Capto™ Phenyl ImpRes and Capto™ Butyl ImpRes resins

HYDROPHOBIC INTERACTION CHROMATOGRAPHY RESINS

Capto™ Phenyl ImpRes and Capto™ Butyl ImpRes are hydrophobic interaction chromatography (HIC) resins developed for the intermediate and polishing steps in a downstream protein purification process (Fig 1). Both chromatography resins extend the well-established Capto™ platform to include high-resolution resins. By combining the high-flow characteristics of Capto™ resins with a smaller particle size, Capto™ Phenyl ImpRes and Capto™ Butyl ImpRes resins deliver both excellent pressure/flow properties and resolution. The ability to run at higher flow velocities and higher bed heights increases flexibility in process design and might enable increased productivity.

Key benefits of Capto™ Phenyl ImpRes and Capto™ Butyl ImpRes resins include:

- High-resolution intermediate and polishing purification based on Capto™ ImpRes base matrix with traditional HIC ligands
- Flexible process design due to a large operational window of flow velocities and bed heights
- Improved productivity and process economy in downstream operations compared with their predecessor resins based on Sepharose™ base matrix
- Excellent chemical stability

Hydrophobic interaction chromatography

HIC separates and purifies biomolecules based on differences in surface hydrophobicity. The technique is versatile and offers specific selectivity. Many proteins and peptides, as well as other hydrophobic biomolecules have sufficient numbers of exposed hydrophobic groups to allow interaction with hydrophobic ligands coupled to chromatographic matrices.

Compared with Reversed Phase Chromatography (RPC) adsorbents, HIC resins display milder elution conditions and consequently better retention of biological activity after separation. HIC is well suited for use in the intermediate or polishing steps of protein purification strategies where chromatographic techniques such as ion exchange and affinity chromatography have been employed. For example, HIC makes an excellent choice for purifying material that has been precipitated with ammonium sulfate or eluted in high salt concentrations during ion exchange.

Fig 1. Capto™ Phenyl ImpRes and Capto™ Butyl ImpRes resins are supplied in various formats ranging from multiwell screening plates to large-scale prepacked columns for GMP manufacturing.

HIC is usually performed in moderate to high concentrations of salts in the starting buffer, promoting binding and helping to stabilize the protein structure. The bound molecules are eluted by decreasing the salt concentration in a linear or stepwise manner. Linear gradient elution is most frequently used when high resolution is needed, and stepwise gradient elution is recommended for sample preparation and concentration. Several factors influence the behavior of proteins and peptides on HIC resins. These include, but are not limited to, sample characteristics, type and concentration of salt, resins porosity and hydrophobicity, flow rate, temperature and pH.

Figure 2 is an overview of Cytiva Capto™ HIC resins. Information about Capto™ Phenyl (high sub), Capto™ Butyl, Capto™ Octyl, and Capto™ Butyl-S resins can be found in data file CY13568.

