# Hydrophobic Interaction Chromatography



# **Proteomix** <sup>®</sup> HIC



Better Surface Chemistry for Better Separation

# Sepax Technologies, Inc.

Sepax Technologies, Inc. develops and manufactures products in the area of chemical and biological separations, biosurfaces and proteomics. Sepax product portfolio includes 1) liquid chromatography columns and media, 2) SPE and Flash chromatography columns and tubes, 3) bulk resin for preparative separation and process chromatography, and 4) natural product and Chinese traditional medicine separation and purification.



# Leader in Biological Separations

Sepax develops and manufactures wide range of biological separation products using both silica and polymeric resins as the support. The selection of particle size is from 1  $\mu$ m to 100  $\mu$ m and pore size from non-porous to 2000 Å. Unique and proprietary resin synthesis and surface technologies have been developed for solving the separation challenges in biological area.

# **Bioseparation Products**

Size Exclusion SRT® SRT®-C Nanofilm® Zenix® Zenix®-C <u>Ion Exchange</u> Proteomix® IEX Antibodix®WCX <u>Hydrophobic Interaction</u> Proteomix®HIC <u>Carbohydrate Separation</u> Carbomix®

### Analytical, Semi-prep and Preparative

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# Proteomix<sup>®</sup> HIC Phases

## Polymer Based Hydrophobic Interaction Chromatography Media and Column

#### General Description

Proteomix® HIC columns are specially designed for high resolution and high efficiency separations of proteins, monoclonal antibodies (mAbs), antibody drug conjugates (ADCs), oligonucleotides and peptides via a hydrophobic interaction chromatography (HIC) mechanism. Utilizing proprietary surface technologies, Proteomix® HIC-NP resin is made of non-porous polystyrenedivinylbenzene (PS/DVB) beads with narrow-dispersed particle size distribution. The PS/DVB bead is modified with alkyl groups or aryl groups that provide hydrophobic interaction with analytes (Figure 1).

Proteomix<sup>®</sup> HIC-NP resin is highly rigid and mechanically stable. In comparison to silica-based HIC phase media, Proteomix® HIC-NP phases have advantages for biomolecule separations with a wide pH range (2-12) and high chemical stability. The nonporous structure and unique chemistry, and narrow particle distribution offer special selectivity, high-resolution separation of proteins such as mAb, ADC and related protein fragments, DNA and oligonucleotides. Proteomix® HIC-NP media is applicable for laboratory discovery, laboratory-scale purification and preparative chromatography for the production of several milligrams to grams of proteins. Sepax also provides polymethacrylate-based Generik MC and Polar MC HIC 30 µm media which offers similar characteristics to the Proteomix® HIC-NP but is designed for process-scale applications from grams to kilograms of proteins.

Figure 1. Structure of Proteomix® HIC resin



#### **Featured Characteristics**

- Highest capacity and resolution
- · High protein recovery with intact biological activity
- High stability and lot-to-lot reproducibility
- High pressure and high temperature tolerance

• Ideal for separation and analysis of hydrophobic proteins, monoclonal antibodies and their derivatives such as antibody drug conjugates, and organic molecules derivatized with polymer branches

#### **Technical Specifications**

Resin Matrix:	Spherical, highly cross-linked PS/DVB
Pore Size:	Nonporous
Particle Size:	1.7, 5 and 10 µm
Phase Structure:	Ethyl, Propyl, Butyl and Phenyl (5, 10 µm) Ethyl and Butyl (1.7 µm)
Separation Mechanism:	Hydrophobic interaction
pH Stability:	2-12
Operating Temperature:	Up to 80 °C
Operating	6000 psi (5, 10 μm)
Pressure:	8000 psi (1.7 μm)
Mobile Phase Compatibility:	Compatible with aqueous solution, a mixture of water and acetonitrile, acetone, methanol, or THF

#### Hydrophobicity Order of the four HIC NP phases

#### HIC Ethyl<Propyl<Butyl<Phenyl



Figure 2. Protein Mixture on Four Proteomix® HIC NP5 Phases

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#### High Stability and Lot-to-Lot Reproducibility

Proteomix® HIC columns are based on PS/DVB resin and all the surface coatings are chemically bonded onto PS/DVB support, which allows exceptionally high stability, resulting in a high number of injections per column life. The columns are compatible with most aqueous buffers, such as ammonium sulfate, sodium acetate, phosphate, Tris as well as a mixture of water and acetone, methanol, acetonitrile and THF.



