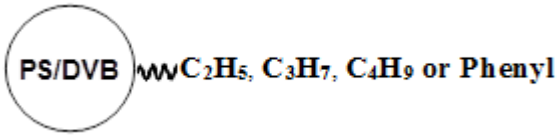
Proteomix® HIC-NP Phases

Polymer Based Hydrophobic Interaction Chromatography Media and Columns

General Description

Proteomix® HIC columns are specially designed for high resolution and high efficiency separations of proteins, oligonucleotides and peptides. Utilizing proprietary surface technologies, *Proteomix*® HIC-NP resin is made of non-porous polystyrenedivinylbenzene (PS/DVB) beads with narrow-dispersed particle size distribution. As shown in Figure 1, the PS/DVB bead is modified with alkyl groups or aryl group that provides hydrophobic interaction with analytes. Proteomix® 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 wide pH range (2-12) and high thermal stability. The nonporous structure and narrow particle distribution offer special selectivity, high resolution separation of proteins such as mAb (monoclonal antibody), ADC (antibody drug conjugate) and related protein fragments, DNA and oligonucleotides. *Proteomix*® HIC-NP media is applicable at laboratory discovery, laboratory-scale purification and process chromatography for the production of a few mgs to kilogram of proteins.

Figure 1. Structure of *Proteomix*® HIC-NP5 resin



Technical Specifications

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

Featured Characteristics

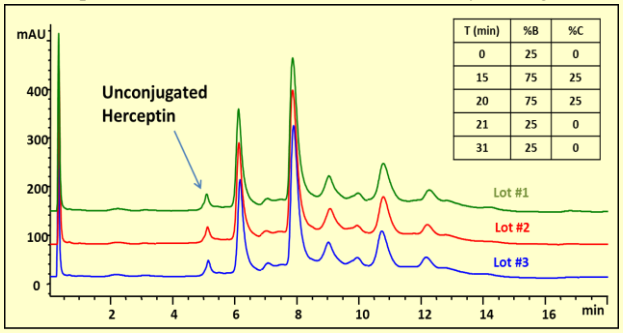
- Highest capacity and resolution
- High lot-to-lot reproducibility
- High protein recovery with intact biological activity
- Negligible non-specific interactions
- Ideal for separation and analysis of hydrophobic proteins, and monoclonal antibodies derivatized with polymer branches
- Suitable for separation and analysis of general biological samples

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 exceptional high stability. The columns are compatible with most aqueous buffers, such as ammonium sulfate, sodium acetate, phosphate, Tris and a mixture of water and acetone, methanol, acetonitrile and THF. When 25 mM sodium phosphate buffer at pH 7.0 was used as the mobile phase to run *the Proteomix*® HICButyl NP5 column, 400 injections or 3 months of usage has negligible deterioration for the columns.

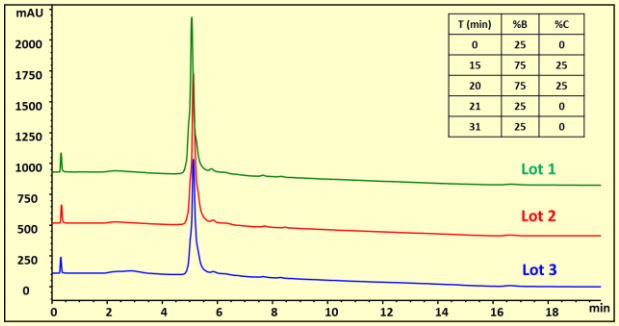
Proteomix® HIC columns provide high lot to lot consistency on the biomolecule separation as shown below in **Figure 2-4**:

Figure 2. *Proteomix*® HIC Butyl-NP5 for Herceptin-cysteine ADC separation-Three resin lot to lot consistency testing



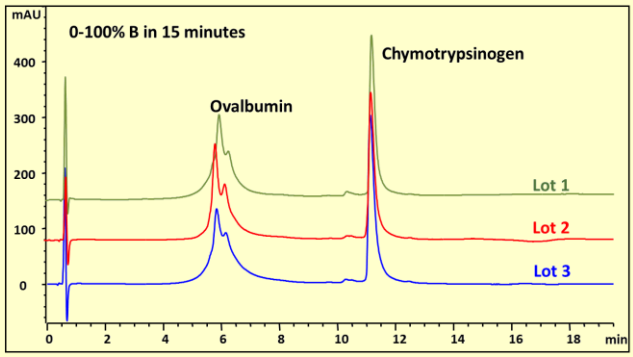
Column: *Proteomix*® HIC Butyl-NP5 (5 µm, 4.6 x 35 mm)
Flow rate: 0.8 mL/min
Detector: UV 214 nm
Temperature: 25 ˚C
Mobile phase: A: 2 M ammonium sulfate in 0.025M sodium phosphate, pH 7.0
B: 0.025 M sodium phosphate pH 7.0
C: 100% IPA
Sample: ADC, 1mg/mL in 1M ammonium sulfate
Injection: 10 µL

Figure 3. *Proteomix*® HIC Butyl-NP5 for Herceptin-mAb separation- Three resin lot to lot consistency testing



Column: *Proteomix*® HIC Butyl-NP5 (5 µm, 4.6 x 35 mm)
Flow rate: 0.8 mL/min
Detector: UV 214 nm
Temperature: 25 ˚C
Mobile phase: A: 2 M ammonium sulfate in 0.025M sodium phosphate, pH 7.0
B: 0.025 M sodium phosphate pH 7.0
C: 100% IPA
Sample: Herceptin, 1mg/mL in 1 M ammonium sulfate
Injection: 10 µL

Figure 4. *Proteomix*® HIC Butyl-NP5 for protein separation- Three resin lot to lot consistency



