

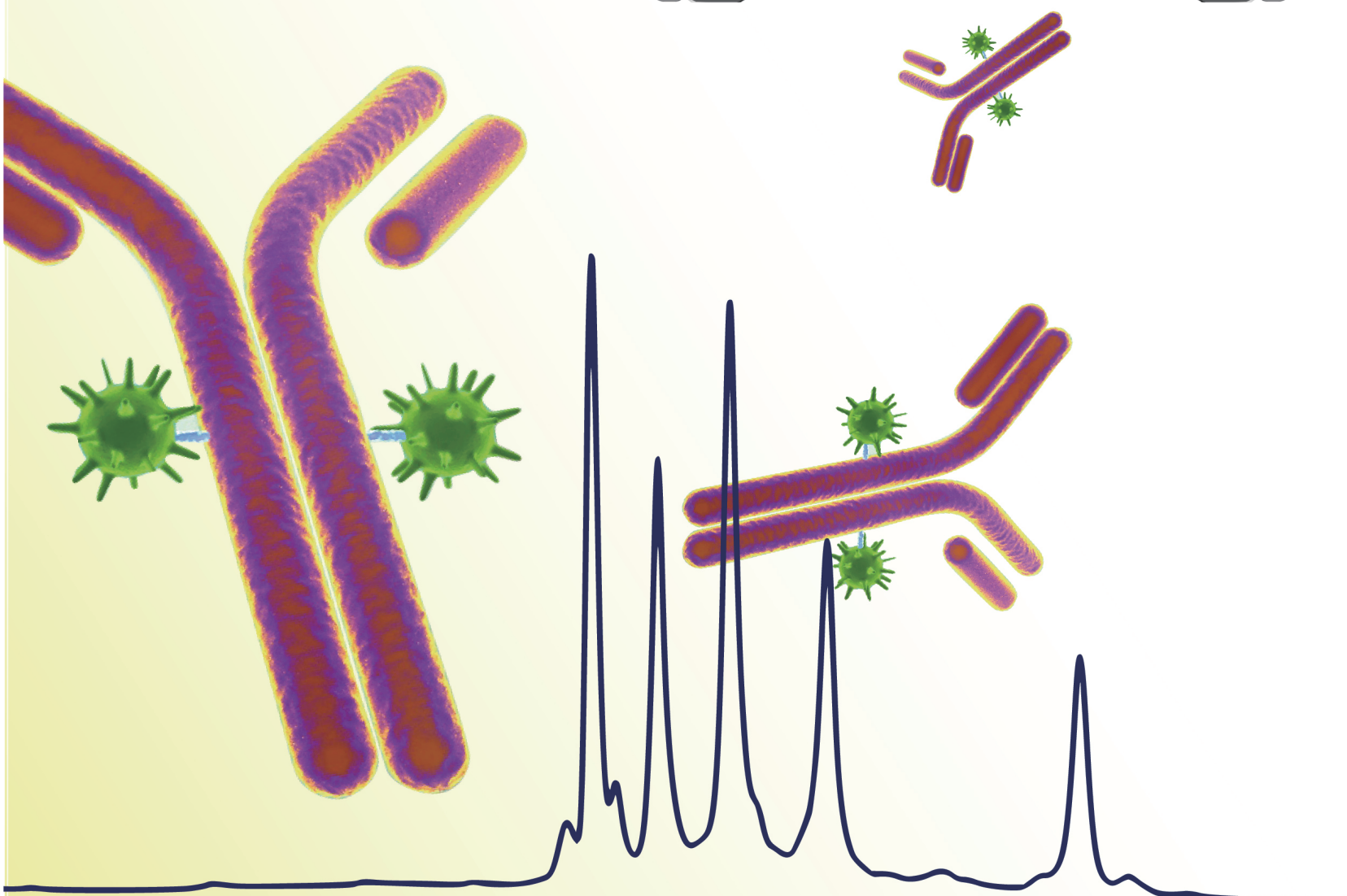
Polymeric Reversed Phase Chromatography

For High Resolution BioMolecule Separation



Sepax Technologies

Proteomix[®] RP



Better Surface Chemistry for Better Separation

SEPAX TECHNOLOGIES

Better Surface Chemistry for Better Separation

Sepax Technologies, Inc. is a Delaware, USA based leading chromatography product manufacturer and service provider specializing in biological separation areas. We offer unique ranges of HPLC columns for bioanalytical characterization and testing, as well as process media for downstream process and purification of MAb, BsMAb, ADC, Proteins, AAV, VLP, DNA, RNA, and various biologic samples. Sepax has a full portfolio of bead technology platforms, linker and organic synthesis chemistry, as well as protein chemistry in different stages of R&D and production pipelines to support the various needs of the biopharmaceutical industry and our customers. Certified to ISO 9001:2015 standards, Sepax focuses on customer & market needs, and is continuing to expand its presence and supply chain around the globe.

SPECIALIZED AND INNOVATIVE CHROMATOGRAPHY EXPERT



Sepax develops and manufactures a wide range of biological separation products using both silica and polymeric resins as the support. The selection of particle size is from 1 μm to 100 μm and pore size is from non-porous to 2000 Å. Available Sepax column dimensions are 2.1, 4.6, 7.8, 10, 21.2, 30, and 50 mm I.D., and 50, 100, 150, 250, and 300 mm length. Sepax also offers custom-made columns. Both stainless steel and PEEK tubing are available. Unique and proprietary resin synthesis and surface technologies have been developed for solving separation challenges in the area of biologics.

Size Exclusion

SRT, SRT-C
Nanofilm
Zenix, Zenix-C
Biomix

Ion Exchange

Proteomix IEX
Antibodix

Reversed Phase

Proteomix RP
Bio-C4
Bio-C8
Bio-C18

Affinity

ProAqa Excel Protein A
Monomix dT20

Hydrophobic Interaction

Proteomix HIC

Analytical, Semi-prep and Preparative

Proteomix® RP Phases

Polymer Based Reversed Phase Chromatography Media and Column

General Description

Proteomix® RP packings consist of porous polystyrene-divinylbenzene (PS/DVB) resins characterized by a narrow particle size distribution. The base matrix comprises a highly cross-linked PS/DVB matrix, serving as the reversed-phase medium for chromatographic separations. The outer surface of these resins is linked with phenyl and various substituted phenyl groups, facilitating hydrophobic interactions. Available in particle sizes of 5 and 10 µm, each size offers a choice of different pore sizes. Compared to traditional silica-based reversed-phase materials, Proteomix® RP phases exhibit enhanced chemical stability across a broad pH spectrum (1-14), alongside superior thermal tolerance. This resilience against temperature fluctuations and wide pH range contributes to their unique selection in analytical and separation processes. Specifically, at certain column temperatures, Proteomix® RP materials demonstrate exceptional selectivity towards protein molecules, including monoclonal antibodies (mAbs), antibody drug conjugates (ADCs), and associated protein fragments, as well as peptides, mRNA, and DNA. Moreover, these media are fully compatible with in-line liquid chromatography-mass spectrometry (LC-MS) analytical techniques, offering a robust platform for advanced proteomic studies.

Featured Characteristics

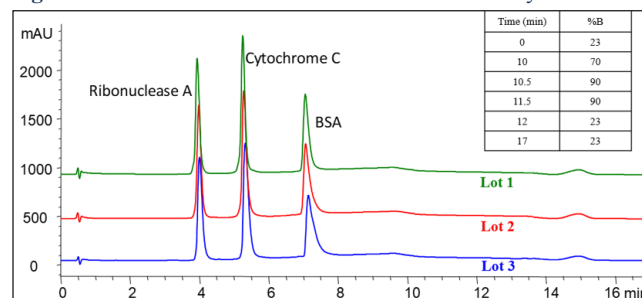
- Excellent chemical stability with wide pH tolerance (pH 1-14), compatible with most commonly used aqueous buffer and organic solvents
- Superior mechanic and thermal stability offering high pressure operation robustness and high temperature tolerance
- High capacity and resolution
- High protein recovery with minimum sample carryover
- Excellent lot-to-lot reproducibility
- Ideal for separation of biomolecules including monoclonal antibodies antibody drug conjugates, proteins, peptides, mRNA, DNA, and other oligonucleotides.

Technical Specifications

Support:	Spherical PS/DVB particles
Phase Structure:	Phenyl and substituted phenyl
Pore Size:	100, 300, 500 and 1000 Å
Particle Size:	5 and 10 µm
Application pH Range:	1-14
Operating Temperature:	Up to 80 °C
Operating Pressure:	Up to 200 bar
Mobile Phase Compatibility:	Compatible with most commonly used aqueous solution and organic solvents such as a mixture of water and acetonitrile, acetone, methanol, or THF

Proteomix® RP -1000 Lot to Lot Consistency

Figure 1. Proteomix® RP-1000 Lot to Lot Consistency



Column:	Proteomix® RP-1000 5 µm, 1000 Å, 2.1 x 50 mm
Flow Rate:	0.35 mL/min
Detector:	UV 214 nm
Temperature:	40 °C
Mobile Phase:	A: 0.1% TFA in water B: 0.1% TFA in 100% ACN
Sample:	BSA Ribonuclease A Cytochrome C (1 mg/mL)
Injection:	5 µL

Applications

Proteomix® RP allows for high efficiency and resolution of biomolecule separation. The different pore sizes of Proteomix® RP can apply to separations of different biological sample mixtures such as peptides, proteins, intact monoclonal antibodies, antibody drug conjugates and their related fragments, mRNA, DNA, and other oligonucleotides.

