



Sepax Technologies

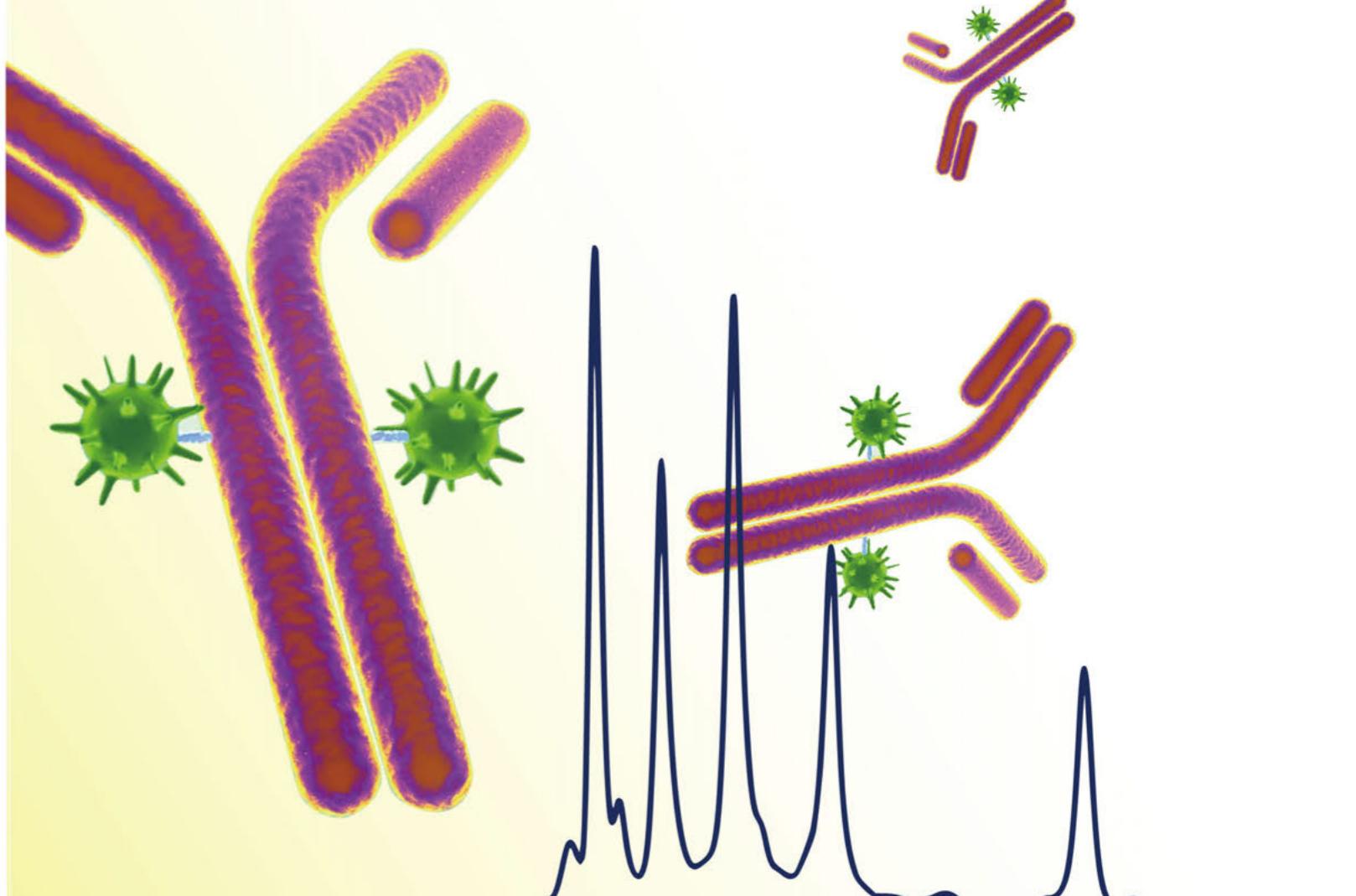
## Polymeric Reversed Phase Chromatography

For High Resolution BioMolecule Separation



Sepax Technologies

## Proteomix® RP



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Better Surface Chemistry for Better Separation

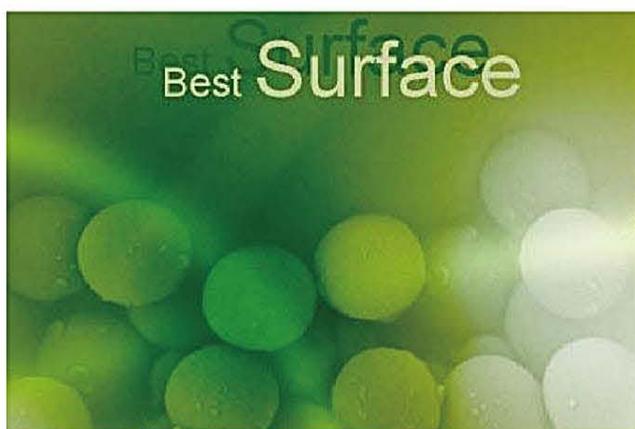
# Sepax Technologies, Inc.

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 µm to 100 µm 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

### Ion Exchange

Proteomix® IEX

Antibodix® WCX

### Hydrophobic Interaction

Proteomix® HIC

### Carbohydrate Separation

Carbomix®

### Analytical, Semi-prep and Preparative

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# Proteomix® RP Phases

## Polymer Based Reversed Phase Chromatography Media and Column

### General Description

Proteomix® RP packings are porous polystyrene divinylbenzene (PS/DVB) resins with narrow-dispersed particle size distribution. The base matrix is made of highly cross-linked PS/DVB which functions as a reversed phase for chromatographic separation. The resin surface is comprised of phenyl and substituted phenyl functional groups that enable hydrophobic interaction. These highly rigid resins are available in the particle sizes of 5 and 10 µm, each with different pore sizes available. In comparison to silica based reversed phase media, Proteomix® RP phases have advantages of stability at a wide pH range (1-14). In addition, Proteomix® RP phases have a relatively high temperature stability when compared to other reversed phase media. At select column temperatures, the Proteomix® RP phases offer unique selectivity for protein separation, such as mAbs (monoclonal antibodies), ADC (antibody drug conjugates) and their related protein fragments. The media is compatible with inline LC-mass spectroscopic analysis.

