GE Healthcare
Life Sciences

Capto™ Phenyl (high sub), Capto Butyl, and Capto Octyl

Capto Phenyl (high sub), Capto Butyl, and Capto Octyl† are hydrophobic interaction chromatography (HIC) media used in the capture and intermediate stages of protein purification. Their combination of high capacity, narrow specification range, high flow rate and low backpressure consistently reduces process cycle times and increases productivity. The Capto product range comprises modern media that meet the demands of large-scale biopharmaceutical manufacturing today.

These Capto HIC media offer the following benefits:

- Improved productivity and process economy in downstream operations
- Very high flow rates and large sample volume processing
- Excellent chemical stability

Fig 1. Capto Phenyl (high sub) and Capto Butyl expand the use of HIC at laboratory and process scales and increase productivity in downstream manufacture.

High-flow agarose and productivity

High throughput in downstream purification requires chromatography media that combine a mechanically strong matrix with a pore structure that allows fast mass transfer and high capacity for target molecules. Capto media are based on a very rigid, high-flow agarose base matrix with an optimized pore structure that offers outstanding pressure/flow properties.

The media are intended for general use in large-scale operations. Their high flow rates allow increased productivity and large-volume processing. Maximum flow rates for Capto media in a one-meter diameter column with a 20 cm bed height extend up to 600 cm/h with a backpressure below 3 bar. Figure 2 compares the pressure/flow performance of a Capto medium with Sepharose™ 6 Fast Flow in a representative large-scale situation. The pressure/flow properties of Capto are significantly better than Sepharose 6 Fast Flow. This improvement is a result of the exceptional mechanical stability of the high-flow agarose base matrix.

When upstream processes are optimized to yield high titers, the need for better downstream productivity increases. By decreasing process times in large-scale chromatographic purifications in general, Capto HIC media increase productivity and improve final process economy.

Capto HIC media have been developed in collaboration with biopharmaceutical manufacturers specifically to improve productivity when processing recombinant proteins.

† Capto Octyl is part of the Custom Designed Media line and is available on request.

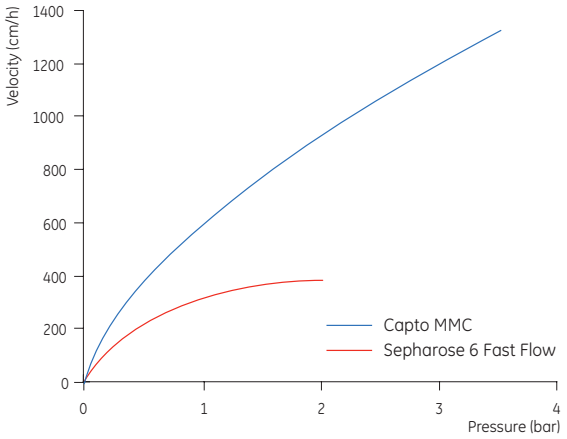


Fig 2. Pressure/flow properties of Capto MMC compared with Sepharose 6 Fast Flow. Running conditions: BPG™ 300 column (30 cm i.d.), open bed at settled bed height equal to 20 cm with water at 20°C. Capto Phenyl (high sub) and Capto Butyl have the same base matrix as Capto MMC.

Capto Octyl

Capto Octyl is a member of the Capto HIC family of media and is available via the Custom Designed Media line (in 25 mL, 1 L, and 5 L packs), as well as in PreDictor™ 96-well plate formats.

Hydrophobic interaction chromatoghraphy

Hydrophobic interaction chromatography is widely used for the purification of peptides and proteins. Substances are separated on the basis of their varying strength of hydrophobic interaction with hydrophobic groups attached to an uncharged base matrix. This technique is usually performed in the presence of moderately high concentrations of anti-chaotropic salts, following the Hofmeister series.

A number of factors influence the chromatographic behavior of proteins and peptides on hydrophobic interaction chromatography media, and several are crucial for developing an optimized purification. Parameters that influence performance (e.g., binding, resolution, selectivity and recovery) include ligand structure, ligand density, base matrix, sample characteristics, ionic strength, type of salt, pH and temperature. Since elution often leaves target molecules in a moderate to low ionic strength state, HIC is a practical step to use after ion exchange (when high salt has been used for elution), after affinity chromatography (to remove aggregates), or before gel filtration. Since loading is performed at high salt concentrations, HIC may be a suitable capture step, for example, after a ammonium sulphate precipitation step.

