

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

HYDROPHOBIC INTERACTION CHROMATOGRAPHY RESINS

Capto™ Phenyl (high sub), Capto™ Butyl, Capto™ Octyl, and Capto™ Butyl-S resins (Fig 1) 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 compared with their predecessor resins based on Sepharose™ base matrix
- 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 shows the pressure/flow performance of a



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

Capto™ resin packed in various large scale column sizes. The pressure/flow properties of Capto™ resins are significantly better than Sepharose™ 6 Fast Flow resins. 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.

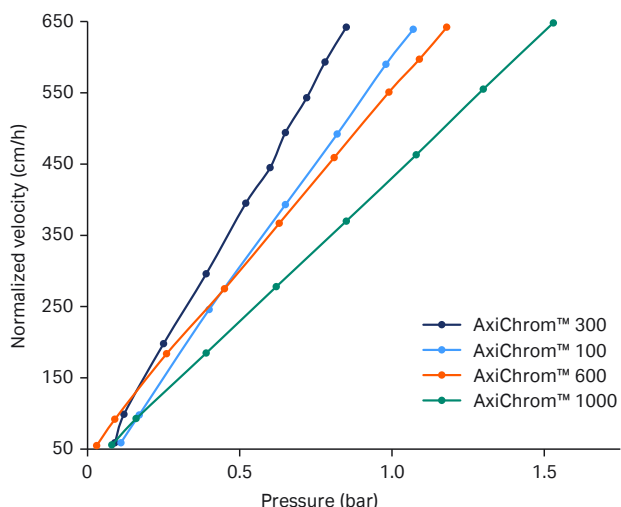


Fig 2. Pressure/flow properties of Capto™ Phenyl (High Sub) resin packed in AxiChrom™ 100, 300, 600, and 1000 columns with 20 cm bed height. Capto™ Butyl, Capto™ Octyl, and Capto™ Butyl-S resins have the same base matrix as Capto™ Phenyl (High Sub) resin.

Capto™ Butyl-S resin

Capto™ Butyl-S resin is the most recent member of the Capto™ HIC resin family. It was developed as a custom design media (CDM) and optimized in cooperation with a process-scale manufacturer of biopharmaceuticals.

- Designed for the binding and elution of relatively strong hydrophobic molecules at comparatively low salt concentrations
- Low risk of denaturation of relatively strong hydrophobic solutes

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.

Of these parameters, the type and concentration of ligand as well as the type and concentration of salt added during the adsorption step are of paramount importance in determining the outcome of a HIC event.

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. Because 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 resins

Figure 3 is an overview of Cytiva Capto™ HIC resins. Information about Capto™ Butyl ImpRes and Capto™ Phenyl ImpRes resins can be found in data file CY13700.