### Featured Characteristics

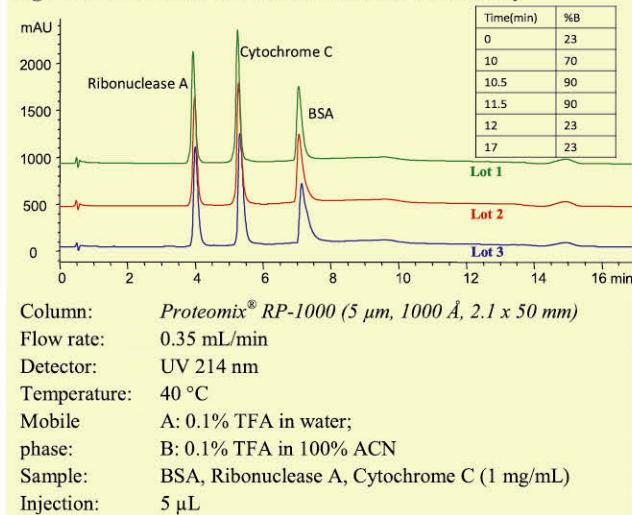
- Excellent chemical stability with wide pH tolerance (pH 1-14), compatible with most commonly used aqueous buffer and organic solvents
- Superior mechanic and thermal stability offering high pressure operation, robustness (up to 200 bar), and high temperature tolerance (up to 80 °C).
- High capacity and resolution
- High protein recovery with minimum sample carry over
- Excellent lot-to-lot reproducibility
- Ideal for separation and analysis of monoclonal antibodies and their derivatives such as antibody drug conjugates, proteins, peptides, oligonucleotides and other biomolecules

### 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

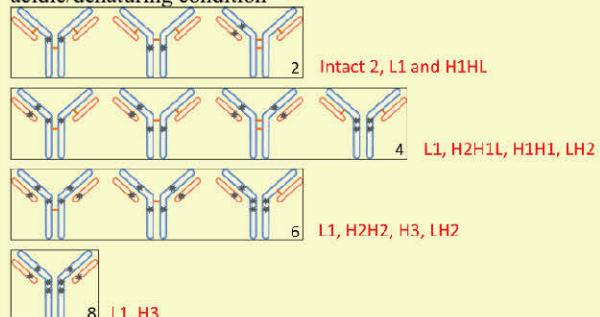


### Applications

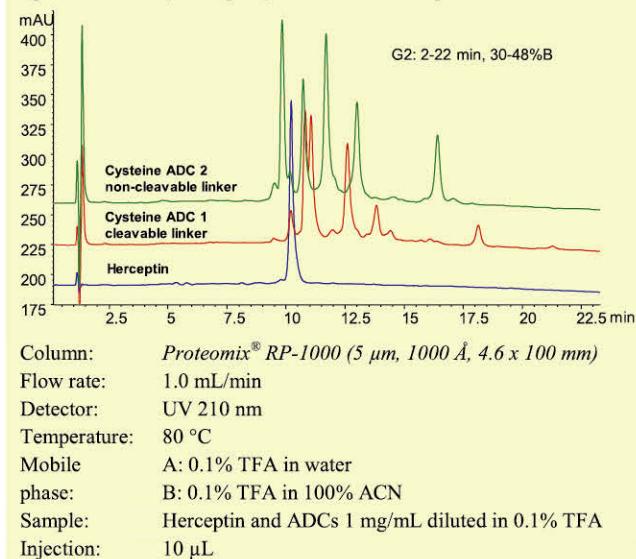
Proteomix® RP allows for high efficiency and resolution of biomolecule separation. The different pore sizes of Proteomix RP can apply to separations of different protein mixtures such as peptides, oligonucleotides, proteins, intact monoclonal antibodies, antibody drug conjugates and their related subunits.

### Antibody and Antibody Drug Conjugates

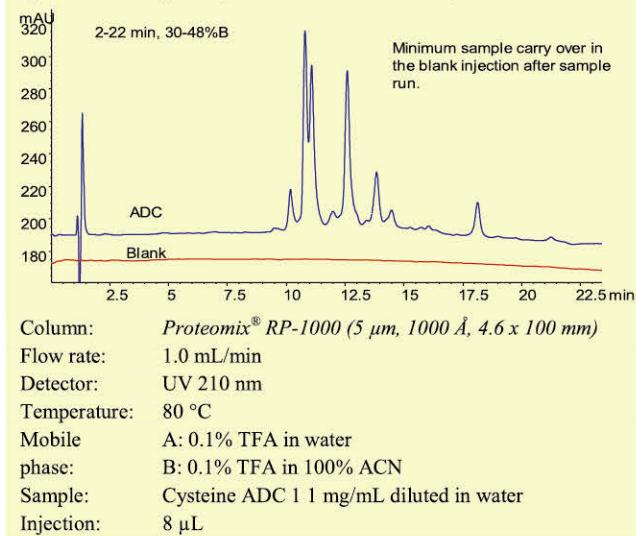
Figure 2. Possible different cysteine ADC isomers under acidic/denaturing condition



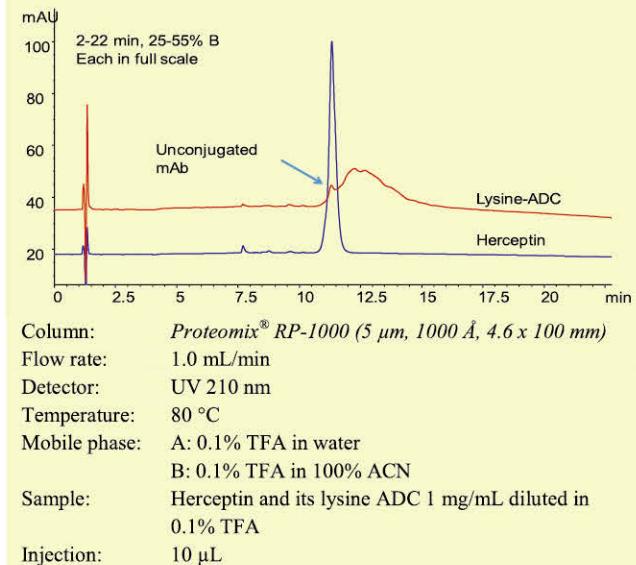
**Figure 3. MAb (Herceptin) and its ADCs Separation**



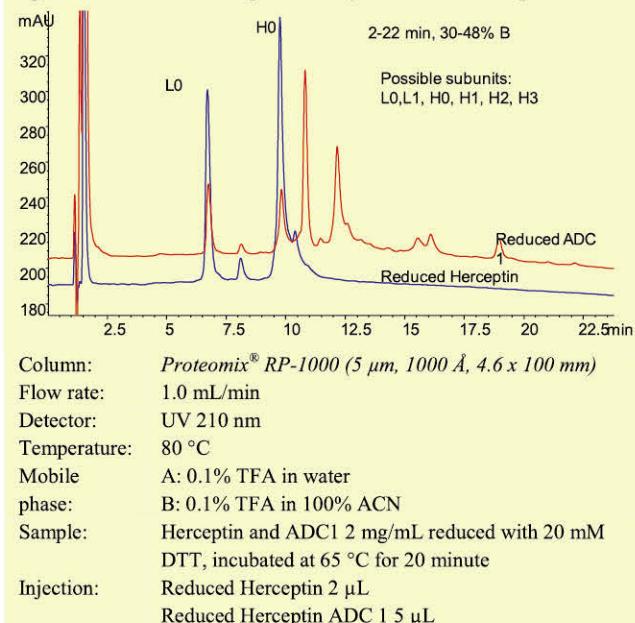
**Figure 4. Herceptin Cysteine ADC1/Blank Separation**



