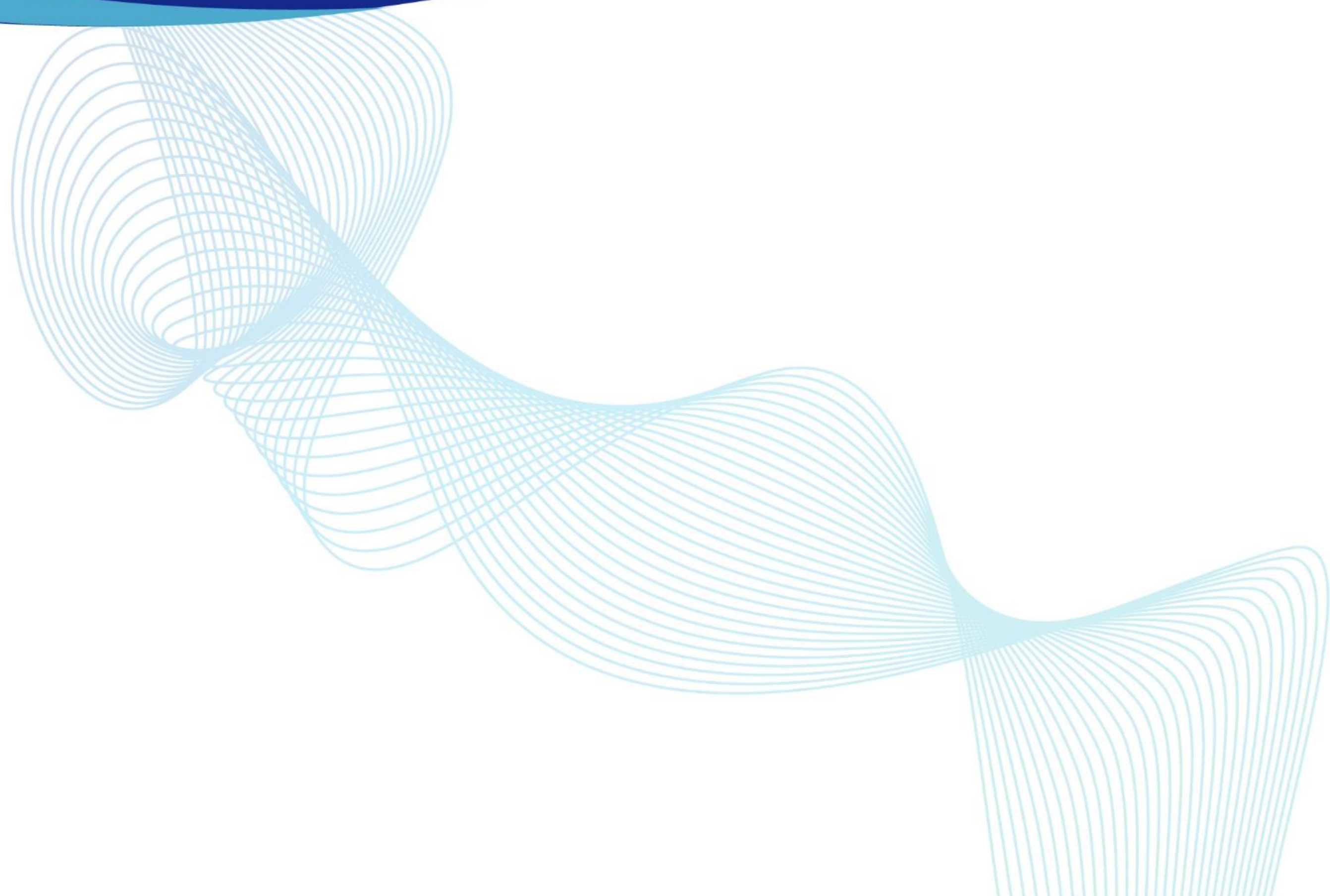
HPP Hydrophobic Interaction Chromatography Resin Product Manual



1. Product Introduction

Hydrophobic interaction chromatography utilizes the principle that proteins are adsorbed on the hydrophobic groups of chromatography resin at high salt concentrations, when the salt concentration is reduced, different proteins are eluted sequentially from weak to strong hydrophobicity to achieve the purpose of separation and purification. Hydrophobic interaction chromatography features mild operating conditions and high resolution. It is one of the most commonly used methods for the separation and purification of biological macromolecules. The HPP hydrophobic chromatography resin developed by Bogen uses highly cross-linked polyacrylate microspheres as the matrix and phenyl (Phenyl) as the ligand. This resin retains the high mechanical strength of the polymer microspheres while provides good compatibility with bioactive macromolecules, is especially suitable for the separation and purification of proteins, enzymes, polysaccharides, nucleic acids, plasmids, etc.