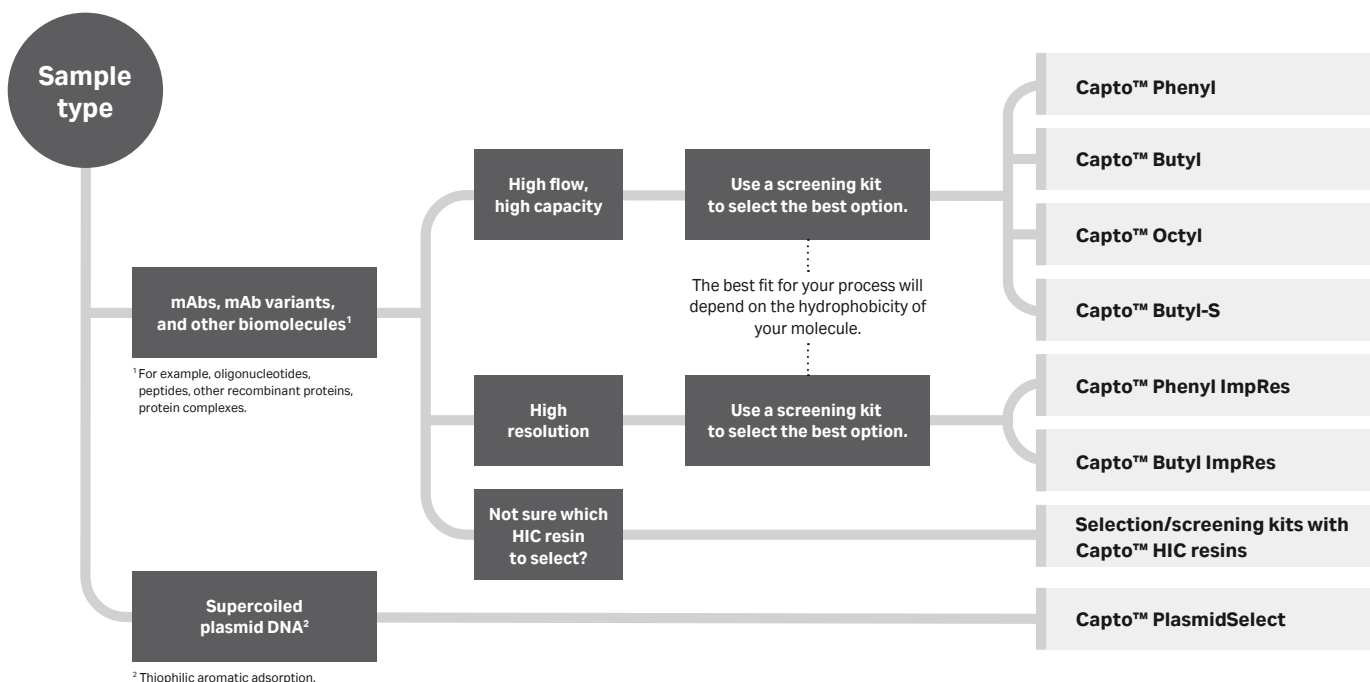


Fig 3. Overview of Capto™ HIC resins.

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, Capto™ Octyl, and Capto™ Butyl-S resins.

Hydrophobicity and selectivity

Figure 4 displays the relative hydrophobicities of Capto™ Phenyl (high sub), Capto™ Butyl, Capto™ Octyl, and Capto™ Butyl-S resins.

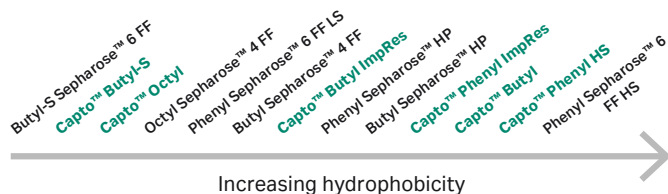


Fig 4. Relative hydrophobic scale of various resins, based on retention of ribonuclease A, lysozyme, and α -Chymotrypsin. Can change with running conditions and proteins. The resins highlighted in bold are the Capto™ HIC resins, which enable optimized productivity compared to Sepharose™ based resins.

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, cleaning-in-place [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.

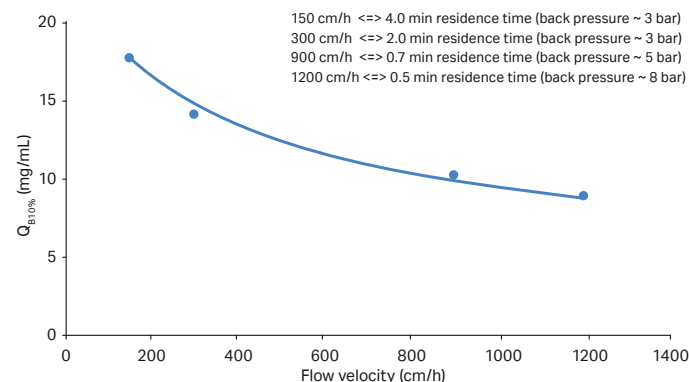


Fig 5. Dynamic binding capacity of Capto™ Phenyl resin as a function of flow velocity. Increasing loading flow velocity from 200 cm/h to 700 cm/h only decreases capacity by 25%, opening up the opportunity to raise productivity by cutting total processing time. Capto™ Phenyl resin packed in a Tricorn™ 5/100 GL column. Binding buffer: 1.2 M $(\text{NH}_4)_2\text{SO}_4$, 100 mM NaH_2PO_4 . Elution buffer: 100 mM NaH_2PO_4 , 10% ethylene glycol.