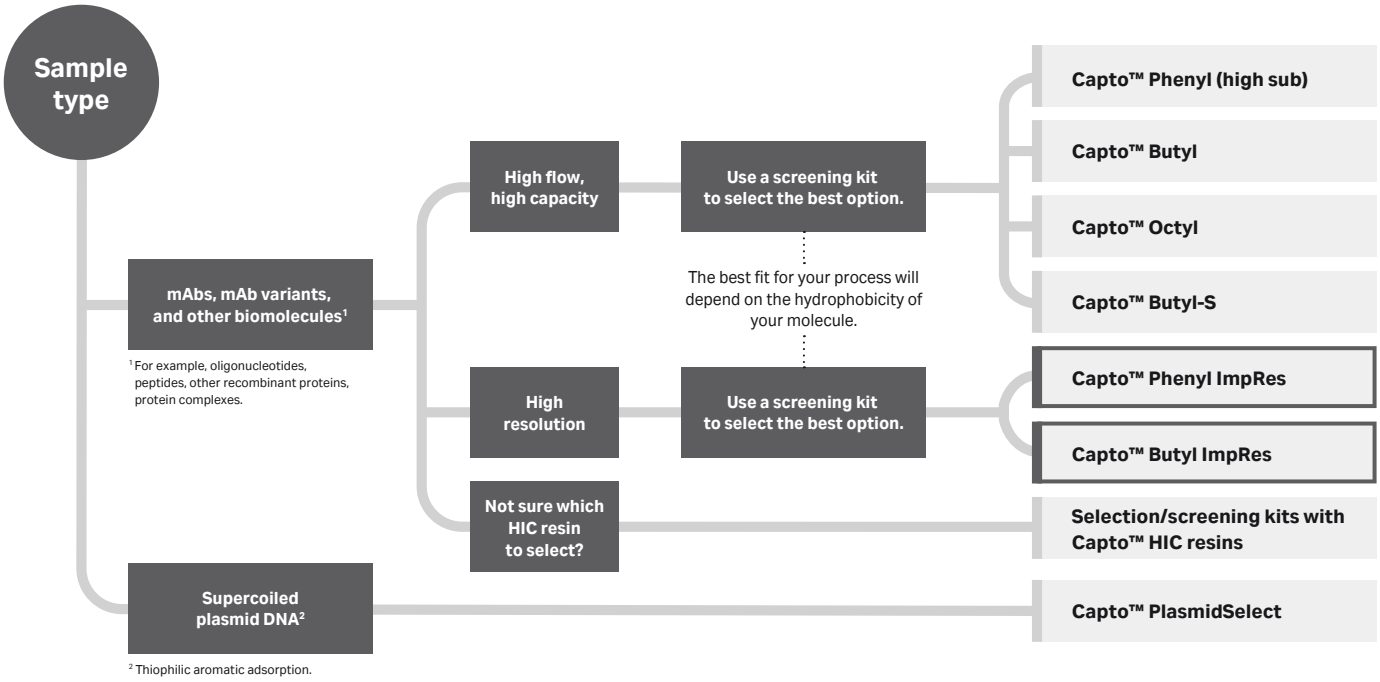

Fig 2. Overview of Capto™ HIC resins.

Resins characteristics

Main resins characteristics for Capto™ Phenyl ImpRes and Capto™ Butyl ImpRes are summarized in Table 1.

Table 1. Main characteristics of Capto™ Phenyl ImpRes and Capto™ Butyl ImpRes HIC resins

	Capto™ Phenyl ImpRes	**Capto™ Butyl ImpRes**
Matrix	High-flow agarose	High-flow agarose
Average particle size ($d_{50,volume}$)*	40 µm	40 µm
Ligand	Phenyl	Butyl
Hydrophobicity†	45 to 50 min retention of lyzosyme	52 to 58 min retention of α-chymotrypsinogen
Flow velocity‡	Up to 220 cm/h in a 1 m diameter column with a bed height of 20 cm at 20°C; measured using process buffers with the same viscosity as water at 300 kPa.	
Binding capacity§	19 mg BSA/mL resin	37 mg BSA/mL resin
pH stability (operational)¶	pH 3 to 13	pH 3 to 13
CIP stability (short term)**	pH 2 to 14	pH 2 to 14
Chemical stability	Stable in commonly used aqueous buffers: 1 M sodium hydroxide††, 1 M acetic acid, 8 M urea, 6 M guanidine hydrochloride, 70% ethanol, 30% isopropanol	
Shelf life	Five years	Five years
Storage conditions	20% ethanol at 4°C to 30°C	20% ethanol at 4°C to 30°C

* $d_{50,volume}$ is the median particle size of the cumulative volume distribution.
† Hydrophobic function according to method described in the *Hydrophobicity* section.
‡ Flow velocity is dependent on the column used.
§ Dynamic binding capacity at 10% breakthrough measured at a residence time of 4 min (150 cm/h) in Tricorn™ 5/100 column with 10 cm bed height. Buffer conditions: 0.1 M sodium phosphate buffer, 1.2 M ammonium sulfate, pH 7.
¶ Long-term stability: pH interval where the resin can be operated without significant change in function.
** Short-term stability: pH interval where the resin can be subjected to cleaning-in place (CIP) without significant change in function.
†† No significant change in function after one month storage in 1 M NaOH at ambient temperature.