Purification protocols for small-scale applications that emphasize resolution naturally differ from those in manufacturing processes, where the focus is on obtaining the highest possible productivity.

Capto HIC chromatography media characteristics

Capto HIC media are based on a highly cross-linked agarose matrix that allows flow velocity up to 600 cm/h in bed heights up to 20 cm. Such high flow velocity permit the rapid processing of sample large volumes with only moderate reductions in binding capacity. Table 1 lists key characteristics of Capto Phenyl (high sub), Capto Butyl and Capto Octyl.

Hydrophobicity and selectivity

Figure 3 displays the relative hydrophobicities of Capto Phenyl (high sub), Capto Butyl and Capto Octyl.

The hydrophobicity of Capto Phenyl (high sub) is similar to that of Phenyl Sepharose 6 Fast Flow (high sub) and that of Capto Butyl is similar to Butyl Sepharose 4 Fast Flow (Fig 4). The differences in selectivities for the model proteins are due to the greater cross-linking of the agarose base matrix of the Capto media.

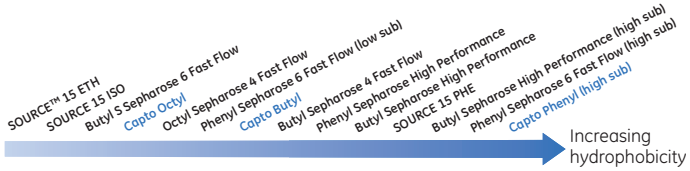


Fig 3. Relative hydrophobic scale, based on retention of RNAs and lysozyme. Can change with running conditions and proteins.

Table 1. Main characteristics of Capto Phenyl (high sub), Capto Butyl and Capto Octyl

	Capto Phenyl (high sub)	Capto Butyl	Capto Octyl
Matrix	Highly cross-linked agarose	Highly cross-linked agarose	Highly cross-linked agarose
Hydrophobic ligand	Phenyl	Butyl	Octyl
Particle size, d_{50v} †	75 µm	75 µm	75 µm
Degree of substitution/mL medium	Approx. 27 µmol/mL	Approx. 53 µmol/mL	Approx. 5 µmol/mL
Dynamic binding capacity at Q_{B10} (BSA)/mL medium	27 mg	27 mg	Not determined
Maximum flow velocity	At least 600 cm/h in a 1 m diameter column with 20 cm bed height at 20°C using process buffers with the same viscosity as water at < 3 bar (0.3 MPa).	At least 600 cm/h in a 1 m diameter column with 20 cm bed height at 20°C using process buffers with the same viscosity as water at < 3 bar (0.3 MPa).	At least 600 cm/h in a 1 m diameter column with 20 cm bed height at 20°C using process buffers with the same viscosity as water at < 3 bar (0.3 MPa).
Long term stability	pH 3 to 13	pH 3 to 13	pH 3 to 13
Short term stability	pH 2 to 14	pH 2 to 14	pH 2 to 14

† d_{50v} is the average particle size of the cumulative volume distribution.

Protein mixture:	Ribonuclease A and Lysozyme
Starting condition:	1.2 M $(NH_4)_2SO_4$, 0.1 M NaH_2PO_4, pH 7
End condition:	0.1 M NaH_2PO_4, pH 7.0
Gradient:	55 min, linear decreasing salt gradient
Flow rate:	0.2 mL/min
Temperature:	23°C
Columns:	Tricorn™ 5/100 columns, bed height 100 mm
System:	ÄKTAFPLC™