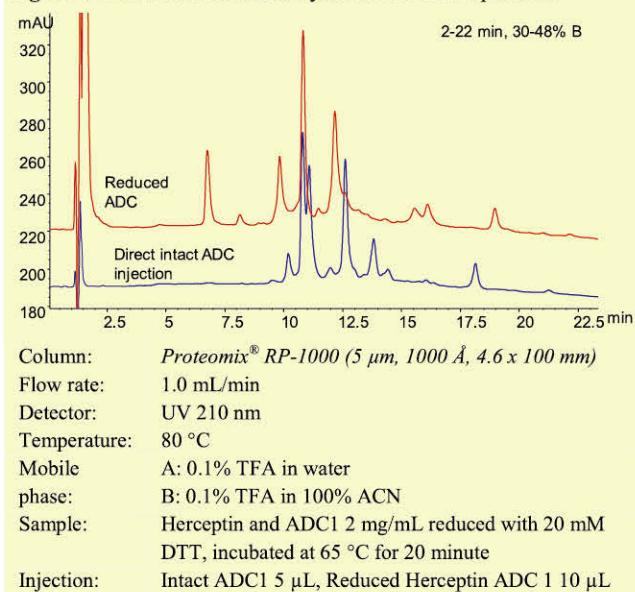
**Figure 5. Herceptin and Lysine-ADC separation**



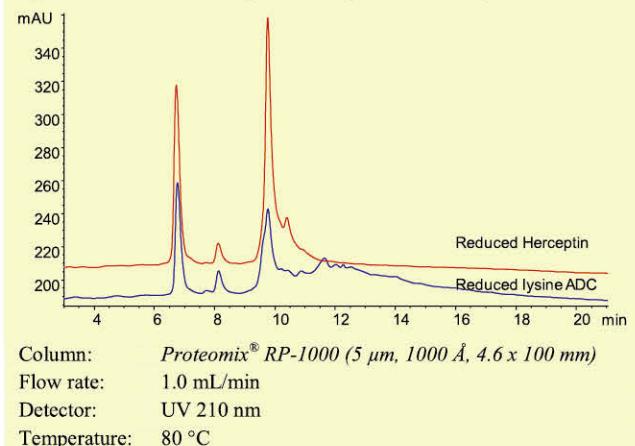
**Figure 6. Reduced Herceptin and Cysteine ADC 1 Separation**



**Figure 7. Intact and Reduced Cysteine ADC 1 Separation**

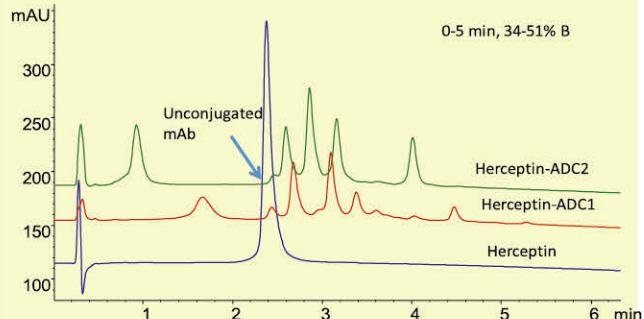


**Figure 8. Reduced Herceptin and Lysine ADC Separation**



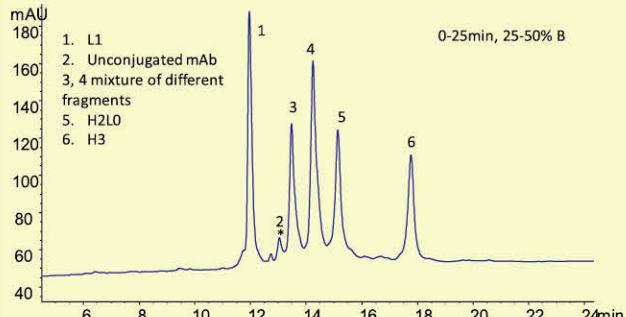
Mobile phase: A: 0.1% TFA in water  
 Sample: B: 0.1% TFA in 100% ACN  
 Herceptin and lysine ADC 2 mg/mL reduced with 20 mM DTT, incubated at 65 °C for 20 minute  
 Injection: Reduced herceptin 2 µL, Reduced lysine ADC 5 µL

**Figure 9.** Herceptin/ADC1/ADC2 Separation-2.1 x 50 mm-Fast Analysis



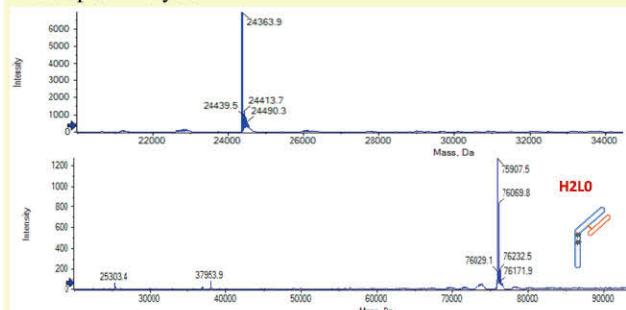
Column: Proteomix® RP-1000 (5 µm, 1000 Å, 2.1 x 50 mm)  
 Flow rate: 0.6 mL/min  
 Detector: UV 210 nm  
 Temperature: 80 °C  
 Column pressure: 70 bar  
 Mobile phase: A: 0.1% TFA in water  
 B: 0.1% TFA in 100% ACN  
 Sample: Herceptin, ADC 1 and ADC 2 diluted in water  
 Injection: 0.5 µL for Herceptin, 1 µL for ADC 1 and ADC 2

**Figure 10.** Herceptin Cysteine ADC2 Separation-2.1 x 50 mm



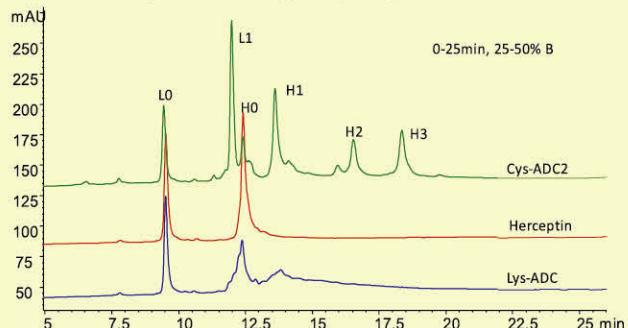
Column: Proteomix® RP-1000 (5 µm, 1000 Å, 2.1 x 50 mm)  
 Flow rate: 0.4 mL/min  
 Detector: UV 210 nm  
 Temperature: 80 °C  
 Column pressure: 45 bar  
 Mobile phase: A: 0.1% TFA in water  
 B: 0.1% TFA in 100% ACN  
 Sample: ADC 2 diluted in water  
 Injection: 3 µL Cysteine ADC 2