Figure 3. Proteomix<sup>®</sup> HIC Butyl-NP5 Life Time Test on Protein Mixture

With a well-controlled synthesis process of surface chemistry, Proteomix<sup>®</sup> HIC phases, the resin production is highly reproducible, leading to consistent column manufacturing, thus high lot-to-lot consistency on the separation of biomolecules like mAb, ADC and proteins (Figure 4-6).





Figure 4. Proteomix® HIC Butyl NP5 for Rituximab-mAb

	(5 μm, 4.6 x 35 mm), PN: 431NP5-4603
Flow rate:	0.8 mL/min
Detector:	UV 214 nm
Temperature:	25 °C
Mobile phase:	A: 2 M ammonium sulfate in 0.1M sodium
	phosphate, pH 7.0
	B: 0.1 M sodium phosphate, pH 7.0
Sample:	Rituximab, 1.3 mg/mL dilute in water
Injection:	4 μL









Sample:	ADC, 1.0 mg/mL in 1.0 M ammonium sulfate
Injection:	10 μL

#### **H**igh Loading Capacity

Loading Capacity is critical for hydrophobic interaction separation and purification.  $Proteomix^{\oplus}$  HIC columns have high loading capacity for biomolecules such as mAb (Figure 7-8).

# Figure 7. MAb Loading Test on *Proteomix®* HIC Butyl NP5 4.6 x 50 mm size column



# Figure 8. MAb Loading Test on *Proteomix®* HIC Butyl NP5 21.2 x 50 mm size column



Column:	Proteomix <sup>®</sup> HIC Butyl NP5
	(5 µm, 21.2 x 50 mm, 17.7 mL resin)
	PN: 431NP5-21205
Flow rate:	20.0 mL/min
Detector:	UV 280 nm
Temperature:	25 °C
Mobile phase:	A: 2 M ammonium sulfate in 0.1 M
	sodium phosphate, pH 7.0
	B: 0.1 M sodium phosphate, pH 7.0

Sample:	2.5 mg/mL mAb in 50% A
Injection:	200, 400 and 1000 µL
Column Pressure:	90 Bar

#### **Applications**

With its unique surface technology the *Proteomix*<sup>®</sup> HIC column offers special selectivity and high-resolution separation of biomolecules such as mAb, ADC and related protein fragments, DNA and oligonucleotides.

#### Separation of ADCs (Antibody Drug Conjugates)

The *Proteomix*<sup>®</sup> HIC Butyl columns provide effective and efficient separation of mAb and Antibody Drug Conjugates with different Drug-to-Antibody Ratio (DAR) species (Figure 9-11).





	(5 μm, 4.6 x 35 mm), PN: 431NP5-4603
Mobile phase:	A: 2 M ammonium sulfate in 0.025 M sodium
	phosphate, pH 7.0
	B: 0.025 M sodium phosphate, pH 7.0
	C: 100% IPA
Flow rate:	0.8 mL/min
Detector:	UV 214 nm
Temperature:	25 °C
Sample:	Herceptin/ADC1/ADC2, 1.0 mg/mL in 25 mM
	sodium phosphate
njection:	10 μL

Figure 10. Herceptin-cysteine ADC separation on *Proteomix®* HIC Butyl NP5 - Competition Comparison with other vendor Butyl-NPR



Column:	Proteomix <sup>®</sup> HIC Butyl NP5
	(5 µm, 4.6 x 35 mm), PN: 431NP5-4603
	Other Vendor Butyl-NPR
Flow rate:	0.8 mL/min
Detector:	UV 214 nm
Temperature:	25 ℃
Mobile phase:	A: 2 M ammonium sulfate in 0.025 M sodium
	phosphate, pH 7.0
	B: 0.025 M sodium phosphate, pH 7.0
	C: 100% IPA
Sample:	ADC, 1.0 mg/mL in 1.0 M ammonium sulfate
Injection:	10 μL