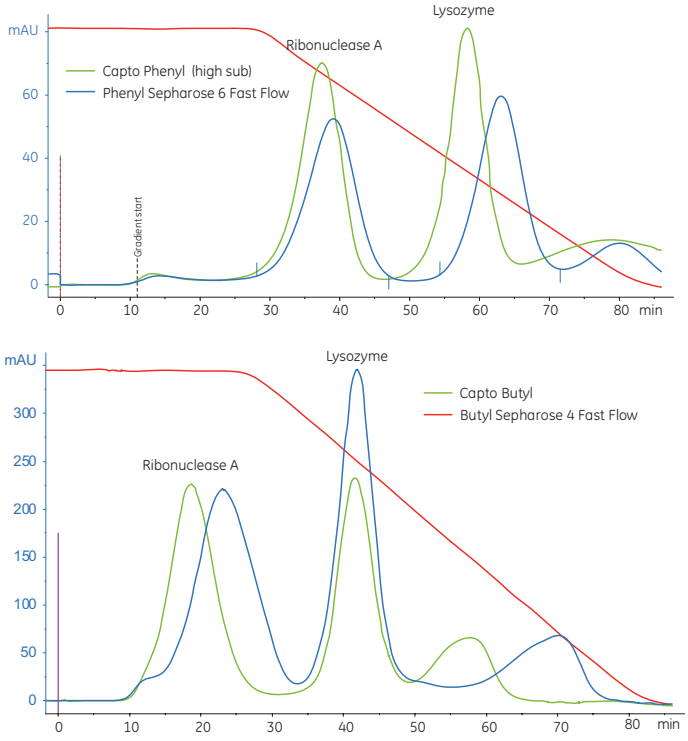
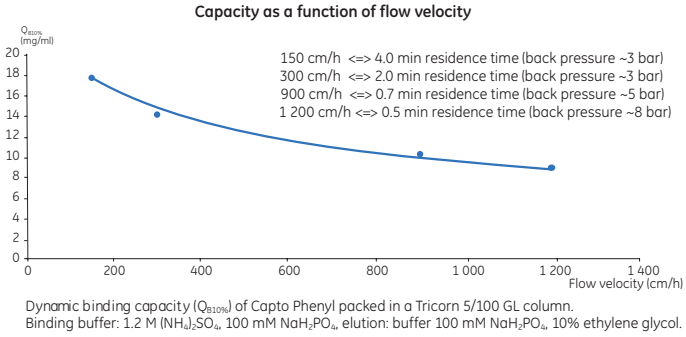


Fig 4. A) Retention of model proteins (Ribonuclease A and lysozyme) on Capto Phenyl (high sub) (red) and Phenyl Sepharose 6 Fast Flow (high sub) (blue). B) Retention of model proteins (Ribonuclease A and lysozyme) on Capto Butyl and Butyl Sepharose 4 Fast Flow.

Significant productivity gains

Increasing flow rates decreases dynamic binding capacity (Q_{B10}). However, a three-fold increase in loading flow velocity, from 200 cm/h to 600 cm/h, results only in a capacity decrease of approximately 25%. To achieve optimal capacity in practice, processes can nevertheless be designed with a lower flow velocity for loading (Fig 5), but then an increased flow velocity over the rest of the chromatographic purification process (i.e., during column packing, conditioning, loading, washing, elution, regeneration, CIP and re-conditioning), thereby reducing total processing time dramatically. The most obvious result is a significant improvement in downstream processing productivity and process economy.



Dynamic binding capacity ($Q_{B10\%}$) of Capto Phenyl packed in a Tricorn 5/100 GL column. Binding buffer: 1.2 M $(NH_4)_2SO_4$, 100 mM NaH_2PO_4, elution: buffer 100 mM NaH_2PO_4, 10% ethylene glycol.

Fig 5. Increasing loading flow velocity from 200 cm/h to 700 cm/h only decreases capacity by 25%, opening up the opportunity to raise productivity by cutting total processing time.

High chemical stability

Both Capto Phenyl (high sub) and Capto Butyl display high chemical stability (Figs 6 and 7 respectively) and can withstand storage at pH 1 to 14 for one week with minimal leakage. The leakage that does occur at low pH comes mainly from the base matrix.

The hydrophobic properties and ligand density of Capto Phenyl (high sub) do not change when stored at pH 13.5 for three months.

The long-term stability for Capto HIC media ranges from pH 3 to 13 and short-term stability from pH 3 to 14.

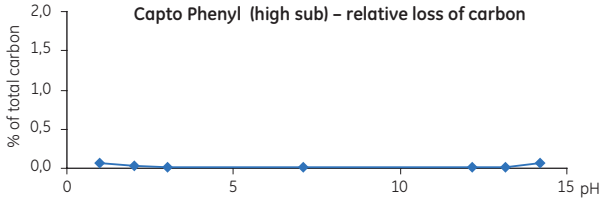


Fig 6. Capto Phenyl (high sub) has high chemical stability and withstands storage at pH 1 to 14 for one week with practically no leakage.