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

	Capto™ Phenyl (high sub)	Capto™ Butyl	Capto™ Octyl	Capto™ Butyl-S
Matrix	Highly cross-linked agarose, spherical			
Particle size, d_{50V} ¹	~ 75 μm	~ 75 μm	~ 75 μm	~75 μm
Hydrophobic ligand	Phenyl	Butyl	Octyl	1-Butylmercaptan
Ligand concentration	~ 27 $\mu\text{mol/mL}$ resin	~ 53 $\mu\text{mol/mL}$ resin	~ 5 $\mu\text{mol/mL}$ resin	10.0–13.5 $\mu\text{mol/mL}$ resin
Dynamic binding capacity, Q_{B10} ²	~ 27 mg BSA/mL resin	~ 27 mg BSA/mL resin	~ 25 mg BSA/mL resin	Not determined
Pressure/flow characteristics	≥ 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) ³			
pH stability, operational ⁴	3 to 13	3 to 13	3 to 13	3 to 13
pH stability, CIP ⁵	2 to 14	2 to 14	2 to 14	2 to 14
Autoclavability	17 min at 121°C in 0.1 M KH_2PO_4 pH 8.0, 10 cycles			

¹ Median particle size of the cumulative volume distribution.

² 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 100 mM NaH_2PO_4 , pH 7.0.

³ 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.

⁴ pH range where resin can be operated without significant change in function.

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

High chemical stability

All Capto™ HIC resins display high chemical stability 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. Figure 6 shows the relative loss of carbon for Capto™ Phenyl (high sub) resin. Very similar results are obtained for Capto™ Butyl, Capto™ Octyl, and Capto™ Butyl-S resins (data not shown).

The operational stability for Capto™ HIC resins ranges from pH 2 to 13 and CIP stability from pH 3 to 14.

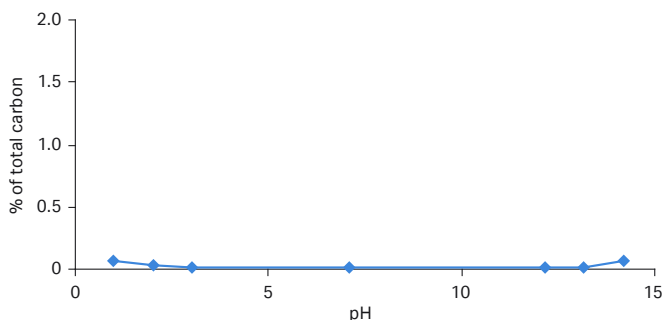


Fig 6. Relative loss of carbon. Capto™ Phenyl (high sub) resin has high chemical stability and withstands storage at pH 1 to 14 for one week with little leakage.

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 multiple formats that are suitable for process development.

Prepacked formats for high-throughput process development (HTPD):

- PreDicator™ 96-well filter plates (96 purifications in parallel under static conditions)
- PreDicator™ RoboColumn™ units (8 purifications in parallel under dynamic conditions)

Prepacked formats for method optimization and parameter screening:

- HiTrap™ columns (1 or 5 mL)
- HiScreen™ columns (4.7 mL)

Kits for resin variability studies:

- Process Characterization Kits (3 bottles of 25 mL, with 3 different ligand densities).

Prepacked formats for scale-up and GMP manufacturing

Capto™ HIC resins are also available in ReadyToProcess™ column formats, which are validated high performance prepacked columns for scale-up and GMP biomanufacturing.

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 buildup of these contaminants and helps maintain 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™ Butyl, Capto™ Phenyl (high sub), and Capto™ Octyl resins are supplied pre-swollen in 20% ethanol. Recommended storage conditions are 20% ethanol at 4°C to 30°C.

Capto™ Butyl-S resin is supplied as a suspension containing 20% ethanol or 2% benzyl alcohol + 0.2 M sodium acetate as preservative. Recommended storage condition is at temperatures between 4°C and 30°C.

Additional reading

Visit our website to explore our application notes showcasing our Capto™ HIC resins.

- [Application note: Increasing productivity in hydrophobic interaction chromatography \(HIC\) using Capto™ resins](#)
- [Application note: Developing a HIC polishing step for removal of mAb aggregates](#)
- [Application note: Optimization of a HIC step with HTPD](#)
- [Packing instruction: How to pack Capto™ HIC resins in AxiChrom™ columns](#)
- [HIC resource center](#)

Ordering information

Multiple resins (screening kits)

