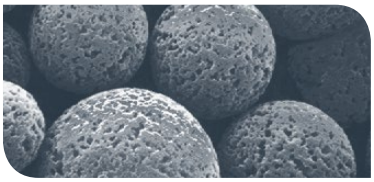

CHROMATOGRAPHY

Macro-Prep® HIC Resin

- Reverse-phase selectivity with maintenance of biological activity
- Efficient capture of proteins from high-salt conditions
- Use for protein, polypeptide, enzyme, and nucleic acid purification
- Particularly suited for HIC operations requiring high throughput and high target recovery

Increase Throughput and Efficiency

Summary

Hydrophobic interaction chromatography (HIC) separates proteins on the basis of relative hydrophobicity. HIC is a natural second step after either ion exchange or salt precipitation since the sample is applied in high-salt buffer. At high ionic strengths, hydrophobic sites of the protein interact with the alkyl groups of the resin. Retention, selectivity, and biological activity are somewhat dependent on pH, type of salt used, and its concentration.

The Macro-Prep Methyl HIC Resin is ideal for purification of proteins with strongly hydrophobic regions. The Macro-Prep t-Butyl HIC Resin is ideal for purification of proteins with few or weakly hydrophobic regions. The two different ligands provide alternative selectivities for easier optimization of separation (Table 1 and Figure 1).

Table 1. Comparison of retention times using Macro-Prep Methyl and t-Butyl ligands.

		Retention Times, min	
Peak	Protein	Methyl	t-Butyl
1	Cytochrome c	7.30	10.41
2	Ovalbumin	32.25	40.22
3	α-amylase	62.81	71.50
4	Ferritin	81.49	90.90

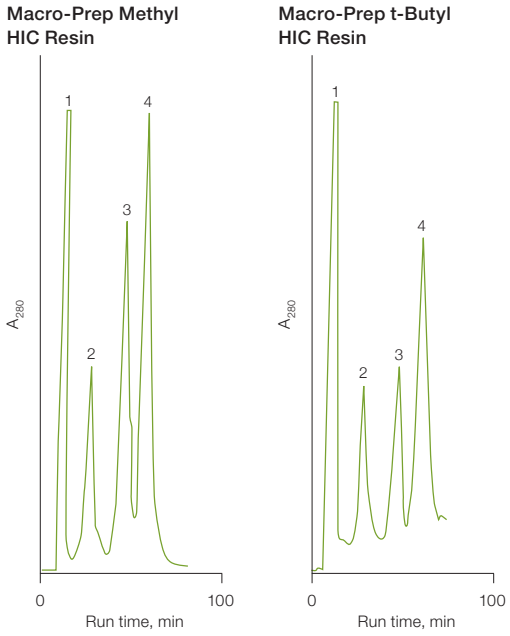

Fig. 1. Sample separation of proteins on Macro-Prep Methyl and t-Butyl Resins. A 50 µl sample (0.6 mg total protein) of cytochrome c (peak 1), ovalbumin (peak 2), α-amylase (peak 3), and ferritin (peak 4) was run on a 1 x 10 cm (4.5 ml) column at a linear flow rate of 38 cm/hr. The sample was eluted with 0.1 M Na_2PO_4, pH 7.0, 1.85 M $(NH_4)_2SO_4$ for 10 min, followed by a gradient from 1.85 to 0 M $(NH_4)_2SO_4$ over 90 min. The retention times for each protein are shown in Table 1 for comparison. A, absorbance.

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The properties of these resins are summarized in Table 2. These methacrylate copolymer resins provide high resolution at very high flow rates. They can be sanitized quickly and efficiently in 0.15% peracetic acid (Figure 2), and are compatible with many common solutions useful in HIC (Table 3). Changes in pH or ionic strength of the buffer do not cause shrinking or swelling of the resin. The rigidity of the Macro-Prep HIC Resins allows high flow rates without bed compression (Figure 3).

Table 2. Properties of Macro-Prep HIC Resins.