Antibody and Antibody Drug Conjugates

Figure 2. Possible Different Cysteine ADC Isomers Under Acidic/denaturing condition

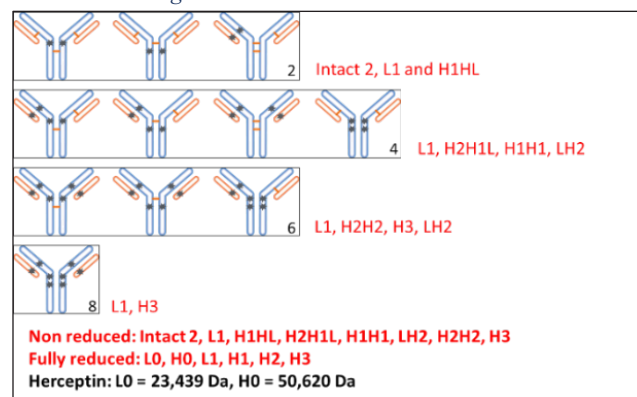
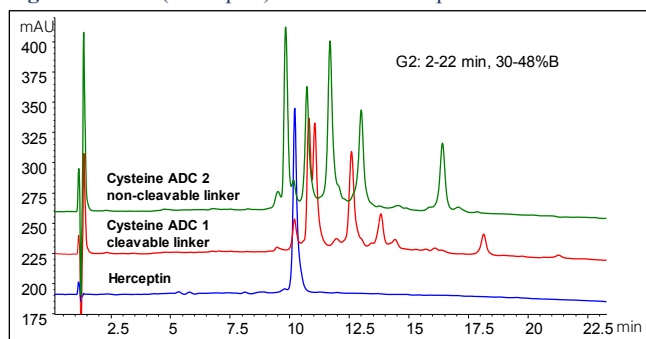
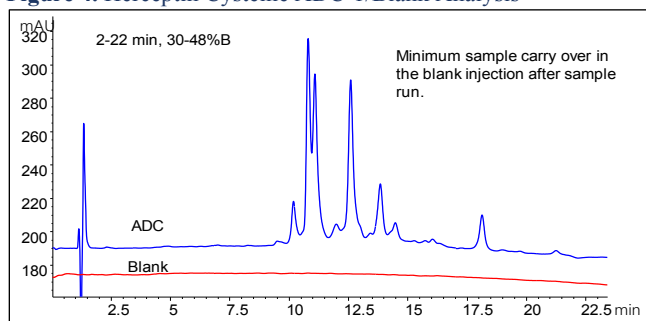


Figure 3. MAb (Herceptin) and its ADCs Separation



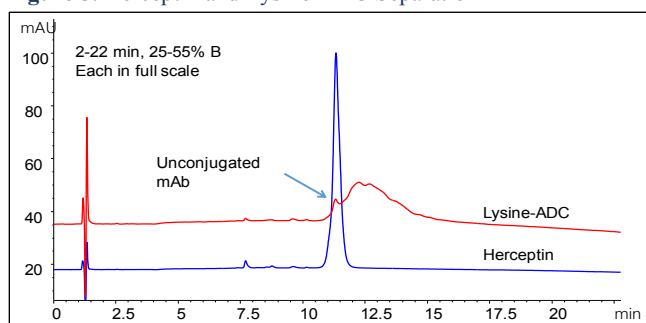
Column: *Proteomix*[®] RP-1000
5 μ m, 1000 Å, 4.6 x 100 mm
Flow Rate: 1.0 mL/min
Detector: UV 210 nm
Temperature: 80 °C
Mobile Phase: A: 0.1% TFA in water
B: 0.1% TFA in 100% ACN
Sample: Herceptin and ADCs 1 mg/mL diluted in 0.1% TFA
Injection: 10 μ L

Figure 4. Herceptin Cysteine ADC-1/Blank Analysis



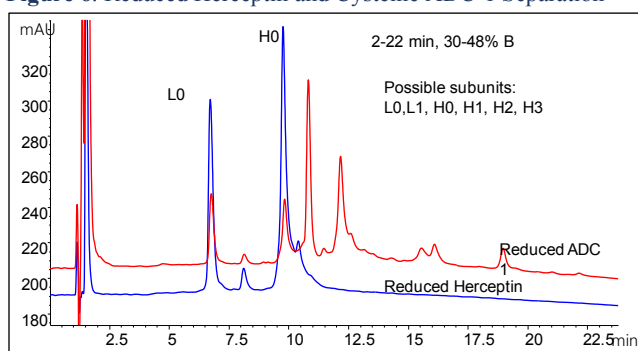
Column: *Proteomix*[®] RP-1000
5 μ m, 1000 Å, 4.6 x 100 mm
Flow Rate: 1.0 mL/min
Detector: UV 210 nm
Temperature: 80 °C
Mobile Phase: A: 0.1% TFA in water
B: 0.1% TFA in 100% ACN
Sample: Cysteine ADC-1 1 mg/mL diluted in water
Injection: 8 μ L

Figure 5. Herceptin and Lysine-ADC Separation



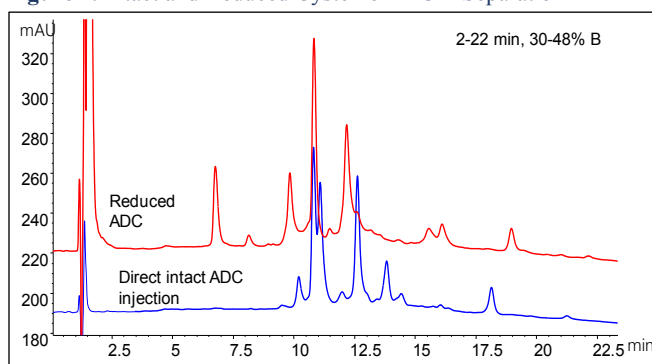
Column: *Proteomix*[®] RP-1000
5 μ m, 1000 Å, 4.6 x 100 mm
Flow Rate: 1.0 mL/min
Detector: UV 210 nm
Temperature: 80 °C
Mobile Phase: A: 0.1% TFA in water
B: 0.1% TFA in 100% CAN
Sample: Herceptin and its lysine ADC 1 mg/mL diluted in 0.1% TFA
Injection: 10 μ L

Figure 6. Reduced Herceptin and Cysteine ADC-1 Separation



Column: *Proteomix*[®] RP-1000
5 μ m, 1000 Å, 4.6 x 100 mm
Flow Rate: 1.0 mL/min
Detector: UV 210 nm
Temperature: 80 °C
Mobile Phase: A: 0.1% TFA in water
B: 0.1% TFA in 100% CAN
Sample: Herceptin and ADC-1 2 mg/mL reduced with 20 mM DTT, incubated at 65 °C for 20 minutes
Injection: Reduced Herceptin, 2 μ L
Reduced Herceptin ADC-1, 5 μ L

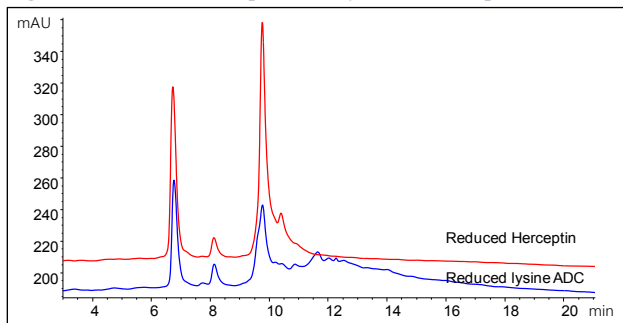
Figure 7. Intact and Reduced Cysteine ADC-1 Separation



Column: *Proteomix*[®] RP-1000
5 μ m, 1000 Å, 4.6 x 100 mm
Flow Rate: 1.0 mL/min
Detector: UV 210 nm
Temperature: 80 °C
Mobile Phase: A: 0.1% TFA in water

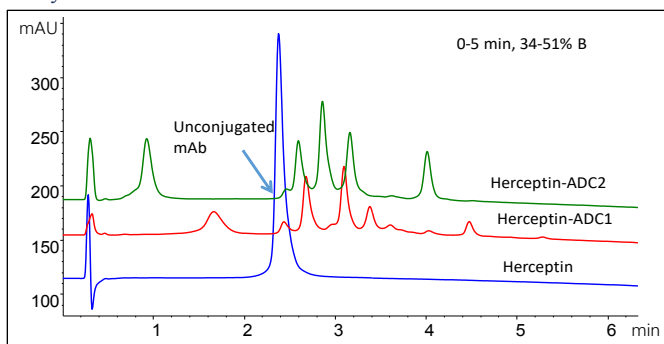
	B: 0.1% TFA in 100% ACN
Sample:	Herceptin and ADC-1 2 mg/mL reduced with 20 mM DTT, incubated at 65 °C for 20 minutes
Injection:	Intact ADC-1, 5 µL, Reduced Herceptin ADC-1, 10 µL

