HIC

CHROMATOGRAPHY
Macro-Prep® HIC Support

$-CH_3$
$-C(CH_3)_3$

Increase Throughput and Efficiency

- **Reverse-phase selectivity with maintenance of biological activity**
- **Efficient capture of proteins from high-salt conditions**

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 support. Retention, selectivity, and biological activity are somewhat dependent on pH, type of salt used, and its concentration.

The Macro-Prep methyl HIC support is ideal for purification of proteins with strongly hydrophobic regions. The Macro-Prep t-butyl HIC support is ideal for purification of proteins with few or weakly hydrophobic regions. The two different ligands provide alternative selectivities for easier optimization of separation (Figure 1 and Table 1). The properties of the supports are summarized in Table 2. These methacrylate copolymer beads 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 support. The superior mechanical and chemical stability of the Macro-Prep HIC supports make them a preferred choice over other HIC supports.

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

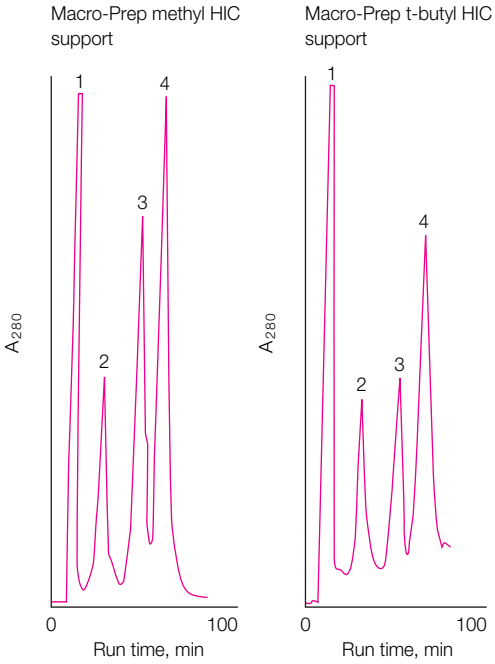

Fig. 1. Sample separation of proteins on Macro-Prep methyl and t-butyl supports. A 50 µl sample (0.6 mg total protein) of cytochrome c (peak 1), ovalbumin (peak 2), α-amylase (peak 3), and ferritin (peak 4) was run on a 1 x 10 cm (4.5 ml) column at a linear flow rate of 38 cm/hr. The sample was eluted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

Table 2. Properties of Macro-Prep HIC supports.

	Methyl	t-Butyl
Type of support	HIC	HIC
Functional group	$-CH_3$	$-C(CH_3)_3$
Binding capacity*	>25 mg/ml	>15 mg/ml
Nominal particle size	50 µm	50 µm
Max. linear flow rate	3,000 cm/h	3,000 cm/h
Autoclavability	121°C	121°C
pH stability	1–14	1–14
Regeneration	70% ethanol	70% ethanol
Sanitization	0.15% peracetic acid	0.15% peracetic acid

* Determined with human serum albumin (HSA)

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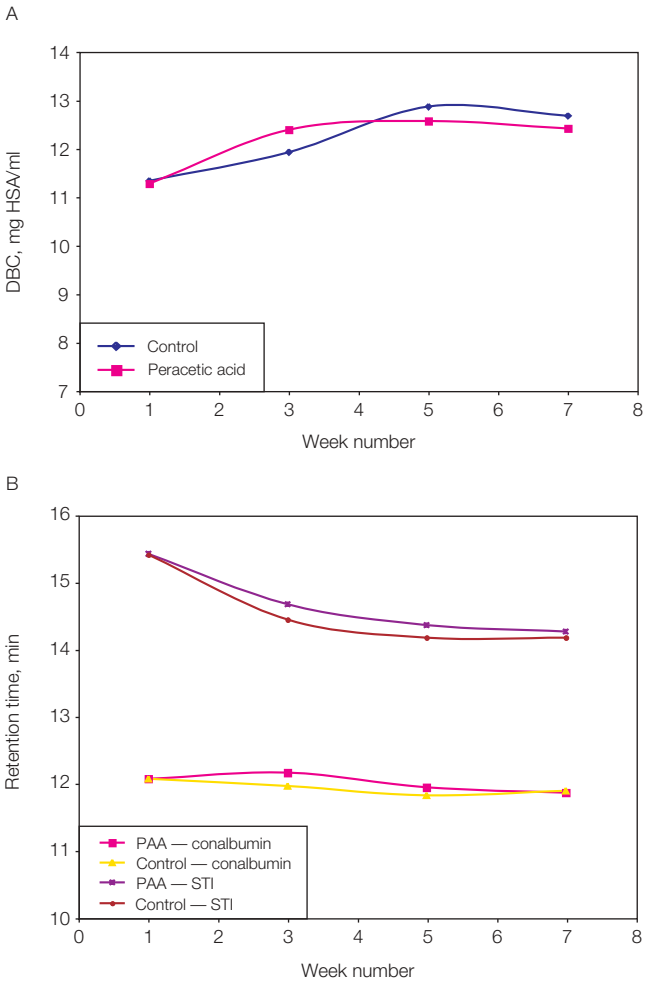

Fig. 2. Macro-Prep HIC supports can be sanitized extensively with 0.15% peracetic acid. A, Dynamic binding capacity; B, retention time.

Table 3. Compatibility of Macro-Prep HIC supports.

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

Recommended Procedure

The Macro-Prep HIC supports 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 the pH range of 4–9 and in 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 Macro-Prep HIC products have manufacturing processes registered with the United States Food and Drug Administration (US FDA) by submission of type II Drug Master Files (DMF). Regulatory support files are available, upon request, to companies entering into clinical trials. 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:9001:1994. For additional information and technical assistance, contact your Bio-Rad representative.

For more information on Bio-Rad's complete line of process chromatography supports and other products for life science research and process-scale production, visit us on the Web at **www.bio-rad.com/process/**

Ordering Information

Catalog #	Description
158-0080	Macro-Prep Methyl HIC Support, 25 ml
156-0080	Macro-Prep Methyl HIC Support, 100 ml
156-0081	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
158-0090	Macro-Prep t-Butyl HIC Support, 25 ml
156-0090	Macro-Prep t-Butyl HIC Support, 100 ml
156-0091	Macro-Prep t-Butyl HIC Support, 500 ml
156-0092	Macro-Prep t-Butyl HIC Support, 5 L
156-0093	Macro-Prep t-Butyl HIC Support, 10 L

Larger volumes are available upon request.

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