Property	Macro-Prep Methyl	Macro-Prep t-Butyl
Hydrophobicity	Weak	Mild
Functional group	Methyl	COO^- and t-butyl
Particle size	50 µm	50 µm
Total ionic capacity	<2 µeq/ml	120 µeq/ml
Dynamic binding capacity	15 mg HSA/ml	25 mg HSA/ml
Recommended maximum linear flow rate	100–600 cm/hr	100–600 cm/hr
Pressure vs. flow performance	<3 bar at 500 cm/hr	<3 bar at 500 cm/hr
pH stability	1–10*	1–10*
Shipping solution	20% ethanol	20% ethanol
Regeneration	70% ethanol	In 1–2 M NaCl/KCl or 70% ethanol
Sanitization	5 column volumes (CV) of 6 M guanidine-HCl, 100 cm/hr or 5 CV of 1% acetic acid in 0.12 M phosphoric acid, pH 1.5, 100 cm/hr	5 CV of 6 M guanidine-HCl, 100 cm/hr, 5 CV of 1% acetic acid in 0.12 M phosphoric acid, pH 1.5, 100 cm/hr, or 5 CV 0.15% peracetic acid
Storage conditions	20% ethanol or 1% acetic acid in 0.12 M phosphoric acid, pH 1.5	20% ethanol or 1% acetic acid in 0.12 M phosphoric acid, pH 1.5
Chemical stability	1% SDS, 8 M guanidine-HCl, 1 M HCl, 100% ethanol	1% SDS, 8 M guanidine-HCl, 1 M HCl, 100% ethanol
Shelf life	5 years	5 years

* The use of basic reagents greater than pH 10 should be evaluated for each application.
HSA, human serum albumin.

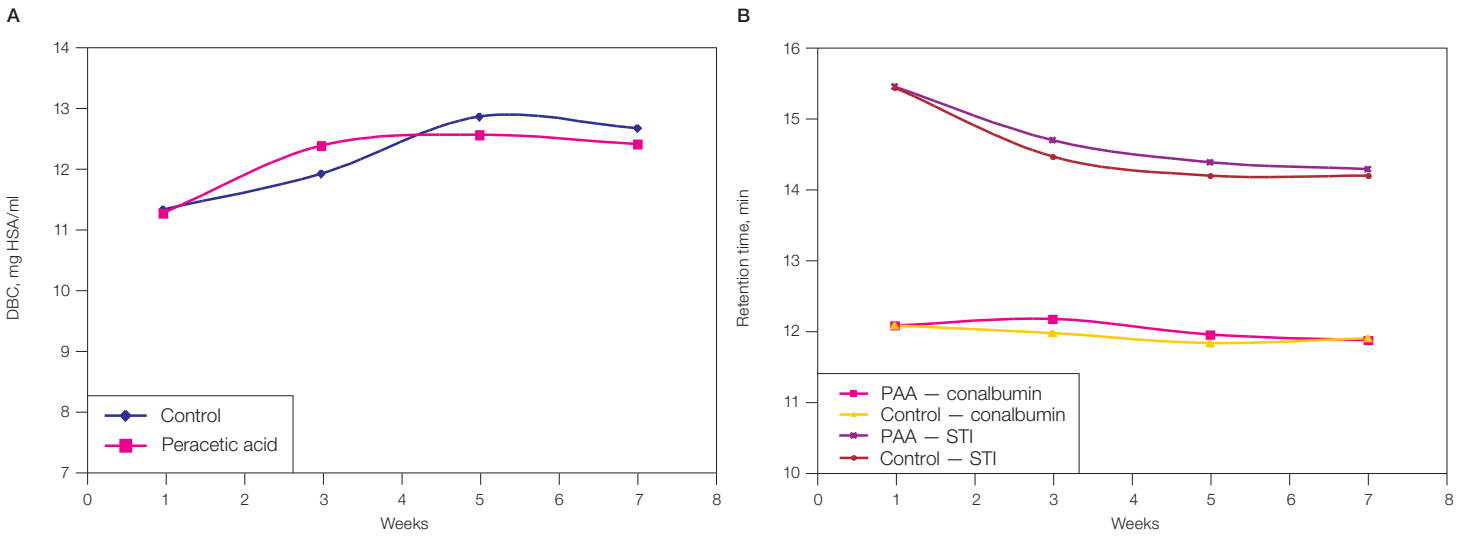

Fig. 2. Macro-Prep HIC Resins can be sanitized extensively with 0.15% peracetic acid (PAA). A, dynamic binding capacity (DBC); **B**, retention time. HSA, human serum albumin; STI, soybean trypsin inhibitor.