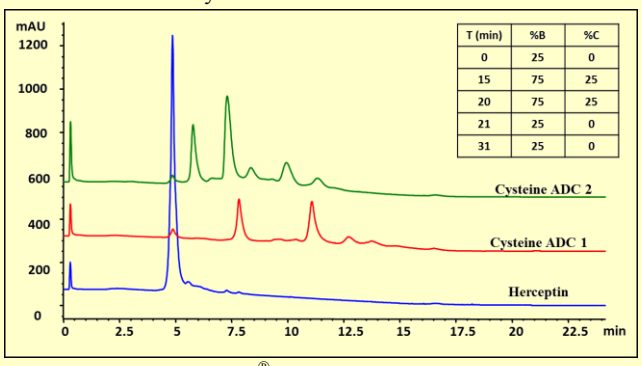
Column: *Proteomix*® HIC Butyl-NP5 (5 µm, 4.6 x 35 mm)
Mobile phase: A: 2M ammonium sulfate in 0.1M sodium phosphate, pH 7.0
B: 0.1M phosphate pH 7.0Flow rate: 0.4 mL/min
Detector: UV 214 nm
Temperature: 25 ˚C
Sample: Ovalbumin 1.0 mg/mL, Chymotrypsinogen 0.5 mg/mL
Injection: 4 µL

Applications

With its unique surface technologies, *Proteomix*® column offers special selectivity and high resolution separation of biomolecules such as mAb (monoclonal antibody), ADC (antibody drug conjugate) and related protein fragments, DNA and oligonucleotides.

Separation of ADCs (Antibody Drug Conjugates)

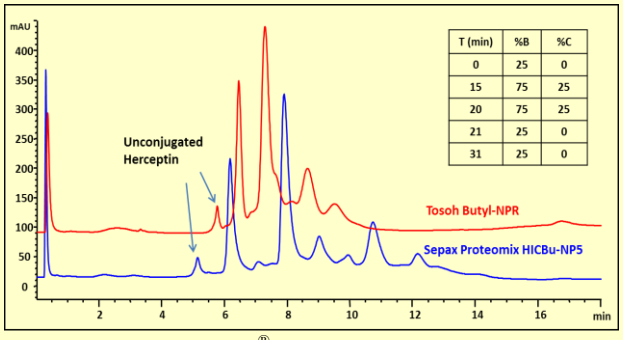
Figure 5. Herceptin and its ADCs separation on *Proteomix*® HIC Butyl-NP5 column



Column: *Proteomix*® HIC Butyl-NP5 (5 µm, 4.6 x 35 mm)
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 mg/mL in 25 mM sodium phosphate
Injection: 10 µL

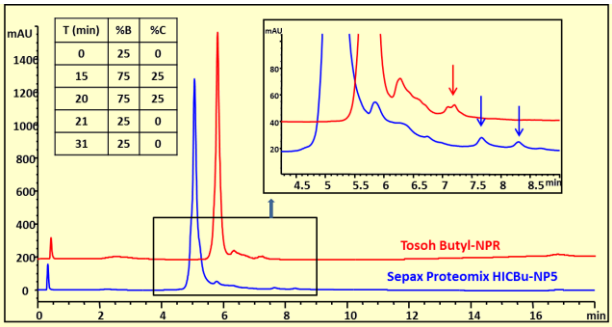
Competitive Comparison

Figure 6. Herceptin-cysteine ADC separation Sepax *Proteomix*® HIC Butyl-NP5 vs. TSKgel butyl-NPR



Column: *Proteomix*® HIC Butyl-NP5 (5 µm, 4.6 x 35 mm)
Flow rate: 0.8 mL/min
Detector: UV 214 nm
Temperature: 25 ℃
Mobile phase: A: 2 M ammonium sulfate in 0.025M sodium phosphate, pH 7.0
B: 0.025 M sodium phosphate pH 7.0
C: 100% IPA
Sample: ADC, 1mg/mL in 1M ammonium sulfate
Injection: 10 µL

Figure 7. Herceptin-Monoclonal antibody separation Sepax *Proteomix*® HIC Butyl-NP5 vs. TSKgel butyl-NPR



Column:	*Proteomix*® HIC Butyl-NP5 (5 µm, 4.6 x 35 mm)
Flow rate:	0.8 mL/min
Detector:	UV 214 nm
Temperature:	25 ˚C
Mobile phase:	A: 2 M ammonium sulfate in 0.025M sodium phosphate, pH 7.0 B: 0.025 M sodium phosphate pH 7.0 C: 100% IPA
Sample:	Herceptin, 1mg/mL in 1M ammonium sulfate
Injection:	5 µL

Disclaimer: TSKgel is registered trademarks of Tosoh Corporations; Comparative separations may not be representative of all applications.

Ordering Information

Proteomix® HIC Butyl-NP5 columns

Column Size (mm)	Particle Size	P/N
4.6 x 35	5 µm	431NP5-4603
4.6 x 100	5 µm	431NP5-4610

Proteomix® HIC Ethyl -NP5 columns

Column Size (mm)	Particle Size	P/N
4.6 x 35	5 µm	432NP5-4603
4.6 x 100	5 µm	432NP5-4610

Proteomix® HIC Phenyl -NP5 columns

Column Size (mm)	Particle Size	P/N
4.6 x 35	5 µm	433NP5-4603
4.6 x 100	5 µm	433NP5-4610

Proteomix® HIC Propyl -NP5 columns

Column Size (mm)	Particle Size	P/N
4.6 x 35	5 µm	434NP5-4603
4.6 x 100	5 µm	434NP5-4610

** Other dimension 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

5 Innovation Way, Suite 100

Delaware 19711 US

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

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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.