Bead size optimized for high-resolution polishing

Capto™ Phenyl ImpRes and Capto™ Butyl ImpRes resins are based on the well-established high-flow agarose matrix, which demonstrates excellent pressure/flow properties. The rigid matrix allows for high flow velocities in modern downstream purification processes. The smaller bead size of 40 µm, employed for Capto™ Phenyl and Butyl ImpRes resins, allows for increased resolution compared with HIC resins based on the larger 75 µm bead employed for Capto™ Phenyl (high sub), Capto™ Butyl, Capto™ Octyl, and Capto™ Butyl-S resins.

Results from a correlation study with six model proteins are illustrated in Figure 3. Phenyl Sepharose™ High Performance resin shows slightly higher resolution compared with Capto™ Phenyl ImpRes resin due to its even smaller particle size (34 µm).

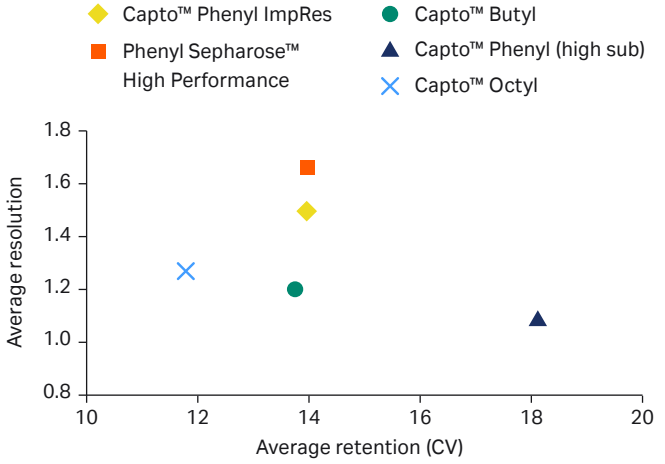

Fig 3. The average resolution of six model proteins plotted against retention volume in column volumes.

Hydrophobicity

Figure 4 displays the relative hydrophobicities of Capto™ Phenyl ImpRes and Capto™ Butyl ImpRes resins.

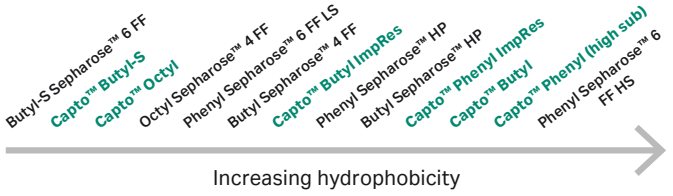

Fig 4. Relative hydrophobic scale of various resins, based on retention of ribonuclease A, lysozyme, and α-chymotrypsin. Can change with running conditions and proteins. The resins highlighted in green are the Capto™ HIC resins, which enable optimized productivity compared to Sepharose™ based resins.

Capacity

The dynamic binding capacity (DBC) at 10% breakthrough ($Q_{b,10}$) was determined by frontal analysis using the following parameters:

Column:	Tricorn™ 5/100
Equipment:	ÄKTA™ system
Residence time:	4 min
Equilibration buffer:	1.2 M ammonium sulfate
Sample:	BSA 3.6 mg/mL dissolved in equilibration buffer
Temperature:	23°C

Results are summarized in Table 2.

Table 2. Comparision of dynamic binding capacities at 10% breakthrough

Resin	$Q_{b,10}$ (mg/mL)
Phenyl Sepharose™ High Performance	21
Capto™ Phenyl ImpRes	19
Butyl Sepharose™ High Performance	39
Capto™ Butyl ImpRes	37

The capacity was determined at several residence times for Capto™ Phenyl ImpRes resin. When decreasing the residence time from 4 min to 1 min (i.e., increasing the flow velocity to a level not suitable for Phenyl Sepharose™ High Performance resin), only a 6% decrease in DBC was observed.