**Figure 11.** Herceptin Cysteine ADC2 Separation-2.1 x 50 mm Mass Spec Analysis



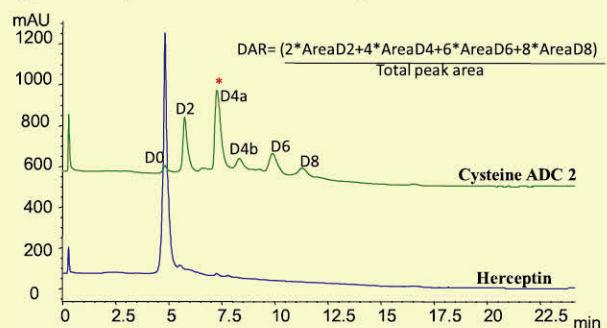
Column: Proteomix® RP-1000 (5 µm, 1000 Å, 2.1 x 50 mm)  
 Flow rate: 0.4 mL/min  
 Detector: UV 210 nm  
 Temperature: 80 °C  
 Column pressure: 70 bar  
 Mobile phase: A: 0.1% TFA in water  
 B: 0.1% TFA in 100% ACN  
 Sample: ADC 2 diluted in water  
 Injection: 3 µL Cysteine ADC 2

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



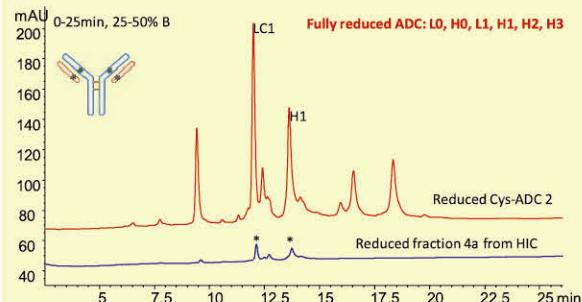
Column: Proteomix® RP-1000 (5 µm, 1000 Å, 2.1 x 50 mm)  
 Flow rate: 0.4 mL/min  
 Detector: UV 214 nm  
 Temperature: 80 °C  
 Column pressure: 45 bar  
 Mobile phase: A: 0.1% TFA in water  
 B: 0.1% TFA in 100% ACN  
 Injection: 1 µL for DTT reduced Herceptin (3 mg/mL), 3 mL Lys-ADC and Cys-ADC (1mg/mL each)

**Figure 13.** Cysteine ADC DAR Analysis on Proteomix® HIC



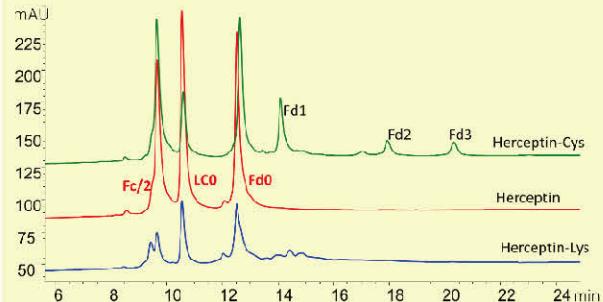
Column: Proteomix® HICBu-NP5 (5 mm, 4.6 x 35 mm)  
 Detector: UV 214 nm  
 Temperature: 25 °C  
 Mobile phase:  
 A: 2 M ammonium sulfate in 0.025 M sodium phosphate, pH 7.0  
 B: 0.025 M sodium phosphate pH 7.0  
 C : 100% IPA  
 Sample: Herceptin ADC2, 1 mg/mL in 25 mM sodium phosphate  
 Injection: 10 µL

**Figure 14.** ADC HIC fraction 4a-DTT reduced fragment separation on Proteomix® RP-1000



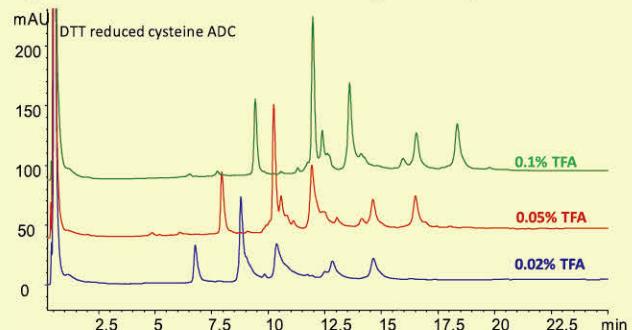
Column: Proteomix® RP-1000 (5 µm, 1000 Å, 2.1 x 50 mm)  
 Flow rate: 0.4 mL/min  
 Detector: UV 214 nm  
 Temperature: 80 °C  
 Column pressure: 45 bar  
 Mobile phase: A: 0.1% TFA in water  
 B: 0.1% TFA in 100% ACN  
 Injection: 30 µL for cysteine ADC separated on HIC, fraction 4a concentrated to 45 µL, reduced with 20 mM DTT

**Figure 15.** mAb/ADC fragment separation - IDeS digested and reduced-Herceptin vs. Herceptin-Lys, Cys-ADC



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

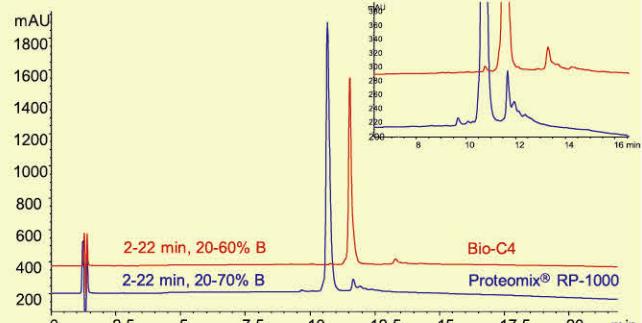
**Figure 16.** TFA Effect on the ADC fragments separation



Column: Proteomix® RP-1000 (5 µm, 1000 Å, 2.1 x 50 mm)  
 Flow rate: 0.4 mL/min  
 Detector: UV 214 nm  
 Temperature: 80 °C  
 Column pressure: 45 bar  
 Mobile phase: A: X % TFA in water  
 B: X % TFA in 100% ACN  
 Injection: 3 µL for DTT reduced Herceptin cysteine ADC 0.1 and 0.05% TFA runs, 2 µL for 0.02% TFA

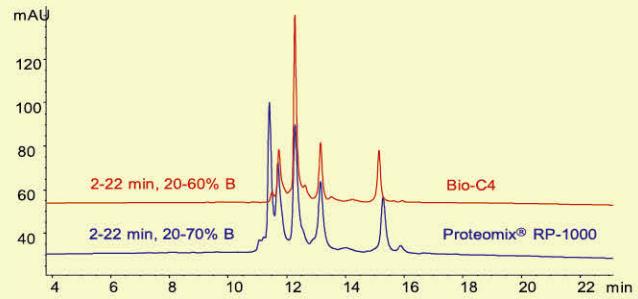
### Silica based C4 vs. polymeric Proteomix® RP-1000