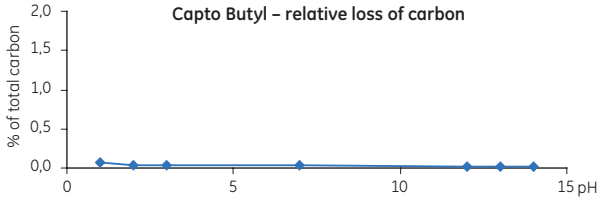


Fig 7. Capto Butyl has high chemical stability and withstands storage at pH 1 to 14 for one week with practically no leakage.

Small-scale format provides fast screening and method development

Using small-scale format to screen for the most suitable chromatography process conditions in the early stages of process development saves both time and sample. Capto HIC media are 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 then permits straightforward scale-up.

Basic characteristics of HiScreen prepacked columns are summarized in Table 2.

Table 2. Characteristics of HiScreen columns

Column volume (CV)	4.7 ml
Column dimensions	0.77 × 10 cm
HiScreen column hardware pressure limit	0.8 MPa, 8 bar, 116 psi

Cleaning and sanitization

Cleaning-in-place (CIP) is a procedure that removes tightly bound impurities and contaminants such as lipids, precipitates, or denatured proteins generated from the sample that may remain in the column after regeneration. Regular CIP also prevents the build up of these contaminants and helps maintain the capacity, flow properties and general performance. A specific CIP protocol should be designed for each process according to the type of contaminants present in the feed stream. General recommendation is to use 1 M sodium hydroxide in Capto HIC media CIP protocols, as well as for sanitization.

Storage

Capto HIC media are supplied pre-swollen in 20% ethanol. Recommended storage conditions are 20% ethanol at 8°C to 30°C.

Ordering information

Product	Quantity	Code no.
Capto Phenyl (high sub)	25 mL	17-5451-01
Capto Phenyl (high sub)	100 mL	17-5451-02
Capto Phenyl (high sub)	1 L	17-5451-03
Capto Phenyl (high sub)	5 L	17-5451-04
PreDictor Capto Phenyl (high sub), 6 µl	4 × 96-well filter plates	17-5451-16
PreDictor Capto Phenyl (high sub), 50 µl	4 × 96-well filter plates	17-5451-17
PreDictor RoboColumn Capto Phenyl (high sub), 200 µl	Eight columns	28-9860-88
PreDictor RoboColumn Capto Phenyl (high sub), 600 µl	Eight columns	28-9861-82
Capto Butyl	25 mL	17-5459-01
Capto Butyl	100 mL	17-5459-02
Capto Butyl	1 L	17-5459-03
Capto Butyl	5 L	17-5459-04
Capto Octyl†	25 ml	17-5465-01
Capto Octyl†	100 mL	17-5465-02
Capto Octyl†	1 L	on request
Capto Octyl†	5 L	on request
PreDictor Capto Butyl, 6 µl	4 × 96-well filter plates	17-5459-16
PreDictor Capto Butyl, 50 µl	4 × 96-well filter plates	17-5459-17
PreDictor RoboColumn Capto Butyl, 200 µl	Eight columns	28-9860-97
PreDictor RoboColumn Capto Butyl, 600 µl	Eight columns	28-9861-83
PreDictor Capto Octyl, 6 µl	4 × 96-well filter plates	17-5465-16
PreDictor Capto Octyl, 50 µl	4 × 96-well filter plates	17-5465-17

† These products are part of our Custom Designed Media. Please contact your local GE Healthcare representative.

Related products

HiScreen Capto Phenyl (high sub)		28-9924-72
HiScreen Capto Butyl		28-9924-73
HiTrap™ Capto Phenyl (high sub)†	5 × 1 mL	17-5451-08
HiTrap Capto Phenyl (high sub)†	5 × 5 mL	17-5451-09
HiTrap Capto Butyl†	5 × 1 mL	17-5459-08
HiTrap Capto Butyl†	5 × 5 mL	17-5459-09
HiTrap Octyl		17-5465-08

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Related literature

Handbook: Hydrophobic Interaction and Reversed Phase Chromatography Principles and methods	11-0012-69
Data File: HiScreen prepacked columns	28-9305-81
Data file: PreDictor™ 96-well filter plates and Assist software	28-9258-39

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