IIIAU	
400	
350	
300	ADC 1 DAR Separation on
250	Sepax Proteomix HIC Butyl
200	NEW 1.7 μm UHPLC Column
150	
100	
50	
0	
2	4 6 8 10 12 14 <sup>m</sup>
Column:	Proteomix <sup>®</sup> HIC Butyl NP1.7
	(1.7 μm, 4.6 x 35 mm), PN: 431NP2-4603
Flow rate:	0.8 mL/min
Detector:	UV 214 nm
Temperature:	25 °C
Mobile phase:	A: 2 M ammonium sulfate in 0.025 M
	sodium phosphate, pH 7.0
	B: 0.025 M sodium phosphate pH 7.0
	C. 100% TPA
Samples	ADC 1 10 mg/mL in 10 M ammonium
Sample:	autor
Tutantan	suitate.
injection:	20 μL

Figure 11. ADC\_1 Separation - Proteomix® HIC Butyl NP1.7

#### Separations of Intact MAbs (Monoclonal Antibodies)

The *Proteomix*<sup>®</sup> HIC Butyl columns provide high resolution and separation of intact mAbs under native conditions. It can also be used for mAb hydrophobicity screening for monoclonal antibody species with differing hydrophobicities.

Figure 12. Separation of four different mAbs on Proteomix<sup>®</sup> HIC Butyl NP5 column



Column:	Proteomix <sup>®</sup> HIC Butyl NP5
	(5 µm, 4.6 x 35 mm), PN: 431NP5-4603
Flow rate:	1.0 mL/min
Detector:	UV 214 nm
Temperature:	25 °C
Mobile phase:	A: 100 mM sodium phosphate buffer, 2 M
	ammonium sulfate, pH 7.0
	B: 100 mM sodium phosphate buffer, pH 7.0
	C: B + 1.0 M NaCl
Injection:	15 μg mAb samples

Figure 13. MAb (Erbitux, Herceptin and Rituximab) Separation on Proteomix<sup>®</sup> HIC Butyl NP1.7



Comparison of the separation of Herceptin and Rituximab between Proteomix<sup>®</sup> HIC Butyl and another vendor's Butyl-NPR column demonstrates that higher resolution can be achieved on both Proteomix<sup>®</sup> HIC Butyl NP 5 and 1.7 µm columns (Figure 14-15).

Figure 14. Herceptin (mAb) separation on *Proteomix*<sup>®</sup> HIC Butyl NP5 - Competition Comparison vs. Other Vendor butyl-NPR





Column:	Proteomix® HIC Butyl NP5
	(5 µm, 4.6 x 35 mm), PN: 431NP5-4603
	Other Vendor Butyl-NPR
Flow rate:	0.8 mL/min
Detector:	UV 214 nm
Temperature:	25 °C
Mobile phase:	A: 2 M ammonium sulfate in 0.025 M sodium
	phosphate, pH 7.0
	B: 0.025 M sodium phosphate, pH 7.0
	C: 100% IPA
Sample:	Herceptin, 1.0 mg/mL in 1.0 M ammonium sulfat
Injection:	5 μL

Figure 15. Rituximb (mAb) separation on Proteomix<sup>®</sup> HIC Butyl-NP1.7 - Competition Comparison vs. Other Vendor butyl-NPR

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#### Impact of Column Length

For higher resolution and separation of difficult samples, longer columns are recommended. The higher aspect ratio can be achieved with our mechanically stable resin allowing for longer contact time and hydrophobic resolving power. A 100 mm length Proteomix® HIC Butyl-NP5 column increases the resolution in comparison to a 35 mm length column (Figure 16).



#### **MAb Oxidation Variants Analysis**

HIC can be used as an orthogonal chromatographic method to assess the populations and potency of the biomolecules in native molecule structure under native conditions. Different forms of mAb oxidation can be monitored in a time course of mAb oxidation process by Proteomix® HIC Butyl columns to gain a better understanding of the composition of species in the drug product. Partially oxidized mAbs can be separated into different peaks at several time points. An 18-hour reaction with t-BHP offers almost complete oxidation, which can be resolved from unoxidized mAb to a great degree (Figure 17-18).