Format	Quantity	Product code
HiTrap™ Capto™ HIC selection kit	5 × 1 mL	29321087
<i>Kit includes the following</i> <i>Capto™ HIC resins: Capto™ Phenyl (high sub), Capto™ Phenyl ImpRes, Capto™ Butyl, Capto™ Butyl ImpRes, and Capto™ Octyl. Prepacked, ready-to-use 1 mL HiTrap™ columns</i>		
PreDicator™ Capto™ HIC Screening Kit	6 µL, 4 × 96 well plates	29711438
	20 µL, 4 × 96 well plates	29711439
<i>Kit includes the following</i> <i>Capto™ HIC resins: Capto™ Phenyl (high sub), Capto™ Phenyl ImpRes, Capto™ Butyl, Capto™ Butyl ImpRes, Capto™ Octyl, and Capto™ Butyl-S</i>		

Capto™ Phenyl (high sub)

Format	Quantity	Product code
Bulk	25 mL	17545101
	100 mL	17545102
	1 L	17545103
	5 L	17545104
	10 L	17545105
	60 L	17545160
HiTrap™ column	5 × 1 mL	17545108
	5 × 5 mL	17545109
HiScreen™ column	1 × 4.7 mL	28992472
PreDicator™ Plate	6 µL, 4 × 96-well plates	17545116
	20 µL, 4 × 96-well plates	29716136
	50 µL, 4 × 96-well plates	17545117
PreDicator™ RoboColumn™ unit	200 µL, 8 columns	28986088
	600 µL, 8 columns	28986182
Process Characterization Kit	3 × 25 mL (3 different ligand densities)	17545170
ReadyToProcess™ column	1 L (80/200)	29316634
	2.5 L (126/200)	29207816
	3.7 L (178/150)	29287578
	5 L (178/200)	29225988
	6.2 L (178/250)	29287579
	7.4 L (251/150)	29287580
	10 L (251/200)	29194484
	12.4 L (251/250)	29287581
	15 L (359/150)	29304091
	20 L (359/200)	29119051
	25 L (359/250)	29304092
	32 L (450/200)	29321929
	57 L (600/200)	29376124

Capto™ Butyl

Format	Quantity	Product code
Bulk	25 mL	17545901
	100 mL	17545902
	1 L	17545903
	5 L	17545904
HiTrap™ column	5 × 1 mL	17545908
	5 × 5 mL	17545909
HiScreen™ column	1 × 4.7 mL	28992473
PreDicator™ Plate	6 µL, 4 × 96-well plates	17545916
	20 µL, 4 × 96-well plates	29716137
	50 µL, 4 × 96-well plates	17545917
PreDicator™ RoboColumn™ unit	200 µL, 8 columns	28986097
	600 µL, 8 columns	28986183
ReadyToProcess™ column	2.5 L (126/200)	29011998
	20 L (359/200)	29594551

Capto™ Octyl

Format	Quantity	Product code
Bulk	25 mL	17546501
	100 mL	17546502
	1 L	17546503
	5 L	17546504
	5 × 1 mL	17546508
HiScreen™ column	1 × 4.7 mL	17546510
PreDicator™ Plate	6 µL, 4 × 96-well plates	17546516
	20 µL, 4 × 96-well plates	29716138
	50 µL, 4 × 96-well plates	17546517
PreDicator™ RoboColumn™ unit	200 µL, 8 columns	29275286
	600 µL, 8 columns	29275287
ReadyToProcess™ column	5 L (178/200)	29436142
	57 L (600/200)	29437660

Capto™ Butyl-S[†]

Format	Quantity	Product code
Bulk – in 20% ethanol	25 mL	17549701
	100 mL	17549702
	1 L	17549703
	5 L	17549704
	5 × 5 mL	17549713
Bulk – in 2 % BnOH + 0.2 M sodium acetate	1 L	17549723
	5 L	17549724
HiTrap™ column	5 × 1 mL	17549711
	1 × 4.7 mL	17549710
HiScreen™ column	1 × 4.7 mL	17549710
ReadyToProcess™ column	10 L (251/200)	29718930

[†] Capto™ Butyl-S is a custom designed resin that is produced on demand.

Related literature

Data file: Capto™ Phenyl ImpRes and Capto™ Butyl ImpRes	CY13700
Data file: HiScreen™ prepacked columns	CY13473
Data file: PreDicator™ 96-well filter plates and Assist software	CY13663
Data file: PreDicator™ RoboColumn™	CY13689
Data file: ReadyToProcess™ columns	CY11724
Handbook: Hydrophobic interaction and reversed phase chromatography	CY11248

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CY13568-04May23-DF