**Figure 17.** mAb 321 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  
 Column pressure: 70 bar  
 Mobile phase: A: 0.1% TFA in water  
 B: 0.1% TFA in 100% ACN  
 Sample: mAb 321 1 mg/mL diluted in 0.1% TFA  
 Injection: 20 µL

**Figure 18.** Herceptin cysteine ADC 2 Separation

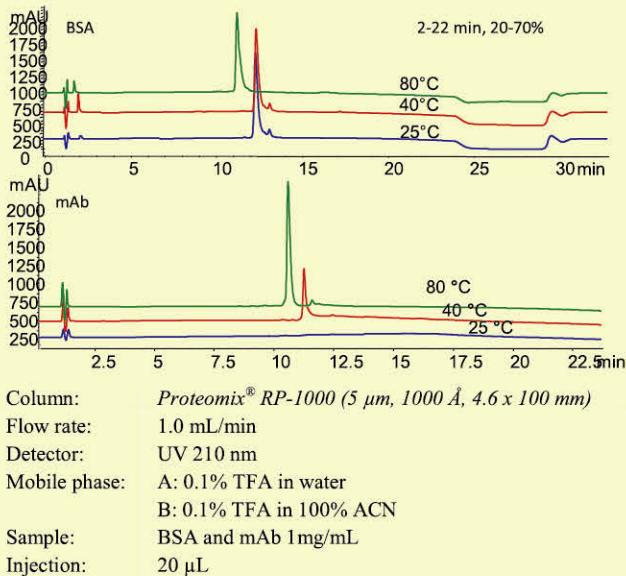


Column:	Proteomix® RP-1000 (5 $\mu$ m, 1000 Å, 4.6 x 100 mm); Bio-C4 (5 $\mu$ m, 300 Å, 4.6 x 100 mm)
Flow rate:	1.0 mL/min
Detector:	UV 210 nm
Temperature:	80 °C
Mobile phase:	A: 0.1% TFA in water
Sample:	B: 0.1% TFA in 100% ACN
Injection:	ADC diluted in 0.1% TFA
	15 $\mu$ L

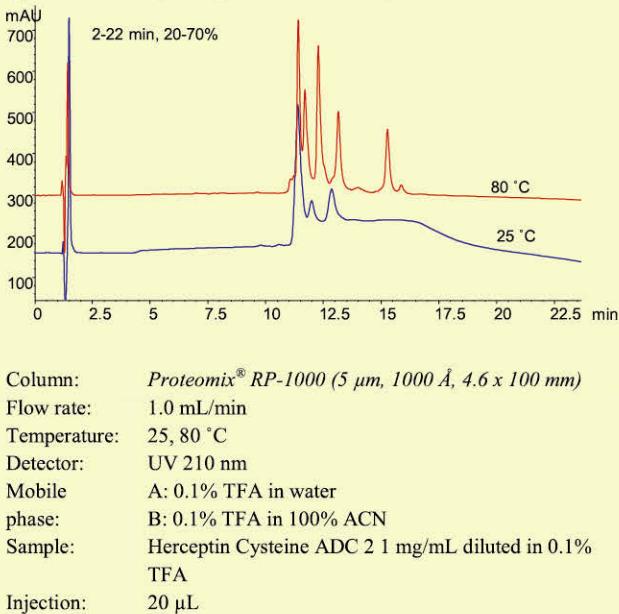
### Temperature effect

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

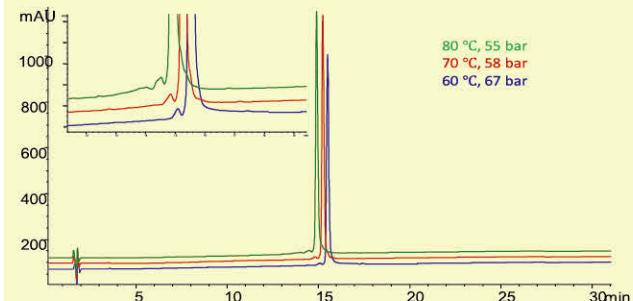
**Figure 19.** BSA and mAb separation with 25°C /40°C / 80°C



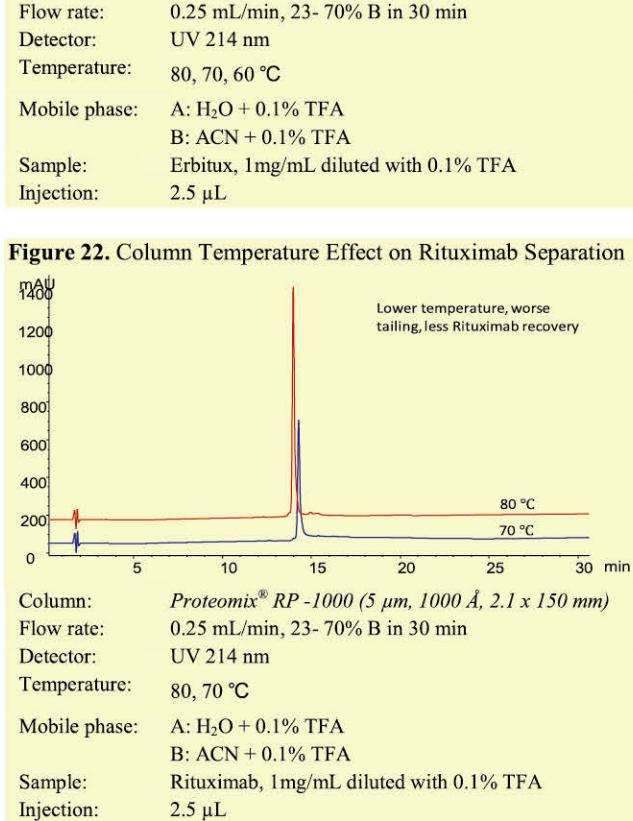
**Figure 20.** Herceptin Cysteine ADC 2 Separation



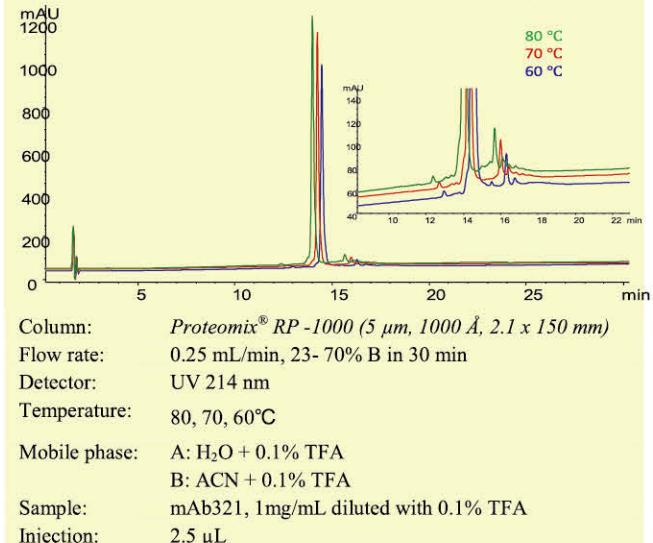
**Figure 21.** Column Temperature Effect on Erbitux Separation