Chemical stability

The chemical stability of Capto™ Phenyl ImpRes and Capto™ Butyl ImpRes resins was determined by a total organic carbon (TOC) leakage analysis after storage in several solutions for one week at 40°C. The results showed that Capto™ Phenyl ImpRes and Capto™ Butyl ImpRes resins exhibit high chemical stability, with only minor carbon leakage at very low pH. Furthermore, the products can withstand storage at pH 14 for one month with no effect on the retention time when run according to the Cytiva standard analytical method.

Operation

Bed heights and flow velocities

The freedom available in process design for a given chromatography resin can be defined as its "window of operation." Figure 5 shows the relationship between column bed height and operating flow velocity for Capto™ ImpRes and Sepharose™ High Performance matrices with Capto™ and Sepharose™ 6 Fast Flow resins included as references. Both resins are composed of smaller average bead sizes (40 µm vs 34 µm) and therefore display high resolution, which is used for the intermediate purification/polishing step in large-scale purification schemes. Sepharose™ 6 Fast Flow and Capto™ resins are composed of comparatively larger average bead sizes (90 µm vs 75 µm) and have a higher throughput but lower resolution than Capto™ ImpRes resin. The size of the area under the pressure-limit curve represents the window of operation, which is the available operating range for the respective resin. As Figure 5 shows, the window of operation of the Capto™ ImpRes resin fits most needs both in terms of bed height and flow velocities.

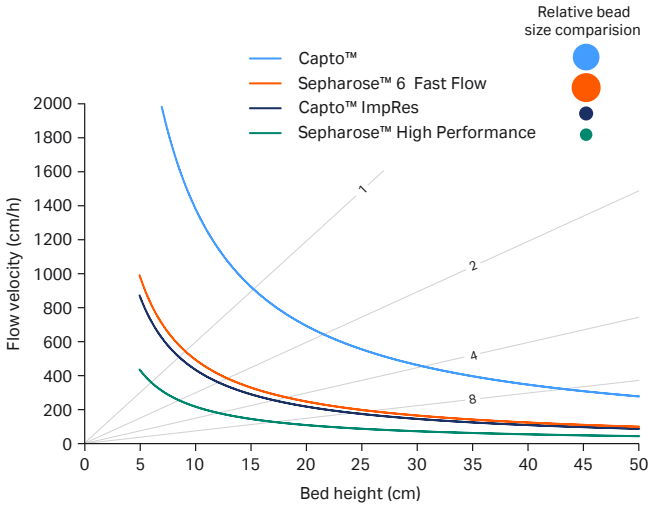

Fig 5. The window of operation (area under the curve) of different resins from Cytiva. Data correspond to a 1 m diameter column at 20°C and viscosity equivalent to water. Gray contours show the residence time in the column in minutes.

Productivity

A more rigid agarose resin allows for increased flow rates as well as the possibility to pack higher column beds, both enabling improved productivity. Increasing flow rate over the whole chromatographic purification process, (i.e., during column packing, conditioning, loading, washing, elution, regeneration, cleaning-in-place, and reconditioning) can reduce total processing time substantially. Using higher column beds with the same diameter, more protein can be purified during the same cycle, which increases throughput. For example, going from 15 cm (Sepharose™ High Performance) to 20 cm (Capto™ ImpRes) results in a 33% increase in resin volume and consequently 33% more protein can be processed per cycle if the capacities of the resins are the same. Altogether, the result of using more rigid chromatography resins, such as Capto™ ImpRes resins, is a significant improvement in downstream process productivity.

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™ Phenyl ImpRes and Capto™ Butyl ImpRes resins are available in multiple formats that are suitable for process development.

