Capto Phenyl ImpRes and **Capto** Butyl ImpRes

HYDROPHOBIC INTERACTION CHROMATOGRAPHY

Capto™ Phenyl ImpRes and Capto Butyl ImpRes are hydrophobic interaction chromatography (HIC) media (resins) developed for the intermediate and polishing steps in a downstream protein purification process (Fig 1). Both chromatography media extend the well established Capto platform to include high-resolution media. By combining the high-flow characteristics of Capto media with a smaller particle size, Capto Phenyl ImpRes and Capto Butyl ImpRes 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 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
- Higher throughput may enable improved productivity and process economy
- Excellent chemical stability
- Security of supply and comprehensive regulatory support

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.

Fig 1. Capto Phenyl ImpRes and Capto Butyl ImpRes are supplied in various formats ranging from 1 mL prepacked HiTrap™ columns to bulk volumes.

Compared with Reversed Phase Chromatography (RPC) adsorbents, HIC media 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.

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 media. These include, but are not limited to, sample characteristics, type and concentration of salt, media porosity and hydrophobicity, flow rate, temperature and pH.

Media characteristics

Main media 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

	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 medium	37 mg BSA/mL medium
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 medium can be operated without significant change in function.
** Short-term stability: pH interval where the medium 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 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 media, allows for increased resolution compared with HIC media based on the larger 75 µm bead employed for Capto Phenyl, Capto Butyl and Capto Octyl.

Results from a correlation study with six model proteins are illustrated in Figure 2. Phenyl Sepharose™ High Performance shows slightly higher resolution compared with Capto Phenyl ImpRes due to its even smaller particle size (34 µm).

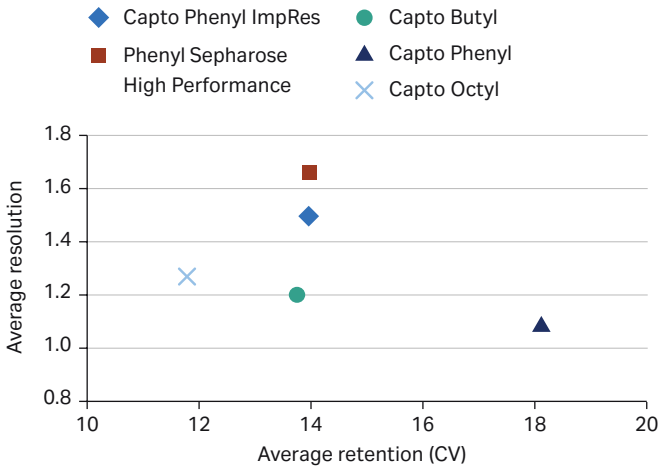

Fig 2. The average resolution of six model proteins plotted against retention volume in column volumes.

Hydrophobicity

Figure 3 displays the relative hydrophobicity of Capto Phenyl ImpRes and Capto Butyl ImpRes compared with other Cytiva HIC media. The relative order may change with running conditions and proteins.

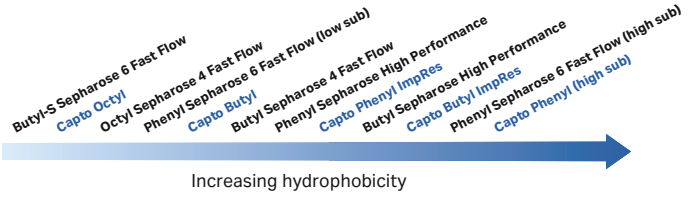

Fig 3. Relative hydrophobicity of HIC media. Products marked in blue are based on high-flow agarose (Capto) for increased productivity.

The hydrophobicity characteristics of Capto Phenyl ImpRes and Capto Butyl ImpRes were analyzed by a selectivity test using three model proteins: ribonuclease A, lysozyme, and α-chymotrypsinogen. The proteins were eluted with a decreasing gradient from 1.7 to 0 M ammonium sulphate over 60 minutes. Capto Phenyl ImpRes was optimized so that the retention times for lysozyme using Capto Phenyl ImpRes and Phenyl Sepharose High Performance were similar. Likewise, Capto Butyl ImpRes was optimized to have a similar retention time to Butyl Sepharose High Performance for α-chymotrypsinogen. The overlay chromatograms shown in Figure 4 show the similarities in retention times for the harmonized model proteins, whereas the differences in retention times for the other two proteins indicate a slight difference in medium selectivity.