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Figure 17. Rituximab oxidation on Proteomix® HIC Butyl NP1.7

Column:	Proteomix <sup>®</sup> HIC Butyl NP1.7
	(1.7 μm, 4.6 x 100 mm), PN: 431NP2-4610
Flow rate:	0.8 mL/min
Detector:	UV 214 nm
Temperature:	25 °C
Pressure:	560 bar
Mobile phase:	A: 100 mM sodium phosphate buffer, 2 M
	ammonium sulfate, pH 7.0
	B: 100 mM sodium phosphate buffer, pH 7.0
MAb	10 mg/mL was diluted to 2.5 mg/mL with
oxidation:	water and 70% t-BHP was added to a final
	5% concentration, incubate in dark and take
	time point, dilute to 2.5 mg/mL in 500 mM
	ammonium sulfate, 25 mM phosphate buffer
Injection:	12.5 µg mAb sample, 25 µg oxidized mAb





Column:	Proteomix <sup>®</sup> HIC Butyl NP5
	(5 μm, 4.6 x 100 mm), PN: 431NP5-4610
Flow rate:	0.8 mL/min
Pressure:	170 bar
System:	UHPLC
Detector:	UV 214 nm
Temperature:	25 °C
Mobile phase:	A: 100 mM sodium phosphate buffer, 2 M
	ammonium sulfate, pH 7.0
	B: 100 mM sodium phosphate buffer, pH 7.0;
MAb	10 mg/mL was diluted to 5 mg/mL with water
oxidation:	and 70% t-BHP was added to a final 5%
	concentration, incubate in dark and take time
	point, dilute to 2.5 mg/mL in 500 mM
	ammonium sulfate, 25 mM phosphate buffer
Injection:	10 μg mAb sample, 20 μg oxidized mAb

#### **Impact of Particle Size**

The smaller particle size of *Proteomix*<sup>®</sup> HIC Butyl NP1.7 makes it more sensitive to capture small changes of biomolecule variants, which provides superior ability to achieve a higher resolution separation of a wide range of protein variants and impurities than the NP 5um column. *Proteomix*<sup>®</sup> HIC Butyl NP1.7 is an excellent choice for highest resolution separations of mAb oxidation and other protein variants analysis.





#### Separations of PEGylated Proteins



Figure 20. PEGylated BSA Separation - Proteomix<sup>®</sup> HIC Butyl NP5

# Ordering Information

### Proteomix<sup>®</sup> HIC Phase Screening Kit

P/N	Phases	Particle Size	Column Size (mm)	
HICKIT-4603	Butyl Phenyl	5 µm	4.6 x 35	
HICKIT-4610	Ethyl Propyl	5 µm	4.6 x 100	

\*Hydrophobicity Order: Ethyl<Propyl<Butyl<Phenyl

#### Proteomix® HIC Butyl

P/N	Particle Size	Column Size (mm)
431NP2-4603	1.7 μm	4.6 x 35
431NP2-4605	1.7 μm	4.6 x 50
431NP2-4610	1.7 μm	4.6 x 100
431NP2-4001C	1.7 μm	4.0 x 10 (Guard)
431NP5-4603	5 µm	4.6 x 35
431NP5-4605	5 µm	4.6 x 50
431NP5-4610	5 µm	4.6 x 100
431NP5-4001C	5 µm	4.0 x 10 (Guard)
431NP5-7805	5 µm	7.8 x 50
431NP5-7810	5 µm	7.8 x 100
431NP510005	5 µm	10.0 x 50
431NP5-10010	5 µm	10.0 x 100
431NP5-10015	5 µm	10.0 x 150
431NP5-21205	5 µm	21.2 x 50
431NP5-21210	5 µm	21.2 x 100
431NP5-21215	5 µm	21.2 x 150
431NP5-30005	5 µm	30.0 x 50
431NP5-30010	5 µm	30.0 x 100
431NP5-30015	5 µm	30.0 x 150

## Proteomix® HIC Ethyl

P/N	Particle Size	Column Size (mm)
432NP2-4603	1.7 μm	4.6 x 35
432NP2-4605	1.7 μm	4.6 x 50
432NP2-4610	1.7 μm	4.6 x 100
432NP2-4001C	1.7 μm	4.0 x 10 (Guard)
432NP5-4603	5 µm	4.6 x 35
432NP5-4605	5 µm	4.6 x 50
432NP5-4610	5 µm	4.6 x 100
432NP5-4001C	5 µm	4.0 x 10 (Guard)
432NP5-7805	5 µm	7.8 x 50
432NP5-7810	5 µm	7.8 x 100
432NP510005	5 µm	10.0 x 50
432NP5-10010	5 µm	10.0 x 100
432NP5-10015	5 µm	10.0 x 150
432NP5-21205	5 μm	21.2 x 50
432NP5-21210	5 µm	21.2 x 100
432NP5-21215	5 µm	21.2 x 150
432NP5-30005	5 µm	30.0 x 50
432NP5-30010	5 µm	30.0 x 100
432NP5-30015	5 µm	30.0 x 150

