

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

## HYDROPHOBIC INTERACTION CHROMATOGRAPHY

Capto™ Phenyl (high sub), Capto Butyl, and Capto Octyl are hydrophobic interaction chromatography (HIC) resins 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 resins that meet the demands of large-scale biopharmaceutical manufacturing today.

These Capto HIC resins offer the following benefits:

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

### High-flow agarose and productivity

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

The resins are intended for general use in large-scale operations. Their high flow rates allow increased productivity and large-volume processing. Maximum flow velocities for Capto resins in a one-meter diameter column with a 20 cm bed height extend up to 600 cm/h with a backpressure below 3 bar (0.3 MPa, 43.5 psi). Figure 2 compares the pressure/flow performance of

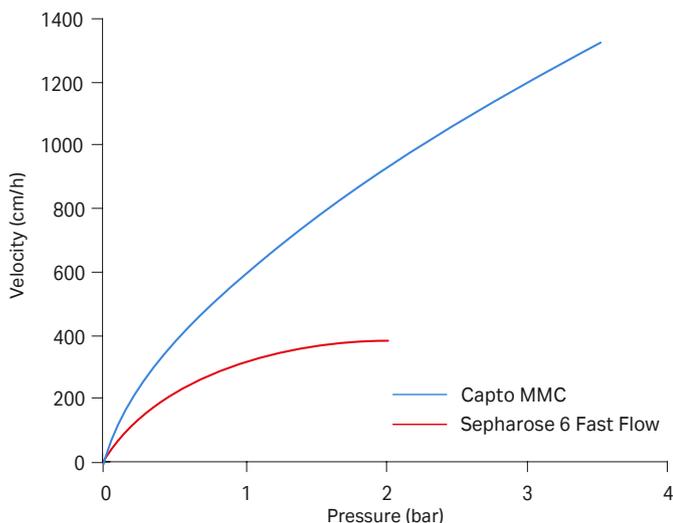


**Fig 1.** Capto Phenyl (high sub) and Capto Butyl expand the use of HIC at laboratory and process scales and increase productivity in downstream manufacture.

a Capto resin 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 resins increase productivity and improve final process economy.

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



**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 resins and is available in 25 mL, 1 L, and 5 L packs, as well as in PreDictor™ 96-well plate formats.

### Hydrophobic interaction chromatography

HIC 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 resins, and several are crucial for developing an optimized purification. Parameters that influence performance (e.g., binding, resolution, selectivity, and recovery) include ligand structure, ligand concentration, base matrix, sample characteristics, ionic strength, type of salt, pH, and temperature.

**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, spherical	
Particle size, $d_{50V}^1$	~ 75 $\mu\text{m}$	~ 75 $\mu\text{m}$	~ 75 $\mu\text{m}$
Hydrophobic ligand	Phenyl	Butyl	Octyl
Ligand concentration	~ 27 $\mu\text{mol/mL}$ resin	~ 53 $\mu\text{mol/mL}$ resin	~ 5 $\mu\text{mol/mL}$ resin
Dynamic binding capacity, $Q_{B10}^2$	~ 27 mg BSA/mL resin	~ 27 mg BSA/mL resin	Not determined
Pressure/flow characteristics	$\geq 600$ cm/h at $< 0.3$ MPa in a 1 m diameter column and 20 cm bed height (at 20°C using process buffers with the same viscosity as water) <sup>3</sup>		
pH stability, operational <sup>4</sup>	3 to 13	3 to 13	3 to 13
pH stability, CIP <sup>5</sup>	2 to 14	2 to 14	2 to 14
Autoclavability	17 min at 121°C in 0.1 M $\text{KH}_2\text{PO}_4$ pH 8.0, 10 cycles		

<sup>1</sup> Median particle size of the cumulative volume distribution.

<sup>2</sup> Dynamic binding capacity at 10% breakthrough by frontal analysis at a mobile phase velocity of 300 cm/h in a Tricorn 5/100 column at 10 cm bed height (2 min residence time) for BSA in 1.5M  $(\text{NH}_4)_2\text{SO}_4$  and 100mM  $\text{NaH}_2\text{PO}_4$ , pH 7.0.

<sup>3</sup> The pressure/flow characteristics describes the relationship between pressure and flow under the set circumstances. The pressure given shall not be taken as the maximum pressure of the resin.

<sup>4</sup> pH range where resin can be operated without significant change in function.

<sup>5</sup> pH range where resin can be subjected to cleaning or sanitization in place without significant change in function.

As 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 size exclusion chromatography. Since loading is performed at high salt concentrations, HIC can be a suitable capture step, for example, after an 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.