Figure 8. Reduced Herceptin and Lysine ADC Separation



Column:	<i>Proteomix</i> [®] RP-1000 5 µm, 1000 Å, 4.6 x 100 mm
Flow Rate:	1.0 mL/min
Detector:	UV 210 nm
Temperature:	80 °C
Mobile Phase:	A: 0.1% TFA in water B: 0.1% TFA in 100% ACN
Sample:	Herceptin and lysine ADC 2 mg/mL reduced with 20 mM DTT, incubated at 65 °C for 20 minutes
Injection:	Reduced Herceptin, 2 µL Reduced lysine ADC, 5 µL

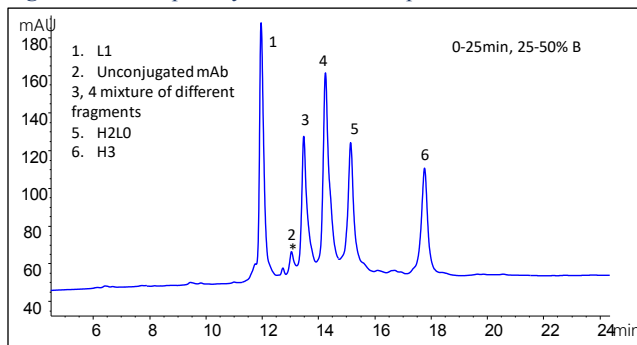
Figure 9. Herceptin/ADC-1/ADC-2 Separation, 2.1 x 50 mm-Fast Analysis



Column:	<i>Proteomix</i> [®] RP-1000 5 µm, 1000 Å, 2.1 x 50 mm
Flow Rate:	0.6 mL/min
Detector:	UV 210 nm
Temperature:	80 °C
Col. Pressure:	70 bar
Mobile Phase:	A: 0.1% TFA in water B: 0.1% TFA in 100% ACN
Sample:	Herceptin, ADC-1 and ADC-2 diluted in water

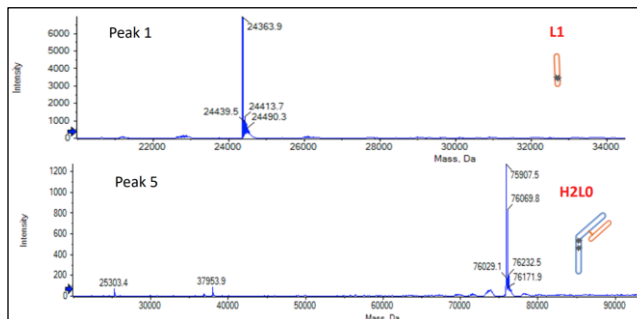
Injection:	0.5 µL for Herceptin, 1 µL for ADC-1 and ADC-2
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Figure 10. Herceptin Cysteine ADC-2 Separation-2.1 x 50 mm



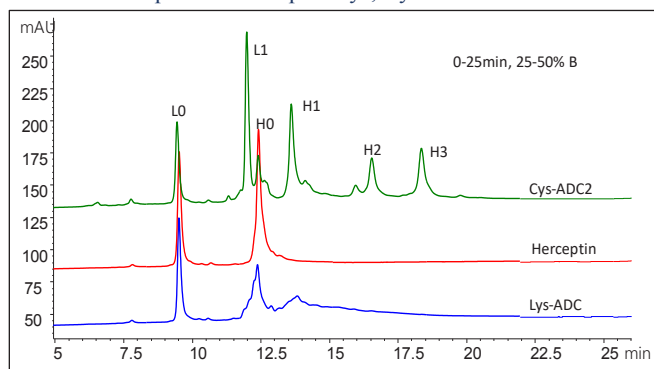
Column:	<i>Proteomix</i> [®] RP-1000 5 µm, 1000 Å, 2.1 x 50 mm
Flow Rate:	0.4 mL/min
Detector:	UV 210 nm
Temperature:	80 °C
Col. Pressure:	45 bar
Mobile Phase:	A: 0.1% TFA in water B: 0.1% TFA in 100% CAN
Sample:	ADC-2 diluted in water
Injection:	3 µL Cysteine ADC-2

Figure 11. Herceptin Cysteine ADC-2 Separation-2.1 x 50 mm for Mass Spec Analysis



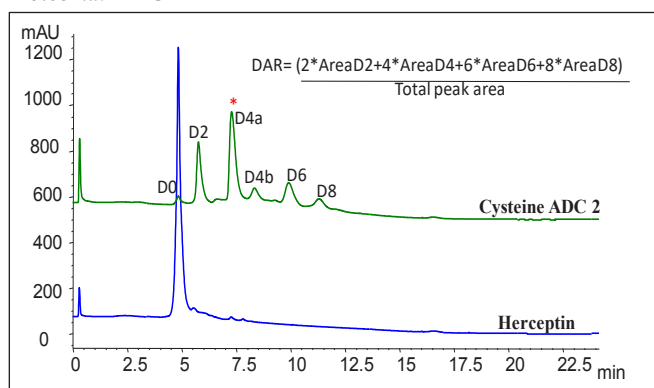
Column:	<i>Proteomix</i> [®] RP-1000 5 µm, 1000 Å, 2.1 x 50 mm
Flow Rate:	0.4 mL/min
Detector:	UV 210 nm
Temperature:	80 °C
Col. Pressure:	45 bar
Mobile Phase:	A: 0.1% TFA in water B: 0.1% TFA in 100% CAN
Sample:	ADC-2 diluted in water
Injection:	3 µL Cysteine ADC-2

Figure 12. MAb/ADC Fragment Separation
Reduced-Herceptin vs. Herceptin-Lys, Cys ADCs



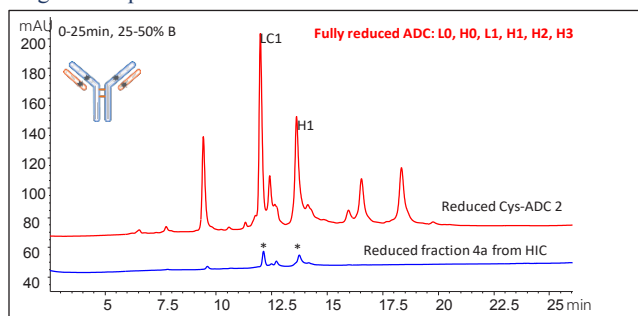
Column:	<i>Proteomix</i> [®] RP-1000 5 μm, 1000 Å, 2.1 x 50 mm
Flow Rate:	0.4 mL/min
Detector:	UV 214 nm
Temperature:	80 °C
Col. Pressure:	45 bar
Mobile Phase:	A: 0.1% TFA in water B: 0.1% TFA in 100% ACN
Injection:	1 μL for DTT reduced Herceptin (3 mg/mL) 3 mL Lys-ADC and Cys-ADC (1 mg/mL ea.)

Figure 13. Cysteine ADC DAR Analysis and Determination on
Proteomix[®] HIC



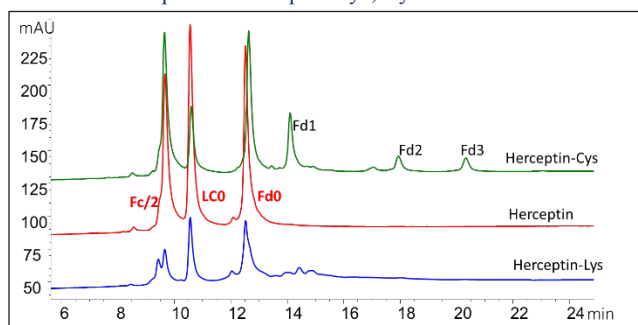
Column:	<i>Proteomix</i> [®] HIC Butyl-NP5 5 μm, 4.6 x 35 mm
Detector:	UV 214 nm
Temperature:	25 °C
Mobile Phase:	A: 2 M ammonium sulfate in 0.025 M sodium phosphate, pH 7.0 B: 0.025 M sodium phosphate pH 7.0 C: 100% IPA
Sample:	Herceptin ADC-2, 1 mg/mL in 25 mM sodium phosphate
Injection:	10 μL

Figure 14. ADC HIC fraction 4a-DTT from Figure 13
Reduced Fragment Separation on *Proteomix*[®] RP-1000



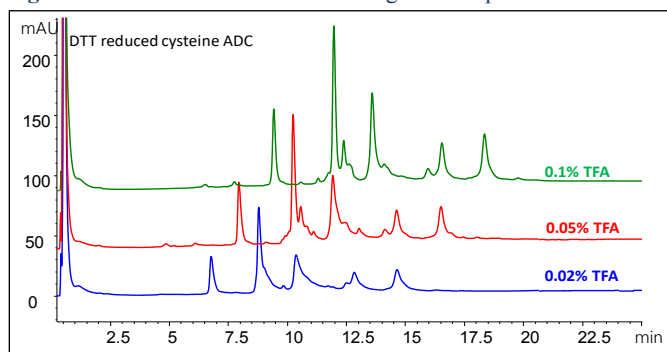
Column:	<i>Proteomix</i> [®] RP-1000 5 μm, 1000 Å, 2.1 x 50 mm
Flow Rate:	0.4 mL/min
Detector:	UV 214 nm
Temperature:	80 °C
Col. Pressure:	45 bar
Mobile Phase:	A: 0.1% TFA in water B: 0.1% TFA in 100% CAN
Injection:	30 μL for cysteine ADC separated on HIC, fraction 4a concentrated to 45 μL, reduced with 20 mM DTT