2. Product Properties

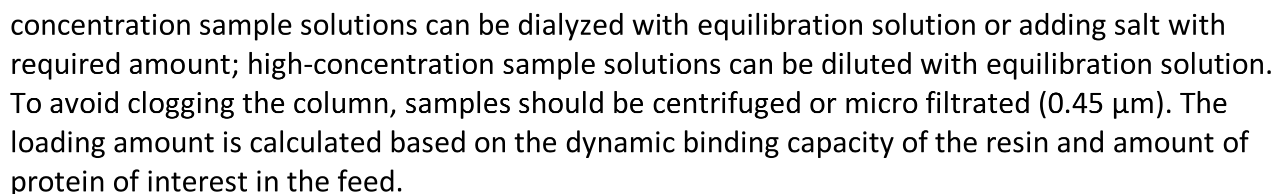
Parameter	Technical specification	
Average particle size	HPP-30	40 µm ± 10 µm
	HPP-60	70 µm ± 20 µm
Pore size	30 nm	
Matrix beads	Polyacrylate microspheres	
Dynamic binding capacity (25℃)	≥45 mg lysozyme /mL	
Pressure upper limit	1 MPa	
pH stability	3-13 (long-term); 1-14 (short-term)	
Chemical stability	Soaked in 1 M NaOH or 0.01 M HCl for 7 days at room temperature, no obvious change in performance observed.	
Storage	4-30 ℃ (20% ethanol)	

3. Operation steps

Hydrophobic interaction chromatography operations usually include steps such as equilibration, loading, washing, elution, and regeneration. Detailed operation methods are as follows (use chromatography column with height of 7.5 cm and I.D. 1.6cm, CV= 15ml as an example):

Equilibration: Use 5 – 10 CV equilibration buffer (Buffer A, such as 50 mM PB+1.0-2.0 M $(NH_4)_2SO_4$, pH7.0, the actual buffer system, salt type and concentration should be screened and optimized according to the stability and hydrophobicity of the target protein and the hydrophobicity of the chromatography resin) at a flow rate of 2 – 5 ml/min to equilibrate the column until the conductivity and pH of the effluent remain stable (consistent with the equilibration solution).

Loading: The buffer of the sample should be as consistent as possible with the equilibration solution. Solid samples can be prepared by dissolving them in a equilibration solution; low-



concentration sample solutions can be dialyzed with equilibration solution or adding salt with required amount; high-concentration sample solutions can be diluted with equilibration solution. To avoid clogging the column, samples should be centrifuged or micro filtrated (0.45 µm). The loading amount is calculated based on the dynamic binding capacity of the resin and amount of protein of interest in the feed.

Washing: Load at a flow rate of 2-5 ml/min and continue washing with equilibration buffer to baseline.

Elution: Elute with elution buffer (Buffer B, such as 50 mM PB, pH7.0) (linear gradient elution or step gradient elution can be used), and collect the effluent.

Regeneration: After each campaign, the chromatography column can be regenerated with 3 CV of low ionic strength buffer at a flow rate of 2-5 ml/min to remove proteins with strong hydrophobic binding (reversible binding), and then rinse with 3 CV of water and equilibrate with 5 CV of equilibration buffer.

Cleaning in place: After the resin is used 5-10 times (the actual number of uses is related to the type and source of the feed material and the experimental requirements), the resin needs to be cleaned in place to remove irreversibly bound proteins and other substances:

(1) For precipitated proteins, hydrophobically bound proteins and lipoproteins, use 1 M NaOH to backwash for 3-4 CV at a flow rate of 1-2 ml/min, and immediately rinse with 3 CV of water and equilibrate with 5 CV of equilibrium buffer;
(2) For strongly hydrophobically bound proteins, lipoproteins and lipids, use 70% ethanol or 30% isopropyl alcohol to backwash for 3-4 CV (when using high-concentration organic solvents, to avoid bubbles, should gradually increasing the organic solvent concentration) and immediately rinse with 3 CV of water and equilibrate with 5 CV of equilibration buffer.

Storage: Store in 20% ethanol at 4-30°C; the resin in the chromatography column can be washed with 20% ethanol and stored at 4-30°C.

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Product information is subject to change without prior notice. Duoning reserves the right of final interpretation.