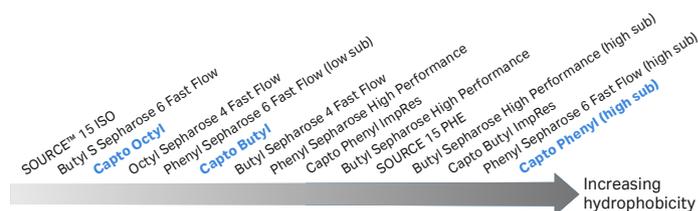
## Capto HIC resin characteristics

Capto HIC resins 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

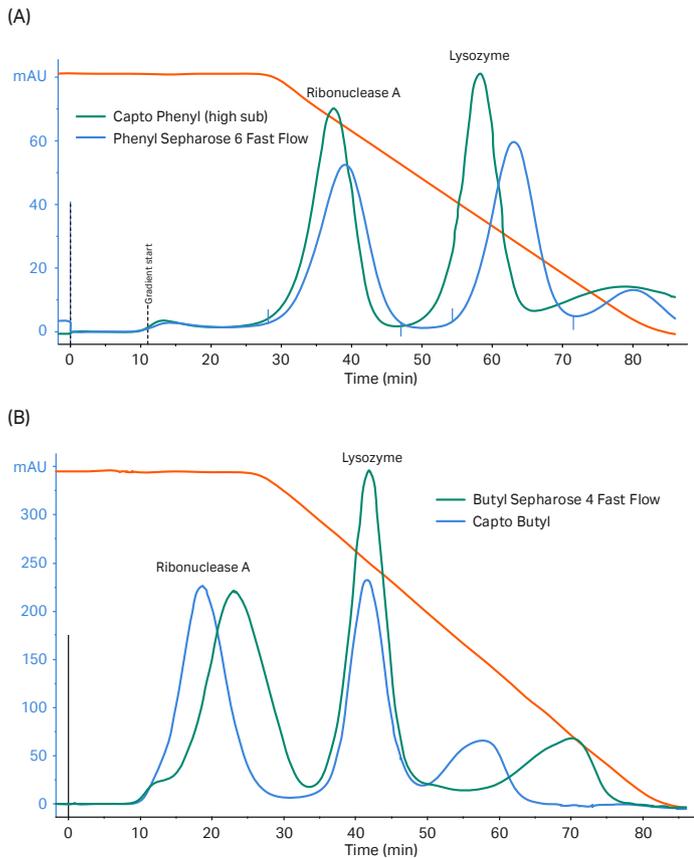
Figure 3 displays the relative hydrophobicities of Capto Phenyl (high sub), Capto Butyl and Capto Octyl.

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 resins.



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

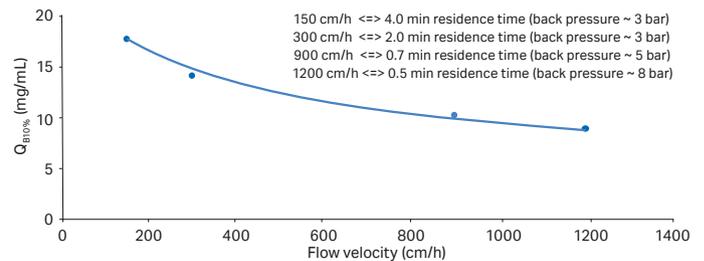
**Protein mixture:** Ribonuclease A and Lysozyme  
**Starting condition:** 1.2 M  $(\text{NH}_4)_2\text{SO}_4$ , 0.1 M  $\text{NaH}_2\text{PO}_4$ , pH 7  
**End condition:** 0.1 M  $\text{NaH}_2\text{PO}_4$ , pH 7.0  
**Gradient:** 55 min, linear decreasing salt gradient  
**Flow rate:** 0.2 mL/min  
**Temperature:** 23°C  
**Columns:** Tricorn™ 5/100 columns, bed height 100 mm  
**System:** ÄKTA<sub>FPLC</sub>



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

## Significant productivity gains

Increasing flow velocities decreases dynamic binding capacity. 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, 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.



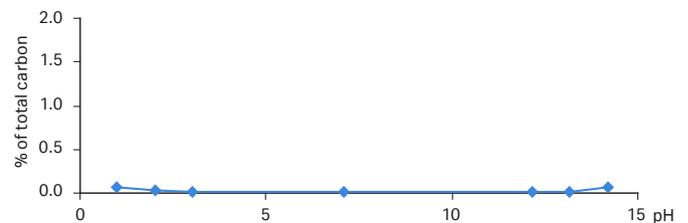
**Fig 5.** Dynamic binding capacity of Capto Phenyl as a function of flow velocity. Increasing loading flow velocity from 200 cm/h to 700 cm/h only decreases capacity by 25%, opening up the opportunity to raise productivity by cutting total processing time. Capto Phenyl packed in a Tricorn 5/100 GL column. Binding buffer: 1.2 M  $(\text{NH}_4)_2\text{SO}_4$ , 100 mM  $\text{NaH}_2\text{PO}_4$ , elution: buer 100 mM  $\text{NaH}_2\text{PO}_4$ , 10% ethylene glycol.