Figure 15. mAb/ADC Fragment Separation - IDeS Digested and
Reduced-Herceptin vs. Herceptin-Lys, Cys-ADC



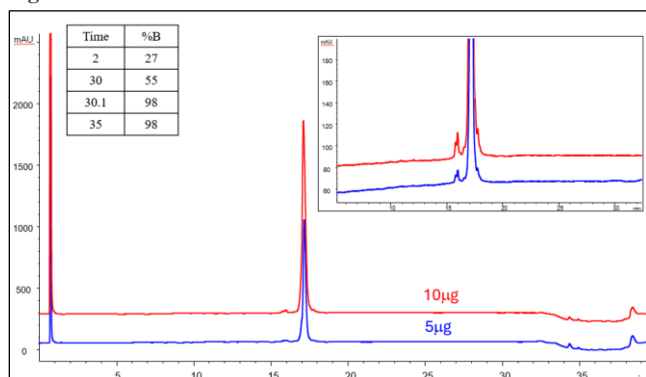
Column:	<i>Proteomix</i> [®] RP-1000 5 μm, 1000 Å, 2.1 x 50 mm
Flow Rate:	0.4 mL/min
Detector:	UV 214 nm
Temperature:	80 °C
Col. Pressure:	45 bar
Mobile Phase:	A: 0.1% TFA in water B: 0.1% TFA in 100% CAN
Injection:	3 μL for IDeS digested Herceptin, Lys-ADC and Cys-ADC (1 mg/mL each) After IDeS digestion, 4M guanidine was added and the samples were reduced with 20 mM DTT for 30 min at 56 °C.

Figure 16. TFA Effect on the ADC Fragments Separation



Column: *Proteomix*[®] RP-1000
5 μ m, 1000 Å, 2.1 x 50 mm
Flow Rate: 0.4 mL/min
Detector: UV 214 nm
Temperature: 80 °C
Col. Pressure: 45 bar
Mobile Phase: A: X % TFA in water
B: X % TFA in 100% ACN
Injection: 3 μ L for DTT reduced Herceptin cysteine ADC 0.1 and 0.05% TFA runs, 2 μ L for 0.02% TFA

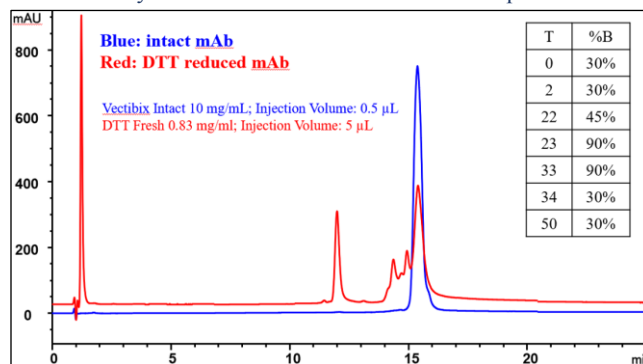
Figure 17. NIST MAb on *Proteomix*[®] RP-1000 2.1 x 100 mm



Column: *Proteomix*[®] RP-1000
5 μ m, 1000 Å, 2.1 x 100 mm
Flow Rate: 1.0 mL/min
Detector: UV 214 nm
Temperature: 80 °C
Mobile Phase: A: Water + 0.1% TFA, ACN + 0.1 % TFA
Sample: NIST MAb 10 mg/mL (pI 9.18, in 12.5 mM histidine, pH 6.0)
Injection: 0.5, 1 μ L



Figure 18. Reversed Phase *Proteomix*[®] RP-1000 Separation After IDES Proteolysis and DTT Reduction on MAb Sample

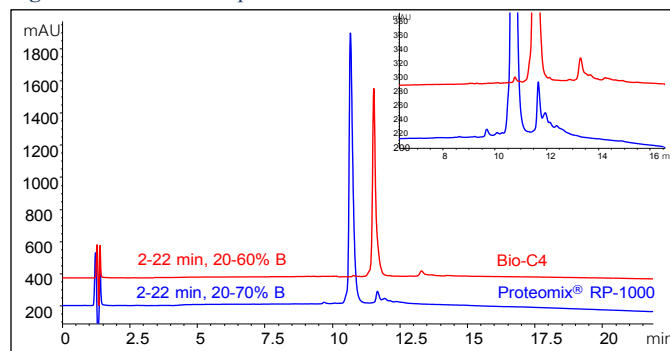


Column: *Proteomix*[®] RP-1000
5 μ m, 1000 Å, 2.1 x 100 mm
Flow Rate: 0.3 mL/min
Detector: UV 214 nm
Temperature: 78 °C
Mobile Phase: A: 0.1% TFA in Water
B: 0.1% TFA in 100% CAN
Gradient: 2-22 min 30%-45% B
Injection: 5 μ g intact and DTT reduced Vectibix (PanitumumAb) IgG2

Silica Based C4 vs. Polymeric *Proteomix*[®] RP-1000

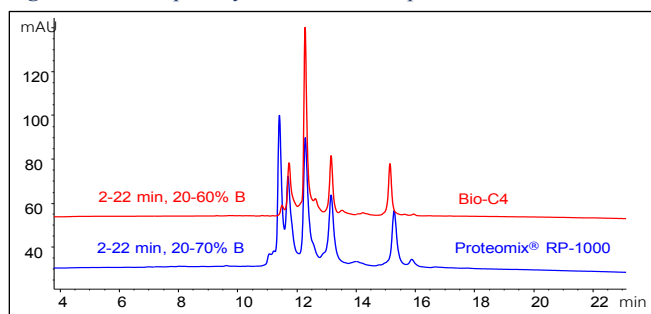
Compared against silica-based RP columns, polymer-based RP column *Proteomix*[®] RP-1000 provides unique selectivity on some biological molecule separation projects especially at elevated temperature conditions.

Figure 19. mAb 321 Separation



Column: *Proteomix*[®] RP-1000
5 μ m, 1000 Å, 4.6 x 100 mm
Bio-C4, 5 μ m, 300 Å, 4.6 x 100 mm
Flow Rate: 1.0 mL/min
Detector: UV 210 nm
Temperature: 80 °C
Col. Pressure: 70 bar
Mobile Phase: A: 0.1% TFA in water
B: 0.1% TFA in 100% CAN
Sample: mAb 321 1 mg/mL diluted in 0.1% TFA
Injection: 20 μ L

Figure 20. Herceptin Cysteine ADC-2 Separation

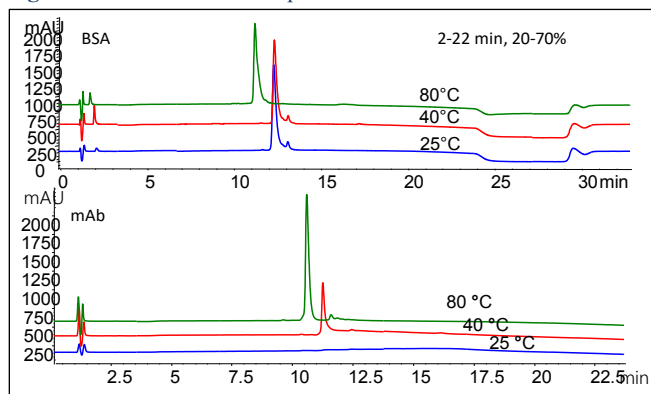


Column:	Proteomix® RP-1000 5 µm, 1000 Å, 4.6 x 100 mm Bio-C4, 5 µm, 300 Å, 4.6 x 100 mm
Flow Rate:	1.0 mL/min
Detector:	UV 210 nm
Temperature:	80 °C
Mobile Phase:	A: 0.1% TFA in water B: 0.1% TFA in 100% ACN
Sample:	ADC diluted in 0.1% TFA
Injection:	15 µL

Temperature Effect

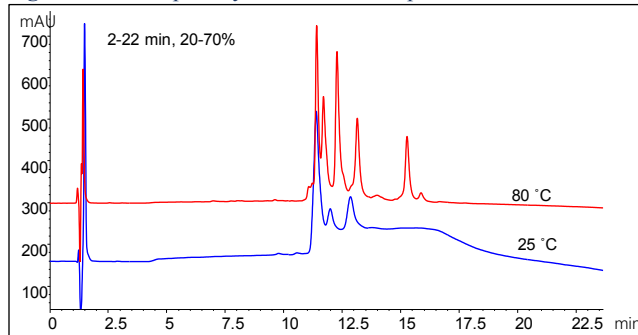
Conditions such as the mobile phase and column temperature play a key role in improving the separations of large intact proteins such as monoclonal antibodies. At elevated temperatures, the viscosity of the mobile phase is reduced, and protein diffusion is enhanced with better sample recovery.