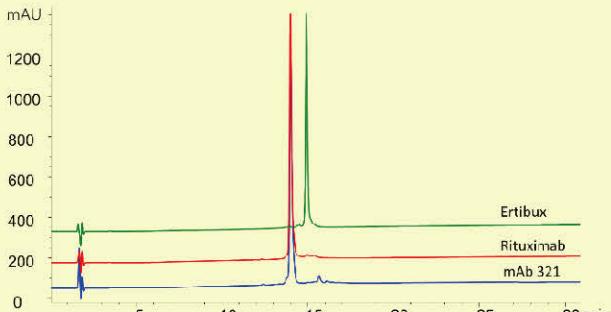


**Figure 22.** Column Temperature Effect on Rituximab Separation

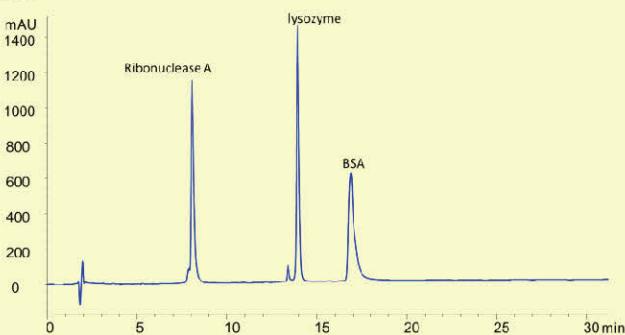


**Figure 23.** Column Temperature Effect on mAb321 Separation

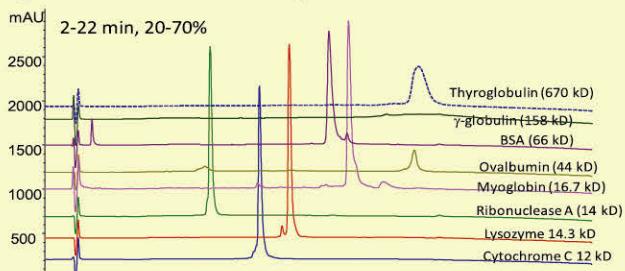


**Figure 24.** mAb321/Erbixux/Rituximab at 80 °C

Column: Proteomix® RP-1000 (5  $\mu$ m, 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<sub>2</sub>O + 0.1% TFA  
B: ACN + 0.1% TFA  
Sample: monoclonal antibody, 1mg/mL diluted with 0.1% TFA  
Injection: 2.5  $\mu$ L

**Figure 25.** Ribonuclease A, BSA and lysozyme at 40 °C-2.1x150 mm

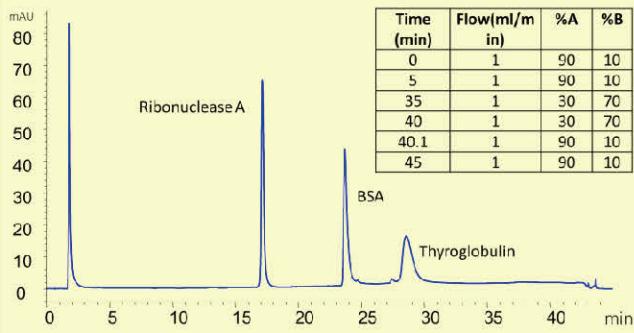
Column: Proteomix® RP-1000 (5  $\mu$ m, 1000 Å, 2.1 x 150 mm)  
Flow rate: 0.25 mL/min, 23- 70% B in 30 min  
Detector: UV 214 nm  
Temperature: 40°C  
Mobile phase: A: H<sub>2</sub>O + 0.1% TFA  
B: ACN + 0.1% TFA  
Sample: 1mg/mL each diluted with 0.1% TFA  
Injection: 5  $\mu$ L

**Figure 26.** Protein standards Separation - 40 °C

Column: Proteomix® RP-1000 (5  $\mu$ m, 1000 Å, 4.6 x 100 mm)  
Flow rate: 1.0 mL/min  
Detector: UV 210 nm

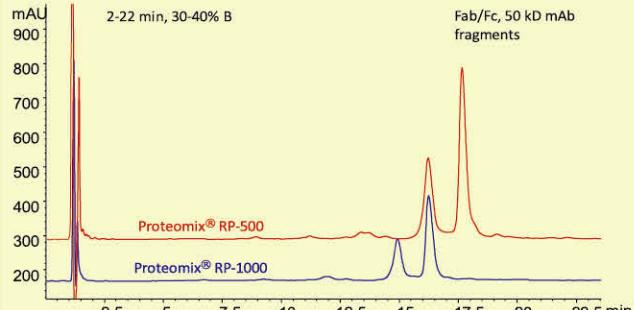
Temperature: 40 °C

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  $\mu$ L

**Figure 27.** Protein Separation

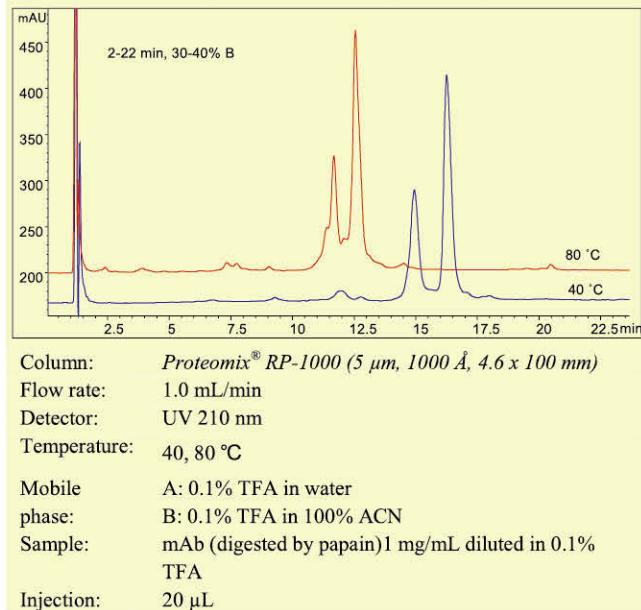
Column: Proteomix® RP-1000 (5  $\mu$ m, 1000 Å, 4.6 x 150 mm)  
Detector: UV 280 nm  
Temperature: 30 °C  
Mobile phase: A: 0.1% TFA in water  
B: 0.1% TFA in acetonitrile  
Sample: 5  $\mu$ L Protein mixture (Thyroglobulin 5.46 mg/ml, BSA 6.3 mg/ml, Ribonuclease A 5.9 mg/ml)

