contact time with the CIP solution (1.5 M NaOH) was 55 hours.

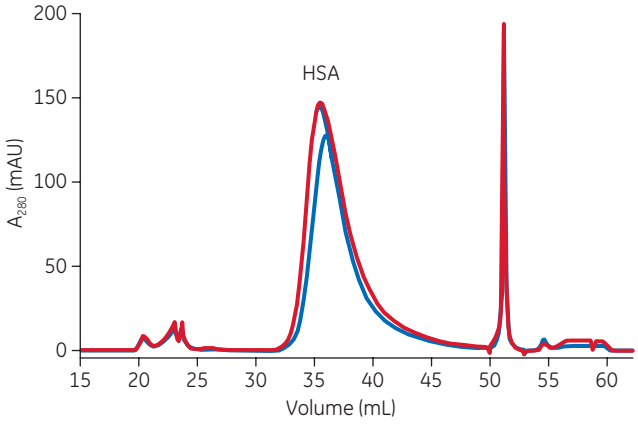


Fig 8. Overlay of 20 chromatograms from the lifetime study of Capto Blue medium. UV curves from the beginning of the study (10 blue curves) were compared with curves from the end of the study (10 red curves). The somewhat smaller HAS elution peaks were not associated with larger protein loss in the flowthrough and were therefore assumed to be caused by method variation. Fresh samples were prepared several times during the study and the sample preparation may have contributed to differences in size of the elution peaks. Similar results were observed for Capto Blue (high sub) (data not shown).

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).

Chemical stability and recommended storage

Capto Blue and Capto Blue (high sub) can be stored at room temperature for three weeks in the storage solutions listed in Table 5. 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 might cause hydrolysis of the base matrix over an extended period of contact and, thus, is not recommended for prolonged storage.

Capto Blue and Capto Blue (high sub) are supplied, preswollen, in a solution of 0.1 M KH_2PO_4 and 20% ethanol, pH 8. The medium should be stored in this solution at 2°C to 8°C.

Table 5. Storage solutions used in the study of chemical stability of Capto Blue and Capto Blue (high sub)

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
1.5 M NaOH

To further investigate alkaline stability, Capto Blue (high sub) was stored in 1.5 M NaOH for up to eight weeks. The stability was evaluated by determination of ligand loss. As can be seen from the results listed in Table 6, Capto Blue (high sub) tolerates exposure to 1.5 M NaOH for up to eight weeks. The results are expected to be applicable also to Capto Blue.

Table 6. Ligand concentration after storage of Capto Blue (high sub) in 1.5 M NaOH for up to eight weeks at room temperature

Sample	Storage (weeks)	Ligand concentration (µmol/mL)
A1	1	21
A2	1	20
B1	2	20
B2	2	20
C1	4	21
C2	4	20
D1	8	20
D2	8	21
E1	Reference	20
E2	Reference	20

Ordering information

Product	Quantity	Code number
Capto Blue	25 mL	17-5448-01
Capto Blue	500 mL	17-5448-02
Capto Blue	5 L	17-5448-04
HiScreen Capto Blue	1 × 4.7 ml	28-9924-74
Capto Blue (high sub)	25 mL	17-5452-01
Capto Blue (high sub)	500 mL	17-5452-02
Capto Blue (high sub)	5 L	17-5452-04

Note: Capto Blue (high sub) is made available through Custom Design Media and is only produced on customer demand. Thus delivery time is typically longer than for standard media, such as Capto Blue. Both media have validated manufacturing methods and are supported with a regulatory support file.

For local office contact information, visit
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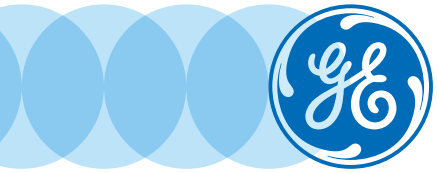

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28-9369-60 AD 10/2014