Figure 21. BSA and mAb Separation with 25 °C / 40 °C / 80 °C



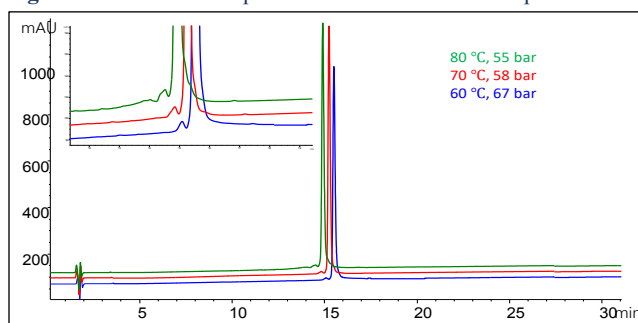
Column:	Proteomix® RP-1000 5 µm, 1000 Å, 4.6 x 100 mm
Flow Rate:	1.0 mL/min
Detector:	UV 210 nm
Mobile Phase:	A: 0.1% TFA in water B: 0.1% TFA in 100% ACN
Sample:	BSA and mAb 1 mg/mL
Injection:	20 µL

Figure 22. Herceptin Cysteine ADC-2 Separation



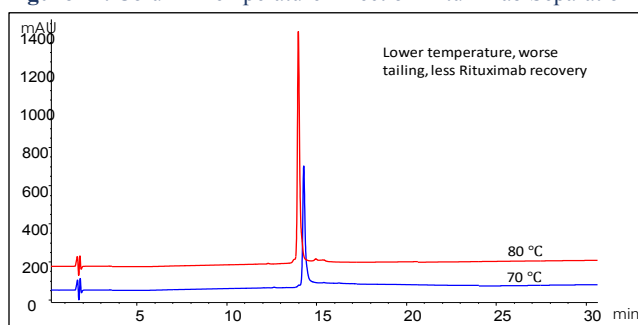
Column:	Proteomix® RP-1000 5 µm, 1000 Å, 4.6 x 100 mm
Flow Rate:	1.0 mL/min
Temperature:	25, 80 °C
Detector:	UV 210 nm
Mobile Phase:	A: 0.1% TFA in water B: 0.1% TFA in 100% CAN
Sample:	Herceptin Cysteine ADC-2, 1 mg/mL diluted in 0.1% TFA
Injection:	20 µL

Figure 23. Column Temperature Effect on Erbitux Separation



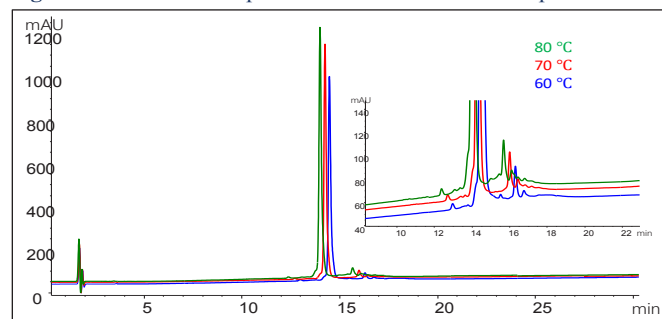
Column:	Proteomix® RP-1000 5 µm, 1000 Å, 2.1 x 150 mm
Flow Rate:	0.25 mL/min, 23- 70% B in 30 min
Detector:	UV 214 nm
Temperature:	80, 70, 60 °C
Mobile Phase:	A: H ₂ O + 0.1% TFA B: ACN + 0.1% TFA
Sample:	Erbitux, 1 mg/mL diluted with 0.1% TFA
Injection:	2.5 µL

Figure 24. Column Temperature Effect on Rituximab Separation



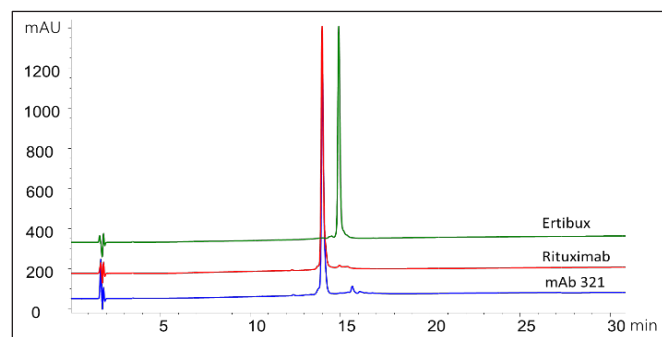
Column:	<i>Proteomix</i> [®] RP-1000 5 µm, 1000 Å, 2.1 x 150 mm
Flow Rate:	0.25 mL/min, 23- 70% B in 30 min
Detector:	UV 214 nm
Temperature:	80, 70 °C
Mobile Phase:	A: H ₂ O + 0.1% TFA B: ACN + 0.1% TFA
Sample:	Rituximab, 1 mg/mL diluted with 0.1% TFA
Injection:	2.5 µL

Figure 25. Column Temperature Effect on mAb321 Separation



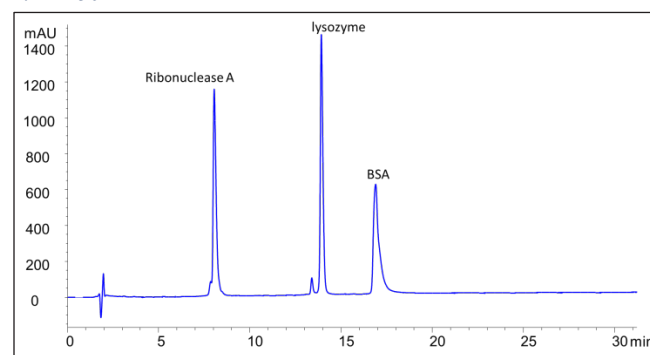
Column:	<i>Proteomix</i> [®] RP-1000 5 µm, 1000 Å, 2.1 x 150 mm
Flow Rate:	0.25 mL/min, 23- 70% B in 30 min
Detector:	UV 214 nm
Temperature:	80, 70, 60 °C
Mobile Phase:	A: H ₂ O + 0.1% TFA B: ACN + 0.1% TFA
Sample:	mAb321, 1 mg/mL diluted with 0.1% TFA
Injection:	2.5 µL

Figure 26. mAb321/Erbitux/Rituximab at 80 °C



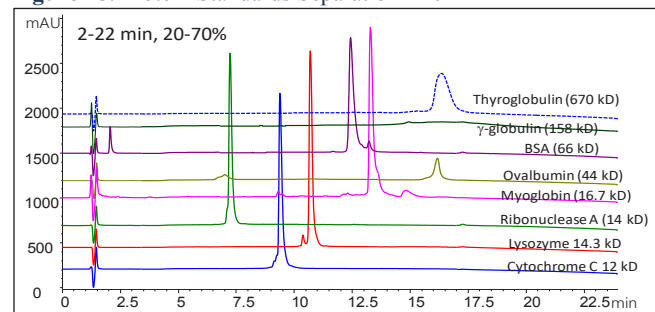
Column:	<i>Proteomix</i> [®] RP-1000 5 µm, 1000 Å, 2.1 x 150 mm
Flow Rate:	0.25 mL/min, 23%-70% B in 30 min
Detector:	UV 214 nm
Temperature:	80 °C
Mobile Phase:	A: H ₂ O + 0.1% TFA B: ACN + 0.1% TFA
Sample:	monoclonal antibody, 1 mg/mL diluted with 0.1% TFA
Injection:	2.5 µL

Figure 27. Ribonuclease A, BSA and Lysozyme at 40 °C
2.1 x 150 mm



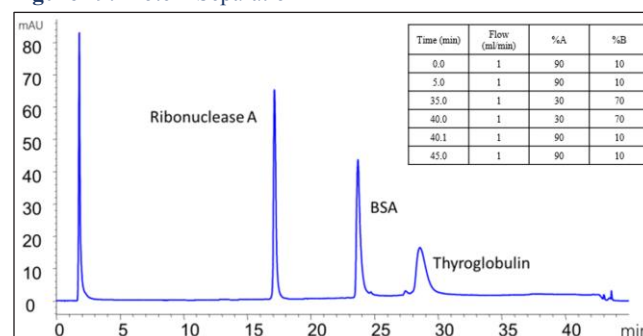
Column:	<i>Proteomix</i> [®] RP-1000 5 µm, 1000 Å, 2.1 x 150 mm
Flow Rate:	0.25 mL/min, 23- 70% B in 30 min
Detector:	UV 214 nm
Temperature:	40 °C
Mobile Phase:	A: H ₂ O + 0.1% TFA B: ACN + 0.1% TFA
Sample:	1 mg/mL each diluted with 0.1% TFA
Injection:	5 µL

Figure 28. Protein Standards Separation - 40 °C



Column:	<i>Proteomix</i> [®] RP-1000 5 µm, 1000 Å, 4.6 x 100 mm
Flow Rate:	1.0 mL/min
Detector:	UV 210 nm
Temperature:	40 °C
Mobile Phase:	A: 0.1% TFA in water B: 0.1% TFA in 100% ACN
Sample:	Proteins 1 mg/mL diluted in 0.1% TFA
Injection:	20 µL

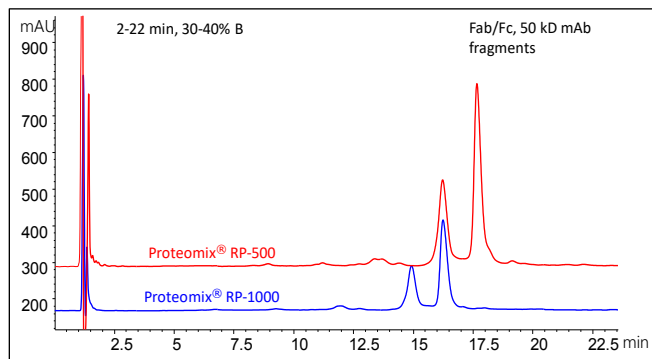
Figure 29. Protein Separation



Column:	<i>Proteomix</i> [®] RP-1000 5 µm, 1000 Å, 4.6 x 150 mm
Detector:	UV 280 nm
Temperature:	30 °C
Mobile Phase:	A: 0.1% TFA in water B: 0.1% TFA in acetonitrile
Sample:	5 µL Protein mixture (Thyroglobulin 5.46 mg/ml, BSA 6.3 mg/ml, Ribonuclease A 5.9 mg/ml)