### Mab Fragments

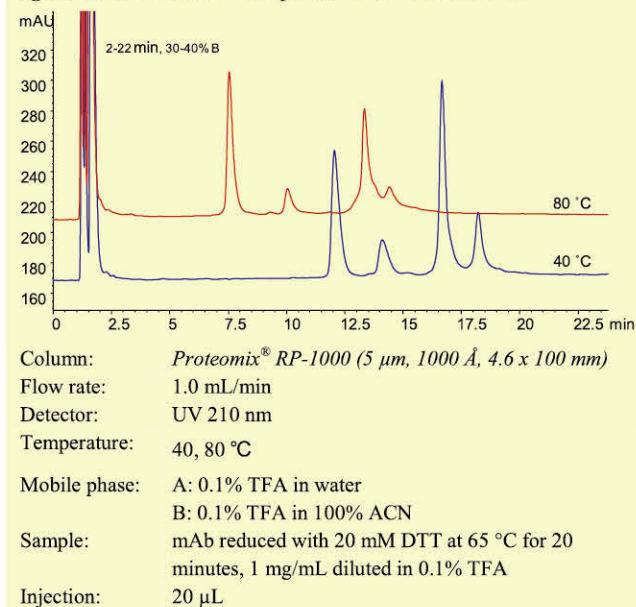
**Figure 28.** Fab/Fc separation on Proteomix® RP-1000 and RP-500 with 40 °C

Column: Proteomix® RP-1000 (5  $\mu$ m, 1000 Å, 4.6 x 100 mm)  
Proteomix® RP-500 (5  $\mu$ m, 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  $\mu$ L

**Figure 29.** Fab/Fc separation with 40 °C and 80 °C

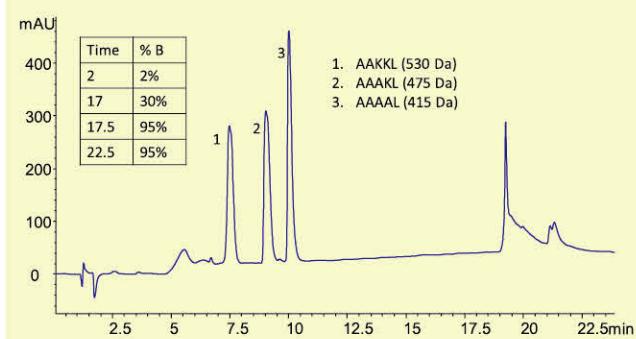


**Figure 30.** Reduced mAb separation: 40 °C and 80 °C



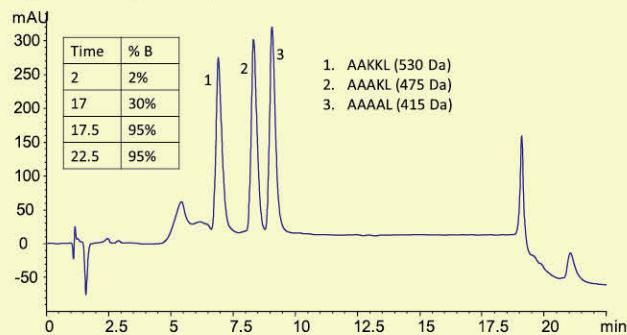
## Peptide Separation

**Figure 31.** Peptide separation on Proteomix® RP-500



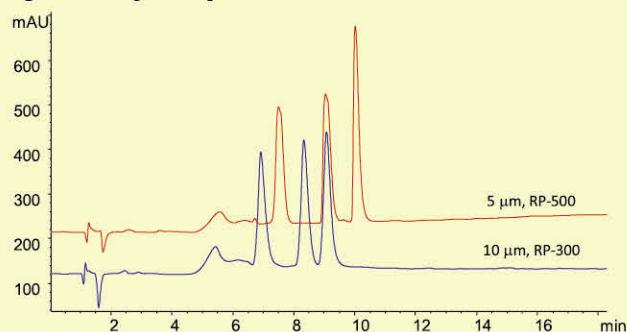
Column:	Proteomix® RP-500 (5 $\mu$ m, 500 Å, 4.6 x 100 mm)
Flow rate:	1.0 mL/min
Detector:	UV 214 nm
Temperature:	40 °C
Pressure:	90-120 bar
Mobile phase:	A: 0.1% TFA in water B: 0.1% TFA in 100% ACN
Sample:	0.7 mg/mL each AAAAL, AAAKL, AAKKL
Injection:	30 $\mu$ L

**Figure 32.** Peptide separation on Proteomix® RP-300

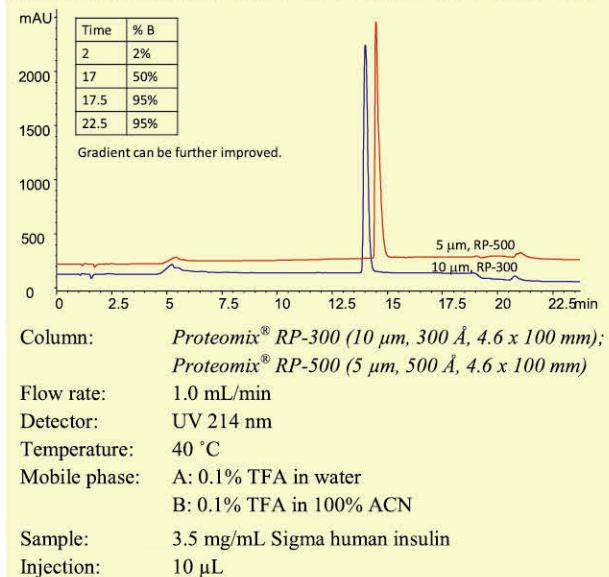


Column:	Proteomix® RP-300 (10 $\mu$ m, 300 Å, 4.6 x 100 mm)
Flow rate:	1.0 mL/min
Detector:	UV 214 nm
Temperature:	40 °C
Mobile phase:	A: 0.1% TFA in water B: 0.1% TFA in 100% ACN
Sample:	0.7 mg/mL each AAAAL, AAAKL, AAKKL
Injection:	30 $\mu$ L