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

- PreDictor™ 96-well filter plates
  (96 purifications in parallel under static conditions)
- PreDictor™ 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™ Phenyl ImpRes and Capto™ Butyl ImpRes resins are also available in ReadyToProcess™ column formats, which are validated high performance prepacked columns for scale-up and GMP biomanufacturing.

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 and that can remain in the column after regeneration. Regular CIP prevents the build-up of these contaminants and also helps maintain the capacity, flow properties, and general performance of the resin. A specific CIP protocol should be designed for each process according to the type of contaminants that are present in the feed stream. General recommendation for CIP and sanitization protocols for all Cytiva HIC resins is to use 1 M NaOH. Use of a water-diluted organic solvent, such as ethanol or isopropanol, can be efficient in breaking strong hydrophobic interactions during CIP.

Storage

Capto™ Phenyl ImpRes and Capto™ Butyl ImpRes resins are supplied as a suspension containing 20% ethanol as preservative. Recommended storage condition is in 20% ethanol 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 *Kit includes the following 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*	5 × 1 mL	29321087
PreDictor™ Capto™ HIC Screening Kit *Kit includes the following Capto™ HIC resins: Capto™ Phenyl (high sub), Capto™ Phenyl ImpRes, Capto™ Butyl, Capto™ Butyl ImpRes, Capto™ Octyl, and Capto™ Butyl-S*	6 µL, 4 × 96 well plates	29711438
	20 µL, 4 × 96 well plates	29711439

Capto™ Phenyl ImpRes

Format	Quantity	Product code
Bulk	25 mL	17548401
	100 mL	17548402
	1 L	17548403
	5 L	17548404
HiTrap™ column	5 × 1 mL	17548411
	5 × 5 mL	17548412
HiScreen™ column	1 × 4.7 mL	17548410
PreDictor™ Plate	6 µL, 4 × 96-well filter plates	29711440
	20 µL, 4 × 96-well filter plates	29711441
PreDictor™ RoboColumn™ unit	200 µL, 8 columns	29701638
	600 µL, 8 columns	17548441
Process Characterization Kit	3 × 25 mL (3 different ligand densities)	17548470
ReadyToProcess™ column	1 L (80/200)	29101697
	1.9 L (126/150)	29609021
	2.5 L (126/200)	29101698
	5 L (178/200)	29642661
	7.4 L (251/150)	29696391
	10 L (251/200)	29101700
	20 L (359/200)	29101702
	32 L (450/200)	29256253
	57 L (600/200)	29649594

Capto™ Butyl ImpRes

Format	Quantity	Product code
Bulk	25 mL	17371901
	100 mL	17371902
	1 L	17371903
	5 L	17371904
HiTrap™ column	5 × 1 mL	17371911
	5 × 5 mL	17371912
HiScreen™ column	1 × 4.7 mL	17371910
PreDictor™ Plate	6 µL, 4 × 96-well filter plates	29711442
	20 µL, 4 × 96-well filter plates	29711443
PreDictor™ RoboColumn™ unit	200 µL, 8 columns	29701637
	600 µL, 8 columns	17371941
Process Characterization Kit	3 × 25 mL (3 different ligand densities)	17371970
ReadyToProcess™ column	1 L (80/200)	29713752
	2.5 L (126/200)	29655954
	5 L (178/200)	29647159
	10 L (251/200)	29138138
	20 L (359/200)	29229399
	32 L (450/200)	29256254
	57 L (600/200)	29474655

Related literature

Data file: Capto™ Phenyl (high sub), Capto™ Butyl, Capto™ Octyl, and Capto™ Butyl-S	CY13568
Data file: HiScreen™ prepacked columns	CY13473
Data file: PreDictor™ 96-well filter plates and Assist software	CY13663
Data file: PreDictor™ RoboColumn™	CY13689
Data file: ReadyToProcess™ columns	CY11724
Handbook: Hydrophobic interaction and reversed phase chromatography	CY11248

cytiva.com

CY13700-11May23-DF

cytiva