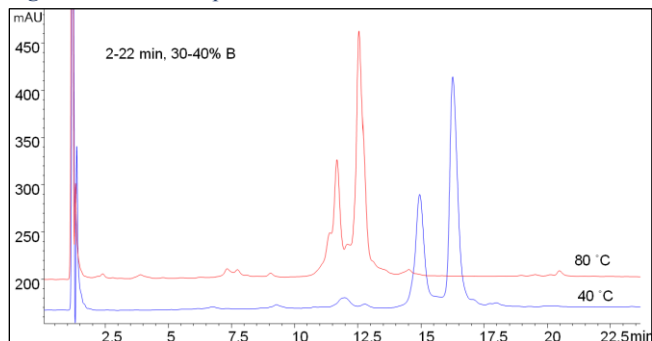
MAb Fragments

Figure 30. Fab/Fc Separation on *Proteomix*[®] RP-1000 and RP-500 with 40 °C



Column:	<i>Proteomix</i> [®] RP-1000 5 µm, 1000 Å, 4.6 x 100 mm <i>Proteomix</i> [®] RP-500 5 µm, 500 Å, 4.6 x 100 mm
Flow Rate:	1.0 mL/min
Detector:	UV 210 nm
Temperature:	40 °C
Mobile Phase:	A: 0.1% TFA in water B: 0.1% TFA in 100% ACN
Sample:	mAb (digested with Papain) 1 mg/mL diluted in 0.1% TFA
Injection:	20 µL

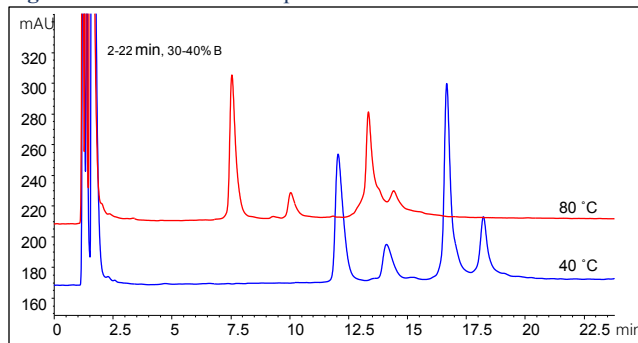
Figure 31. Fab/Fc Separation with 40 °C and 80 °C



Column:	<i>Proteomix</i> [®] RP-1000 5 µm, 1000 Å, 4.6 x 100 mm
Flow Rate:	1.0 mL/min
Detector:	UV 210 nm

Temperature:	40, 80 °C
Mobile Phase:	A: 0.1% TFA in water B: 0.1% TFA in 100% CAN
Sample:	mAb (digested by papain) 1 mg/mL diluted in 0.1% TFA
Injection:	20 µL

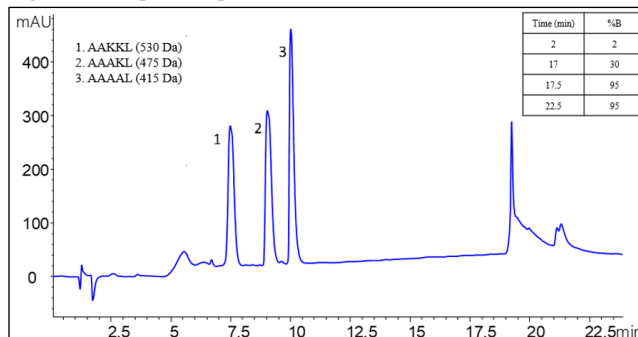
Figure 32. Reduced mAb Separation: 40 °C and 80 °C



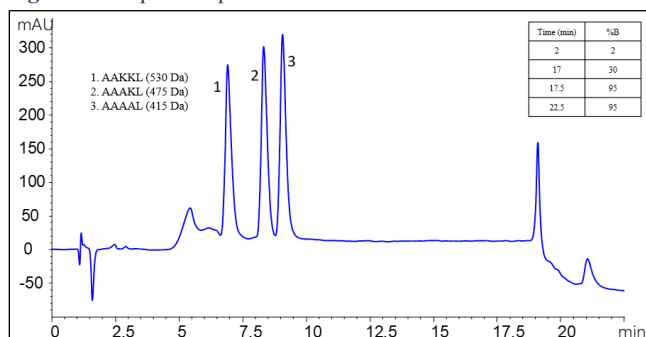
Column:	<i>Proteomix</i> [®] RP-1000 5 µm, 1000 Å, 4.6 x 100 mm
Flow Rate:	1.0 mL/min
Detector:	UV 210 nm
Temperature:	40, 80 °C
Mobile Phase:	A: 0.1% TFA in water B: 0.1% TFA in 100% ACN
Sample:	mAb reduced with 20 mM DTT at 65 °C for 20 minutes, 1 mg/mL diluted in 0.1% TFA
Injection:	20 µL

Peptide Separation

Figure 33. Peptide Separation on *Proteomix*[®] RP-500



Column:	<i>Proteomix</i> [®] RP-500 5 µm, 500 Å, 4.6 x 100 mm
Flow Rate:	1.0 mL/min
Detector:	UV 214 nm
Temperature:	40 °C
Pressure:	90-120 bar
Mobile Phase:	A: 0.1% TFA in water B: 0.1% TFA in 100% ACN
Sample:	0.7 mg/mL each AAAAL, AAKKL, AAKKL
Injection:	30 µL

Figure 34. Peptide Separation on *Proteomix*[®] RP-300

Column: *Proteomix*[®] RP-300
10 μ m, 300 Å, 4.6 x 100 mm

Flow Rate: 1.0 mL/min

Detector: UV 214 nm

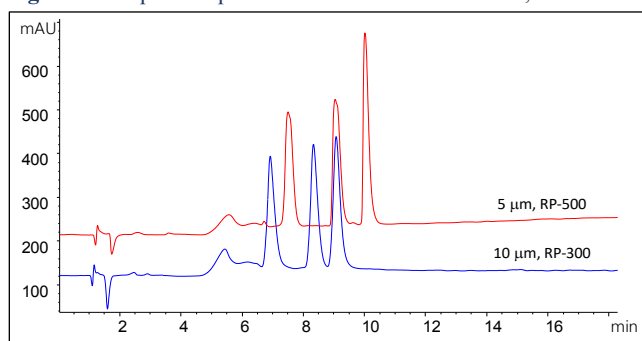
Temperature: 40 °C

Pressure: 33-40 bar

Mobile Phase: A: 0.1% TFA in water
B: 0.1% TFA in 100% ACN

Sample: 0.7 mg/mL each
AAAAL, AAKKL, AAKKL

Injection: 30 μ L

Figure 35. Peptide Separation on *Proteomix*[®] RP-300, RP-500

Column: *Proteomix*[®] RP-300
10 μ m, 300 Å, 4.6 x 100 mm

Proteomix[®] RP-500
5 μ m, 500 Å, 4.6 x 100 mm

Flow Rate: 1.0 mL/min

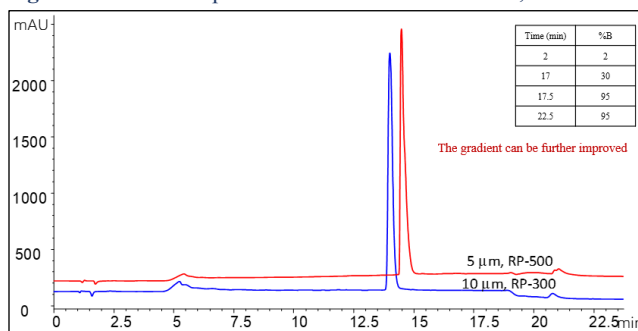
Detector: UV 214 nm

Temperature: 40 °C

Mobile Phase: A: 0.1% TFA in water
B: 0.1% TFA in 100% ACN

Sample: 0.7 mg/mL
each AAAAL, AAKKL, AAKKL

Injection: 30 μ L

Figure 36. Insulin Separation on *Proteomix*[®] RP-300, RP-500

Column: *Proteomix*[®] RP-300
10 μ m, 300 Å, 4.6 x 100 mm

Proteomix[®] RP-500
5 μ m, 500 Å, 4.6 x 100 mm

Flow Rate: 1.0 mL/min

Detector: UV 214 nm

Temperature: 40 °C

Mobile Phase: A: 0.1% TFA in water
B: 0.1% TFA in 100% ACN

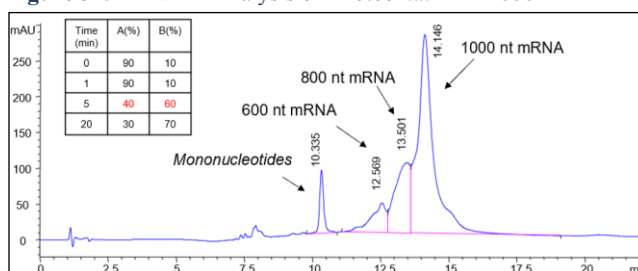
Sample: 3.5 mg/mL Sigma human insulin

Injection: 10 μ L

Oligonucleotides Separation

Ion-paired reversed phase HPLC (IP-RP-HPLC) method can be used for the characterization of oligonucleotides, such as its integrity analysis including the determination of product related impurities and the percentage of fragment.