**Figure 33.** Peptide separation on Proteomix® RP-300, RP-500

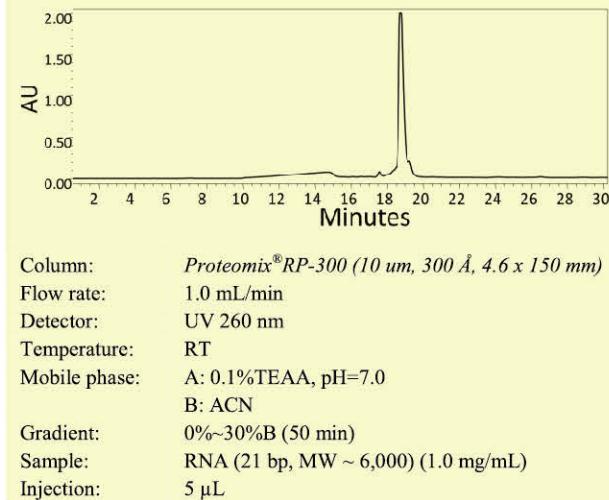


**Figure 34.** Insulin separation on Proteomix® RP-300, RP-500

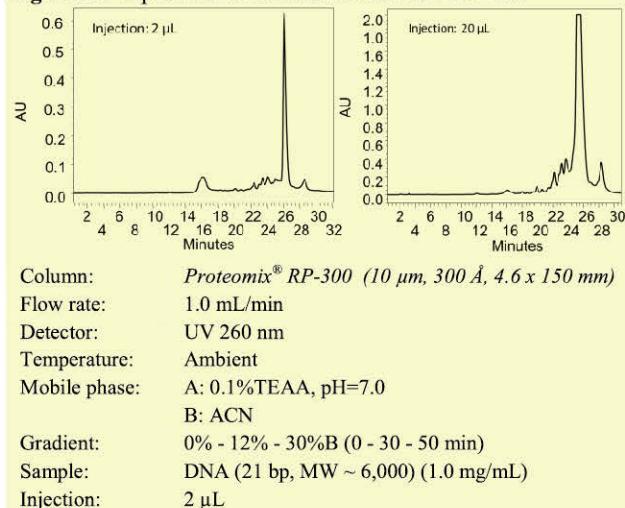


### Oligonucleotides Separation

**Figure 35.** Separation of RNA on Proteomix® RP-300

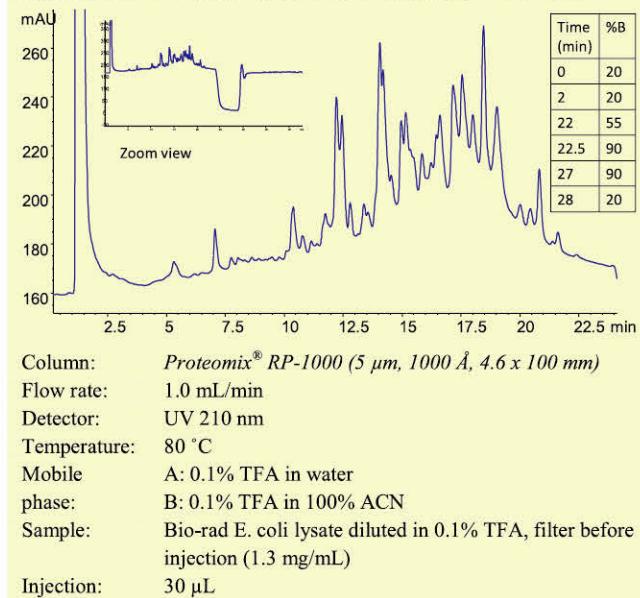


**Figure 36.** Separation of DNA on Proteomix® RP-300

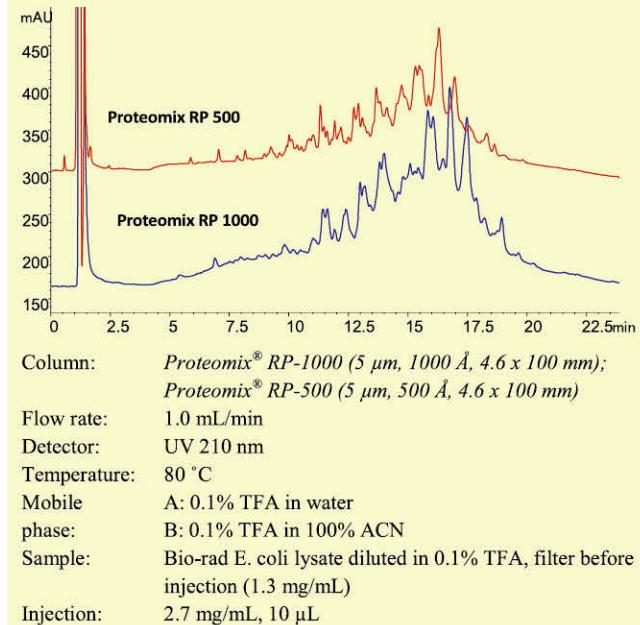


### E. Coli lysate Separation

**Figure 37.** E. Coli lysate separation on Proteomix® RP-1000



**Figure 38.** Bio-Rad E. Coli lysate separation on Proteomix® RP (1000  $\text{\AA}$  vs. 500  $\text{\AA}$ )



## **Ordering Information**

### **Proteomix® RP Column**

PN#	Column Size	Particle Size	Pore Size
465300-4605	4.6x50mm	5 µm	300 Å
465300-4610	4.6x100mm	5 µm	300 Å
465300-2105	2.1x50mm	5 µm	300 Å
465500-4605	4.6x50mm	5 µm	500 Å
465500-4610	4.6x100mm	5 µm	500 Å
465500-2105	2.1x50mm	5 µm	500 Å
465950-4605	4.6x50mm	5 µm	1000 Å
465950-4610	4.6x100mm	5 µm	1000 Å
465950-2105	2.1x50mm	5 µm	1000 Å
469300-4605	4.6x50mm	10 µm	300 Å
469300-4610	4.6x100mm	10 µm	300 Å
469300-2105	2.1x50mm	10 µm	300 Å
469500-4605	4.6x50mm	10 µm	500 Å
469500-4610	4.6x100mm	10 µm	500 Å
469500-2105	2.1x50mm	10 µm	500 Å
469950-4605	4.6x50mm	10 µm	1000 Å
469950-4610	4.6x100mm	10 µm	1000 Å
469950-2105	2.1x50mm	10 µm	1000 Å

\*Additional dimensions are also available

### **How to Order**

Please contact the Sepax Sales Department:

Phone: (302)366-1101 1-877-SEPAX-US

Fax: (302)366-1151

Email: [sales@sepax-tech.com](mailto:sales@sepax-tech.com)

5 Innovation Way, Suite 100  
Delaware 19711 USA

### **Discounts**

Sepax Technologies offers the best discounts depending on the volume of your purchase. Please contact the Sepax Sales team for more information regarding discounts.

### **Opening a Sepax Account**

Call the Sepax Sales Department to provide your business information to set up an account, 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 the 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 the customer receiving the order.
- Non-eligible returns include products that have been contaminated, treated, or tested, with isotopes, radioactive chemicals, or any other types of hazardous material. This applies to semi-prep and prep columns, custom products, bulk resins/materials, and demo purchases.
- Prior authorization is required for all returns. Please contact your local sales manager for prior authorization and a Return Authorization (RA) number.
- 15% restocking charge will be made on all returns.
- Shipping costs are non-refundable. The customer is liable for the cost of returning the item to Sepax Technologies, Inc. A refund will only be processed upon receipt of the returned product.
- Returns and refunds are to be made via the same method of purchase, i.e. through a 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.