Table 3. Compatibility of Macro-Prep HIC Resins.

	Methyl	t-Butyl
1% SDS	Yes	Yes
8 M guanidine-HCl	Yes	Yes
1 M HCl	Yes	Yes
100% ethanol	Yes	Yes

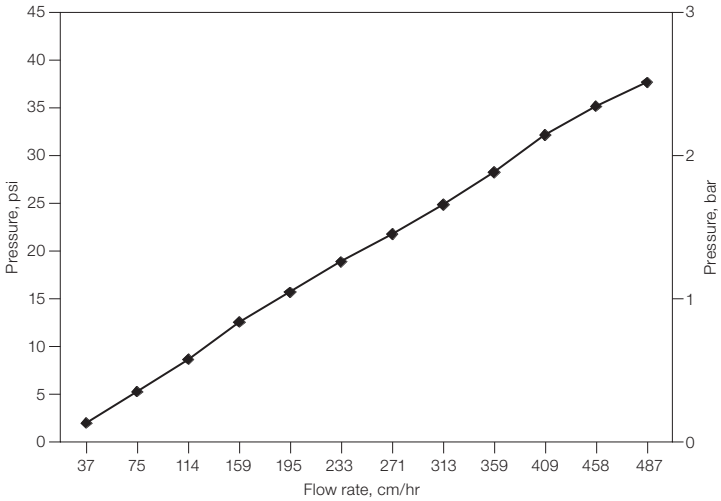

Fig. 3. Pressure vs. flow for Macro-Prep Resins. Resin bed dimensions were 14 cm diameter by 25 cm length.

Recommended Procedure

The Macro-Prep HIC Resins are easy to use. Rinse out the ethanol solution with 2–3 bed volumes of double distilled water and equilibrate in starting buffer. Samples are typically loaded on the column in a pH range of 4 to 9 and a salt concentration of 0.5 to 2 M ammonium sulfate or NaCl. Protein binding increases with both ligand hydrophobicity and salt concentration. In theory, less salt is required to salt out a protein when the pH is close to its isoelectric point. Temperature influences the hydrophobic interaction; protein binding is increased at elevated temperatures (40°C) and reduced at low temperatures (4°C). The salt concentration is gradually decreased to elute bound protein. Elution is followed by stripping and regeneration steps of choice. For more detailed information, refer to the instruction manual.

Technical Assistance

The Bio-Rad Life Sciences Group and its design, development, and manufacture of chemicals and analytical instruments have been assessed and registered by National Quality Assurance Limited against the provisions of BS EN ISO:9001:2000. For additional information and technical assistance, contact your Bio-Rad representative.

Visit **bio-rad.com/web/Macro-PrepHIC** for more information about Bio-Rad's Macro-Prep HIC Resins and to request a free sample.

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Ordering Information

Catalog #	Description
Bulk Resin	
1580080	**Macro-Prep Methyl HIC Support**, 25 ml
1560080	**Macro-Prep Methyl HIC Support**, 100 ml
1560081	**Macro-Prep Methyl HIC Support**, 500 ml
156-0082	**Macro-Prep Methyl HIC Support**, 5 L
156-0083	**Macro-Prep Methyl HIC Support**, 10 L
1580090	**Macro-Prep t-Butyl HIC Support**, 25 ml
1560090	**Macro-Prep t-Butyl HIC Support**, 100 ml
1560091	**Macro-Prep t-Butyl HIC Support**, 500 ml
156-0093	**Macro-Prep t-Butyl HIC Support**, 10 L

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France *33 01 47 95 69 65* ***Germany*** *49 89 31 884 0* ***Hong Kong*** *852 2789 3300* ***Hungary*** *36 1 459 6100* ***India*** *91 124 4029300*
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