In the below study (Figure 37), a single-stranded, 1000 nucleotides sized mRNA sample were analyzed by *Proteomix*[®] RP-1000 column under 50 °C.

Figure 37. mRNA-2 Analysis on *Proteomix*[®] RP-1000

Column: *Proteomix*[®] RP-1000
5 μ m, 1000 Å, 2.1 x 100 mm

Flow Rate: 0.3 mL/min

Detector: UV 260 nm

Temperature: 50 °C

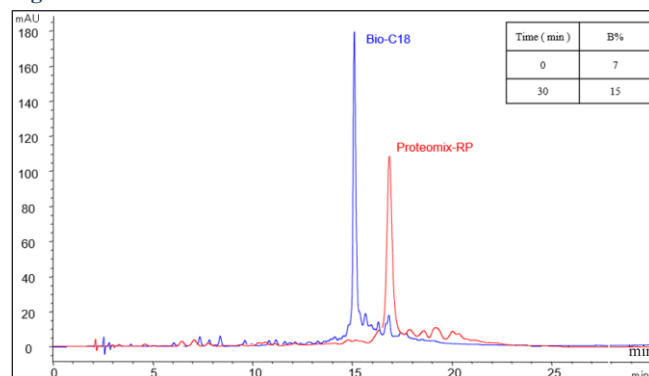
Col. Pressure: 95 bar

Mobile Phase: A: 100 mM TEAA
B: 100 mM TEAA / 25% ACN

Sample: mRNA-2

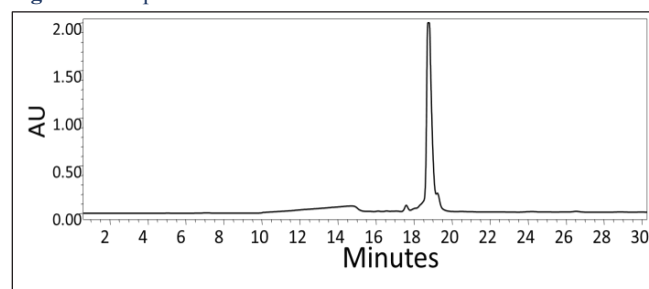
Injection: 10 μ L

Figure 38. *Proteomix*[®] RP-1000 RNA Ladder



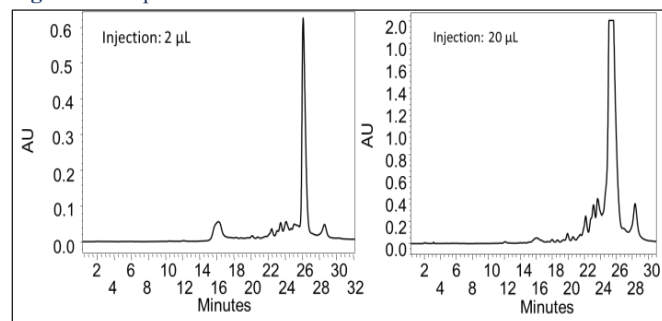
Column: *Proteomix*[®] RP-1000
5 μ m, 1000 Å, 4.6 x 150 mm
Flow Rate: 1.0 mL/min
Detector: UV 260 nm
Temperature: 70 °C
Mobile Phase: A: 100 mM TEAA (pH 6.94)
B: ACN
Sample: Low Range and High Range RNA (Thermo)
Injection: 3 μ L

Figure 39. Separation of RNA on *Proteomix*[®] RP-300



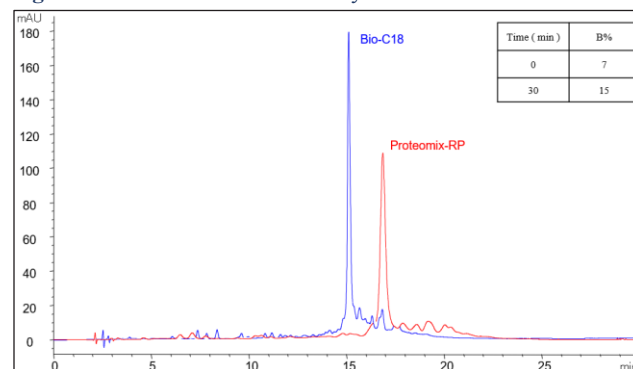
Column: *Proteomix*[®] RP-300
10 μ m, 300 Å, 4.6 x 150 mm
Flow Rate: 1.0 mL/min
Detector: UV 260 nm
Temperature: RT
Mobile Phase: A: 0.1%TEAA, pH=7.0
B: ACN
Gradient: 0%~30%B (50 min)
Sample: RNA (21 bp, MW ~ 6,000) (1.0 mg/mL)
Injection: 5 μ L

Figure 40. Separation of DNA on *Proteomix*[®] RP-300



Column: *Proteomix*[®] RP-300
10 μ m, 300 Å, 4.6 x 150 mm
Flow Rate: 1.0 mL/min
Detector: UV 260 nm
Temperature: Ambient
Mobile Phase: A: 0.1%TEAA, pH=7.0
B: ACN
Gradient: 0% - 12% - 30%B (0 - 30 - 50 min)
Sample: DNA (21 bp, MW ~ 6,000) (1.0 mg/mL)
Injection: 2 μ L

Figure 41. DNA Primer 25 nt Analysis on *Proteomix*[®] RP

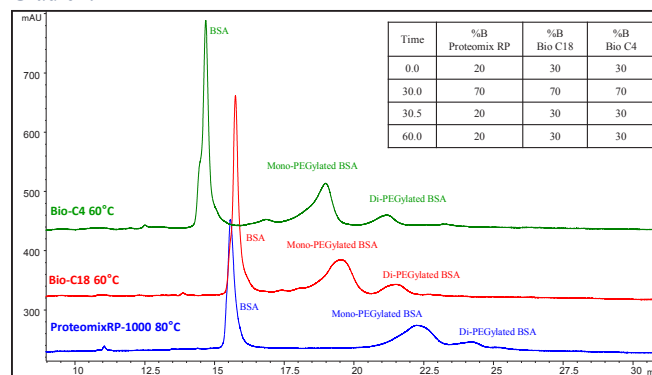


Column: *Proteomix*[®] RP-300
5 μ m, 300 Å, 4.6 x 150 mm
Bio-C18, 5 μ m, 300 Å, 4.6 x 150 mm
Flow Rate: 0.8 mL/min
Detector: UV 260 nm
Temperature: 30 °C
Mobile Phase: A: 100 mM TEAA (pH 7.0)
B: ACN
Sample: 25 nt link1 about 200 μ g/mL in water
Injection: 20 μ L

PEGylated Protein Separation

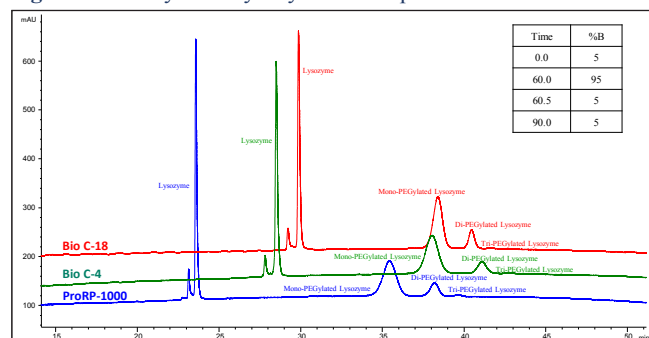
In this study, silica-based Bio-C4, Bio-C18 as well polymer based *Proteomix*[®] RP-1000 columns were used for the characterization of PEGylated proteins such as PEGylated BSA, and PEGylated Lysozyme (Figure 42-43).