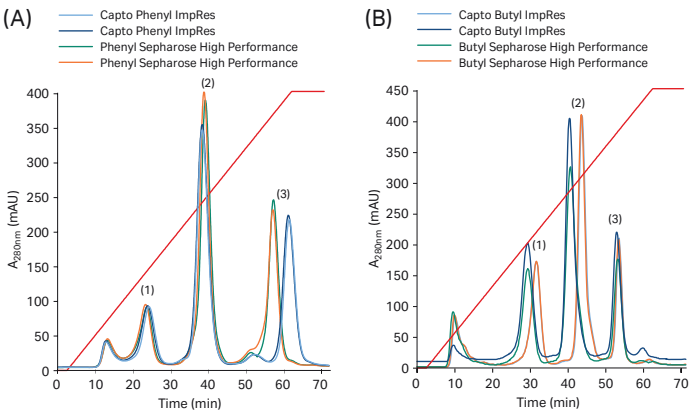

Fig 4. Selectivity test using three model proteins, ribonuclease A (1), lysozyme (2), and α-chymotrypsinogen (3). (A) Comparison of Capto Phenyl ImpRes with Phenyl Sepharose High Performance. (B) Comparison of Capto Butyl ImpRes with Butyl Sepharose High Performance.

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: ÄKTAexplorer™ 10
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

Medium	$Q_{b,10}$ (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. 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), only a 6% decrease in DBC was observed.

Chemical stability

The chemical stability of Capto Phenyl ImpRes and Capto Butyl ImpRes 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 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 medium 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 included as references. Both media 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 media are composed of comparatively larger average bead sizes (90 µm vs 75 µm) and have a higher throughput but lower resolution than Capto ImpRes. The size of the area under the pressure-limit curves represents the window of operation, which means the available operating range for the respective medium. As Figure 5 shows, the window of operation of the Capto ImpRes media fits most needs both in terms of bed height and flow velocities.

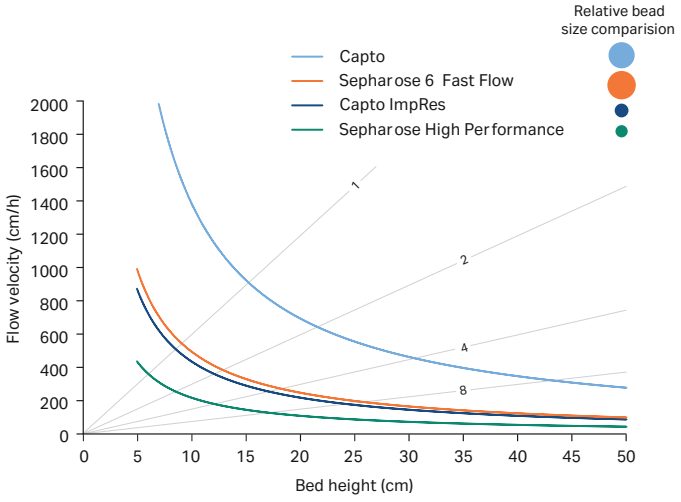

Fig 5. The window of operation (area under the curve) of different media from Cytiva. Data correspond to a 1 m diameter column at 20°C and viscosity equivalent to water. Gray contours show the residence time in the column in minutes.

Productivity

A more rigid agarose medium 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 gel volume and consequently 33% more protein can be processed per cycle if the capacity of the media is the same. Altogether, the result of using more rigid chromatography media, such as Capto ImpRes media, is a significant improvement in downstream process productivity.

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 medium. 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 media 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 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.

Small-scale format provides fast screening and method development

In the early stages of process development, using a small-scale format to screen for the most suitable chromatography process conditions saves both time and sample. Capto Phenyl ImpRes and Capto Butyl ImpRes are available in small, prepacked HiTrap (1 and 5 mL) and HiScreen™ (4.7 mL) column formats (Fig 1). 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 process scale-up.

Ordering information

Product	Quantity	Code number
Capto Phenyl ImpRes	25 mL	17-5484-01
Capto Phenyl ImpRes	100 mL	17-5484-02
Capto Phenyl ImpRes	1 L	17-5484-03
Capto Phenyl ImpRes	5 L	17-5484-04
Capto Butyl ImpRes	25 mL	17-3719-01
Capto Butyl ImpRes	100 mL	17-3719-02
Capto Butyl ImpRes	1 L	17-3719-03
Capto Butyl ImpRes	5 L	17-3719-04
HiScreen Capto Phenyl ImpRes	4.7 mL	17-5484-10
HiTrap Capto Phenyl ImpRes	5 × 1 mL	17-5484-11
HiTrap Capto Phenyl ImpRes	5 × 5 mL	17-5484-12
HiScreen Capto Butyl ImpRes	4.7 mL	17-3719-10
HiTrap Capto Butyl ImpRes	5 × 1 mL	17-3719-11
HiTrap Capto Butyl ImpRes	5 × 5 mL	17-3719-12

Related literature	Code number
HiScreen prepacked columns, Data file	28-9305-81
Hydrophobic Interaction and Reversed Phase Chromatography Principles and Methods, Handbook	11-0012-69

cytiva.com/capto

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