## High chemical stability

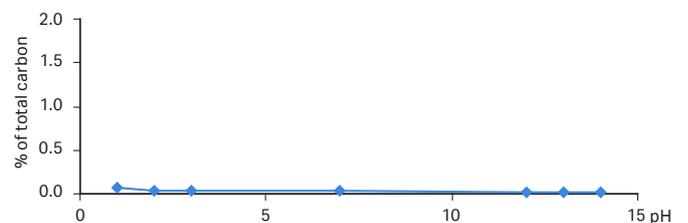
Both Capto Phenyl (high sub) and Capto Butyl display high chemical stability (Figs 6 and 7 respectively) and can withstand storage at pH 1 to 14 for one week with minimal leakage. The leakage that does occur at low pH comes mainly from the base matrix.

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

The operational stability for Capto HIC resins ranges from pH 2 to 13 and cleaning-in-place (CIP) stability from pH 3 to 14.



**Fig 6.** Relative loss of carbon. 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.** Relative loss of carbon. 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 resins 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	8 bar (0.8 MPa, 116 psi)

## Cleaning and sanitization

CIP is a procedure that removes tightly bound impurities and contaminants such as lipids, precipitates, or denatured proteins generated from the sample that might 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 1.0 M sodium hydroxide in Capto HIC resin CIP protocols, as well as for sanitization.

## Storage

Capto HIC resins are supplied pre-swollen in 20% ethanol. Recommended storage conditions are 20% ethanol at 4°C to 30°C.

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CY13568-28Aug20-DF

## Ordering information

Product	Quantity	Code number
Capto Phenyl (high sub)	25 mL	17545101
Capto Phenyl (high sub)	100 mL	17545102
Capto Phenyl (high sub)	1 L	17545103
Capto Phenyl (high sub)	5 L	17545104
Capto Phenyl (high sub)	10 L	17545105
Capto Phenyl (high sub)	60 L	17545160*
PreDictor Capto Phenyl (high sub), 6 µL	4 × 96-well filter plates	17545116
PreDictor Capto Phenyl (high sub), 50 µL	4 × 96-well filter plates	17545117
PreDictor RoboColumn™ Capto Phenyl (high sub), 200 µL	Eight columns	28986088
PreDictor RoboColumn Capto Phenyl (high sub), 600 µL	Eight columns	28986182
Capto Butyl	25 mL	17545901
Capto Butyl	100 mL	17545902
Capto Butyl	1 L	17545903
Capto Butyl	5 L	17545904
Capto Octyl	25 mL	17546501
Capto Octyl	100 mL	17546502
Capto Octyl	1 L	on request
Capto Octyl	5 L	on request
PreDictor Capto Butyl, 6 µL	4 × 96-well filter plates	17545916
PreDictor Capto Butyl, 50 µL	4 × 96-well filter plates	17545917
PreDictor RoboColumn Capto Butyl, 200 µL	Eight columns	28986097
PreDictor RoboColumn Capto Butyl, 600 µL	Eight columns	28986183
PreDictor Capto Octyl, 6 µL	4 × 96-well filter plates	17546516
PreDictor Capto Octyl, 50 µL	4 × 96-well filter plates	17546517

\* Pack size available upon request.

## Related products

HiScreen Capto Phenyl (high sub)	28992472
HiScreen Capto Butyl	28992473
PreDictor Capto HIC screening	50 µL 29305795
HiTrap™ Capto HIC Selection Kit	5 × 1 mL 29321087
HiTrap Capto Phenyl (high sub)	5 × 1 mL 17545108
HiTrap Capto Phenyl (high sub)	5 × 5 mL 17545109
HiTrap Capto Butyl	5 × 1 mL 17545908
HiTrap Capto Butyl	5 × 5 mL 17545909
HiTrap Capto Octyl	5 × 1 mL 17546508

## Related literature

Handbook: Hydrophobic Interaction and Reversed Phase Chromatography principles and methods	11001269
Selection guide: Hydrophobic Interaction Chromatography (HIC)	29022223
Data file: HiScreen prepacked columns	28930581
Data file: PreDictor 96-well filter plates and Assist software	28925839