### Proteomix® HIC Phenyl

P/N	Particle Size	Column Size (mm)
433NP5-4603	5 µm	4.6 x 35
433NP5-4605	5 µm	4.6 x 50
433NP5-4610	5 µm	4.6 x 100
433NP5-4001C	5 µm	4.0 x 10 (Guard)
433NP5-7805	5 µm	7.8 x 50
433NP5-7810	5 µm	7.8 x 100
433NP510005	5 µm	10.0 x 50
433NP5-10010	5 µm	10.0 x 100
433NP5-10015	5 µm	10.0 x 150
433NP5-21205	5 µm	21.2 x 50
433NP5-21210	5 µm	21.2 x 100
433NP5-21215	5 µm	21.2 x 150
433NP5-30005	5 µm	30.0 x 50
433NP5-30010	5 µm	30.0 x 100
433NP5-30015	5 µm	30.0 x 150

# Proteomix<sup>®</sup> HIC Propyl

P/N	Particle Size	Column Size (mm)
434NP5-4603	5 µm	4.6 x 35
434NP5-4605	5 µm	4.6 x 50
434NP5-4610	5 µm	4.6 x 100
434NP5-4001C	5 µm	4.0 x 10 (Guard)
434NP5-7805	5 µm	7.8 x 50
434NP5-7810	5 µm	7.8 x 100
434NP510005	5 µm	10.0 x 50
434NP5-10010	5 µm	10.0 x 100
434NP5-10015	5 µm	10.0 x 150
434NP5-21205	5 µm	21.2 x 50
434NP5-21210	5 µm	21.2 x 100
434NP5-21215	5 µm	21.2 x 150
434NP5-30005	5 µm	30.0 x 50
434NP5-30010	5 µm	30.0 x 100
434NP5-30015	5 µm	30.0 x 150

\* 10  $\mu m$  particle size columns and other column dimensions available upon request





# How to Order

Please contact Sepax Sales Department:

Phone: (302)366-1101 1-877-SEPAX-US Fax: (302)366-1151 Email: sales@sepax-tech.com

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#### Discounts

Sepax Technologies offers best discounts determined by the volume of the purchase. Please contact the Sepax Sales Department for your maximum discount.

#### **Opening a Sepax Account**

Call the Sepax Sales Department and supply your business information, and billing and shipping address to set up a Sepax account. Open account terms are subject to credit approval.

#### **Payment Term**

Terms of payment are net 30 days. Mastercard®, Visa®, and American Express® are accepted. There is no minimum order.

#### **Return Policy**

#### Shipping

If items are damaged in transit, simply follow these instructions:

- If shipment is visibly damaged on arrival, do not accept it until the delivery person has endorsed it with a statement for the extent of damage.
- Notify us immediately of the damaged shipment in order for us to make the appropriate adjustment and/or provide you with return instructions.

#### Returns

 Sepax accepts eligible returns within 15 days of customer receiving order.

- Non-eligible returns include products contaminated, treated, or tested, with isotope, radioactive chemical, or any other types of hazardous material, semi-prep and prep columns, custom products, bulk resins/materials, and demo purchase.
- Prior authorization required for all returns. Please contact your local sales manager for prior authorization and Return Authorization Number.
- 15% restocking charge will be made on all returns.
- Shipping costs are non-refundable. Customer pays for all shipping related costs sending return product back to Sepax. Refund will only be processed upon receipt of the returned product.
- Return and refund to be made with same method of purchase, i.e. through distributor if purchased through distributor.

#### Warranty

Sepax Technologies warrants its products to be free from manufacturing defects for 90 days after the shipment. Sepax will accept for return or replacement any product which fails to meet the stated specifications. This warranty shall not apply to any defect, failure or damage caused by improper use or improper or inadequate maintenance and care. This warranty is exclusive and no other warranty, whether written or oral is expressed or implied. Sepax specifically disclaims the implied warranties of merchantability and fitness for a particular purpose. Under no circumstance shall Sepax be liable for direct, indirect or consequential damages arising from the use of its products. The maximum liability that Sepax will assume should be no more than the invoice price of the product.



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