Figure 42. PEGylated BSA – Comparison RP Columns
Gradient 2



Column:	Bio-C4, 5 μ m, 4.6 x 250 mm Bio-C18, 5 μ m, 4.6 x 250 mm <i>Proteomix</i> [®] RP-1000, 5 μ m, 4.6 x 100 mm
Flow Rate:	0.5 mL/min
Detector:	UV 214 nm
Temperature:	60, 80 $^{\circ}$ C
Mobile Phase:	A: Water + 0.1% TFA B: Acetonitrile + 0.1% TFA
Sample:	2 mg/mL PEGylated BSA
Injection:	10 μ g

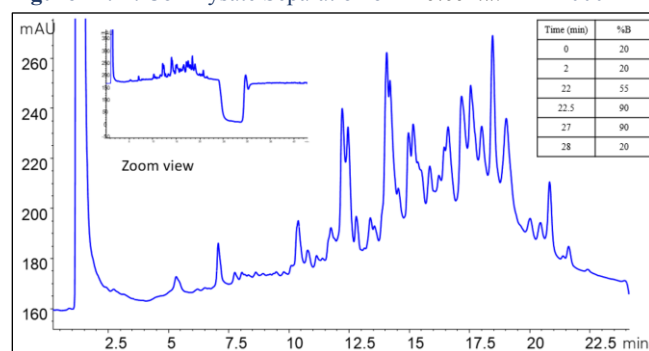
Figure 43. PEGylated Lysozyme – Comparison RP Columns



Column:	Bio-C4, 5 μ m, 4.6 x 250 mm Bio-C18, 5 μ m, 4.6 x 250 mm <i>Proteomix</i> [®] RP-1000, 5 μ m, 4.6 x 100 mm
Flow Rate:	0.5 mL/min
Detector:	UV 214 nm
Temperature:	60, 80 $^{\circ}$ C
Mobile Phase:	A: Water + 0.1% TFA B: Acetonitrile + 0.1% TFA
Sample:	2 mg/mL PEGylated Lysozyme
Injection:	10 μ L

E. Coli Lysate Separation

Figure 44. E. Coli Lysate Separation on *Proteomix*[®] RP-1000



Column:	<i>Proteomix</i> [®] RP-1000 5 μ m, 1000 $\text{\AA}$, 4.6 x 100 mm
Flow Rate:	1.0 mL/min
Detector:	UV 210 nm
Temperature:	80 $^{\circ}$ C
Mobile Phase:	A: 0.1% TFA in water B: 0.1% TFA in 100% ACN

Sample:	Bio-Rad E. coli lysate diluted in 0.1% TFA, filter before injection (1.3 mg/mL)
Injection:	30 μ L

Ordering Information

***Proteomix*[®] RP Column (5 μ m, 300 $\text{\AA}$)**

Part Number	Column Size (mm)	Particle Size	Pore Size
465300-2105	2.1 x 50	5 μ m	300 $\text{\AA}$
465300-2110	2.1 x 100	5 μ m	300 $\text{\AA}$
465300-2115	2.1 x 150	5 μ m	300 $\text{\AA}$
465300-4605	4.6 x 50	5 μ m	300 $\text{\AA}$
465300-4610	4.6 x 100	5 μ m	300 $\text{\AA}$
465300-4615	4.6 x 150	5 μ m	300 $\text{\AA}$
465300-10005	10 x 50	5 μ m	300 $\text{\AA}$
465300-10015	10 x 150	5 μ m	300 $\text{\AA}$
465300-10025	10 x 250	5 μ m	300 $\text{\AA}$
465300-21205	21.2 x 50	5 μ m	300 $\text{\AA}$
465300-21215	21.2 x 150	5 μ m	300 $\text{\AA}$
465300-21225	21.2 x 250	5 μ m	300 $\text{\AA}$
465300-30005	30 x 50	5 μ m	300 $\text{\AA}$
465300-30015	30 x 150	5 μ m	300 $\text{\AA}$
465300-30025	30 x 250	5 μ m	300 $\text{\AA}$

***Proteomix*[®] RP Column (5 μ m, 500 $\text{\AA}$)**

Part Number	Column Size (mm)	Particle Size	Pore Size
465500-2105	2.1 x 50	5 μ m	500 $\text{\AA}$
465500-2110	2.1 x 100	5 μ m	500 $\text{\AA}$
465500-2115	2.1 x 150	5 μ m	500 $\text{\AA}$
465500-4605	4.6 x 50	5 μ m	500 $\text{\AA}$
465500-4610	4.6 x 100	5 μ m	500 $\text{\AA}$
465500-4615	4.6 x 150	5 μ m	500 $\text{\AA}$
465500-10005	10 x 50	5 μ m	500 $\text{\AA}$
465500-10015	10 x 150	5 μ m	500 $\text{\AA}$
465500-10025	10 x 250	5 μ m	500 $\text{\AA}$
465500-21205	21.2 x 50	5 μ m	500 $\text{\AA}$
465500-21215	21.2 x 150	5 μ m	500 $\text{\AA}$
465500-21225	21.2 x 250	5 μ m	500 $\text{\AA}$
465500-30005	30 x 50	5 μ m	500 $\text{\AA}$
465500-30015	30 x 150	5 μ m	500 $\text{\AA}$
465500-30025	30 x 250	5 μ m	500 $\text{\AA}$

Proteomix® RP Column (5 µm, 1000 Å)

Part Number	Column Size (mm)	Particle Size	Pore Size
465950-2105	2.1 x 50	5 µm	1000 Å
465950-2110	2.1 x 100	5 µm	1000 Å
465950-2115	2.1 x 150	5 µm	1000 Å
465950-4605	4.6 x 50	5 µm	1000 Å
465950-4610	4.6 x 100	5 µm	1000 Å
465950-4615	4.6 x 150	5 µm	1000 Å
465900-10005	10 x 50	5 µm	1000 Å
465900-10015	10 x 150	5 µm	1000 Å
465900-10025	10 x 250	5 µm	1000 Å
465900-21205	21.2 x 50	5 µm	1000 Å
465900-21215	21.2 x 150	5 µm	1000 Å
465900-21225	21.2 x 250	5 µm	1000 Å
465900-30005	30 x 50	5 µm	1000 Å
465900-30015	30 x 150	5 µm	1000 Å
465900-30025	30 x 250	5 µm	1000 Å

Proteomix® RP Column (10 µm, 300 Å)

Part Number	Column Size (mm)	Particle Size	Pore Size
469300-4605	4.6 x 50	10 µm	300 Å
469300-4610	4.6 x 100	10 µm	300 Å
469300-4615	4.6 x 150	10 µm	300 Å
469300-10005	10 x 50	10 µm	300 Å
469300-10015	10 x 150	10 µm	300 Å
469300-10025	10 x 250	10 µm	300 Å
469300-21205	21.2 x 50	10 µm	300 Å
469300-21215	21.2 x 150	10 µm	300 Å
469300-21225	21.2 x 250	10 µm	300 Å
469300-30005	30 x 50	10 µm	300 Å
469300-30015	30 x 150	10 µm	300 Å
469300-30025	30 x 250	10 µm	300 Å

Proteomix® RP Column (10 µm, 500 Å)

Part Number	Column Size (mm)	Particle Size	Pore Size
469500-4605	4.6 x 50	10 µm	500 Å
469500-4610	4.6 x 100	10 µm	500 Å
469500-4615	4.6 x 150	10 µm	500 Å
469500-10005	10 x 50	10 µm	500 Å
469500-10015	10 x 150	10 µm	500 Å
469500-10025	10 x 250	10 µm	500 Å
469500-21205	21.2 x 50	10 µm	500 Å

469500-21215	21.2 x 150	10 µm	500 Å
469500-21225	21.2 x 250	10 µm	500 Å
469500-30005	30 x 50	10 µm	500 Å
469500-30015	30 x 150	10 µm	500 Å
469500-30025	30 x 250	10 µm	500 Å

Proteomix® RP Column (10 µm, 1000 Å)

Part Number	Column Size (mm)	Particle Size	Pore Size
469950-4605	4.6 x 50	10 µm	1000 Å
469950-4610	4.6 x 100	10 µm	1000 Å
469950-4615	4.6 x 150	10 µm	1000 Å
469900-10005	10 x 50	10 µm	1000 Å
469900-10015	10 x 150	10 µm	1000 Å
469900-10025	10 x 250	10 µm	1000 Å
469900-21205	21.2 x 50	10 µm	1000 Å
469900-21215	21.2 x 150	10 µm	1000 Å
469900-21225	21.2 x 250	10 µm	1000 Å
469900-30005	30 x 50	10 µm	1000 Å
469900-30015	30 x 150	10 µm	1000 Å
469900-30025	30 x 250	10 µm	1000 Å

Accessories**Precolumn Filter for Analytical Columns**

For Analytical Columns with Particle Size of 5 µm
 Part Number: 102000-P355 PEEK Precolumn Filter & Frits (2 µm)
 Part Number: 102001-P355 PEEK Refill Frits (2 µm), 5 units/pk
 Part Number: 102000-S355 SS Precolumn Filter & Frits (2 µm)
 Part Number: 102001-S355 SS Refill Frits (2 µm), 5 units/pk

**Precolumn Filter for Preparative Column IDs ≥ 21.2 mm**

Part Number: 102020-21200 SS Precolumn Filter & Frits (2 µm)
 Part Number: 102020-00001 SS Refill Frits (2 µm), 5 units/pk

**Cartridge & Holder**

Packed with Resin. Part Number is dependent to phase.
Replacement Cartridge Holder
 Part Number: 102000-2001, Holder for 2.1mm ID column
 Part Number: 102000-4001, Holder for 4.6 mm ID column

**PEEK Column Coupler**

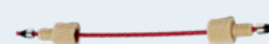
Part Number: 102002-COUPLER

**PEEK/SS coupler (for Columns ID > 7.8 mm)**

Part Number: 102003-COUPLER

**PEEK Coupler with Flexible Tubing**

(10cm length, 10-32 fitting with 1/16 OD)
 Part Number: 102006-COUPLER



How to Order

Please contact Sepax Sales Department:

Phone: 302-366-1101 or 1-877-SEPAX-US

Fax: 302-366-1151

Email: sales@sepax-tech.com

5 Innovation Way
Newark, Delaware 19711, USA

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Payment Term

Payment terms are net 30 days. Credit card payment are accepted. There is no minimum order.

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

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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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Published in March 2024



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