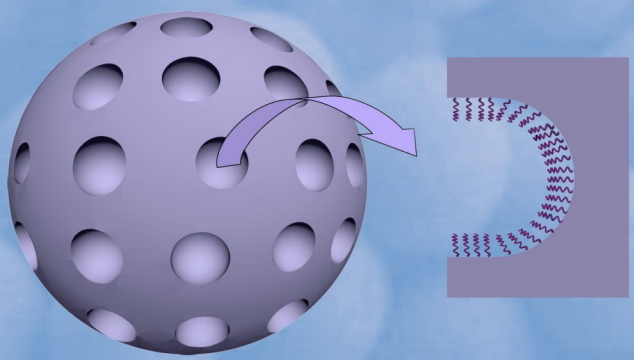
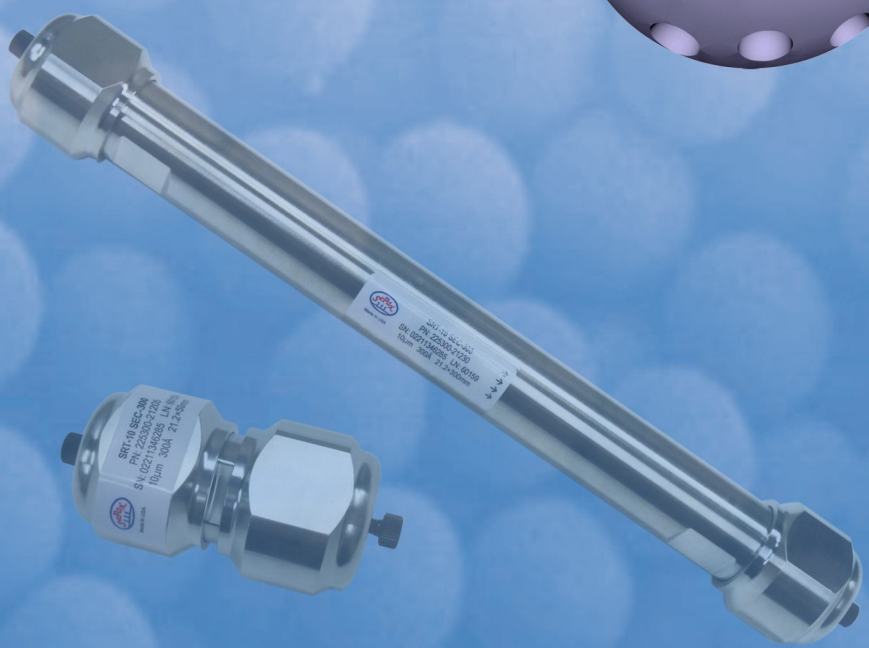
Preparative Size Exclusion Chromatography

Sepax Technologies

SRT®-10 (10 µm)





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 separation challenges in biological area.

Bioseparation Products

Size Exclusion

SRT®, SRT®-C

Nanofilm®

Zenix™, Zenix™-C

Ion-exchange

Proteomix®

Glycomix™

Antibody Separation

Antibodix™

Carbohydrate Separation

Carbomix®

Analytical, Semi-prep and Preparative

SRT®-10 SEC Bulk Media and Columns

High Resolution and High Loading Semi-prep and Preparative Size Exclusion Separation

General Description

Utilizing proprietary surface technologies, SRT-10 SEC phases are made of uniform, hydrophilic, and neutral nanometer thick films chemically bonded on high purity and mechanically stabilized 10 μm silica. The well-controlled surface chemistry results in excellent lot-to-lot reproducibility. Our unique bonding chemistry, coupled with the maximized bonding density, allows SRT-10 phases to provide high stability and negligible non-specific interactions. SRT-10 packings have large pore volume, resulting in high separation resolution. The narrowly dispersed, spherical silica particles of the SRT-10 SEC-300 and 500 have nominal pore sizes at 300 and 500 Å, respectively. Typical applications for SRT-10 columns include separation and detection of biological molecules and water-soluble polymers in aqueous buffers.

Featured Characteristics

- Particle size: 10 μm
- Pore size selection: 300 and 500 Å
- pH stability 2-8.5
- High capacity and resolution
- High stability over low and high concentration salt
- Excellent lot-to-lot reproducibility
- High protein recovery with intact biological activity
- Chemically bonded stationary phase resulting in negligible non-specific interactions
- Ideal for separation of biological molecules: proteins, nucleic acids, oligonucleotides, peptides and virus
- Ideal for separation of natural polymers, e.g. polysaccharides, water soluble synthetic polymers, and nanomaterials, e.g. nanoparticles

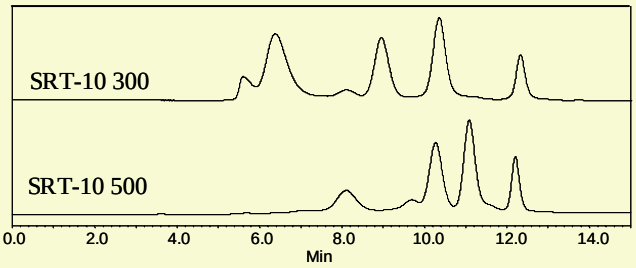
Pore size vs. MW exclusion limit

Phases (10 μm)	Pore Size	Protein MW Exclusion Limit (D)
SRT-10 SEC-300	300 Å	1,250,000
SRT-10 SEC-500	500 Å	5,000,000

High Resolution

Benefiting from the rigid silica particle with high pore volume and uniform hydrophilic coating, SRT-10 packings achieved high efficiency and high resolution. Figure 1 shows the separation profiles of four proteins (thyroglobulin, BSA dimmer, BSA and ribonuclease A) and uracil with the molecular weight in the range of 660,000 D – 120 D.

Figure 1. Comparison of the separation profiles of a protein mixture by SRT-10 SEC-300 and 500 columns

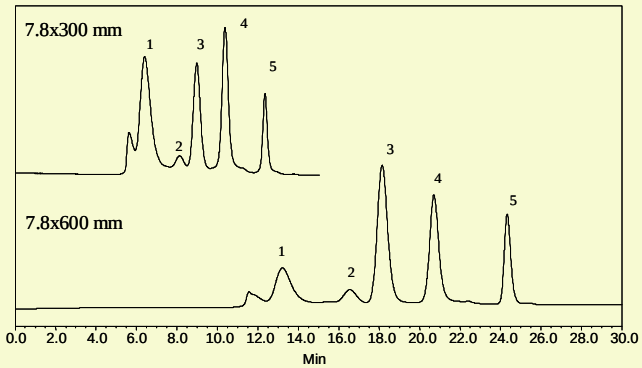


Columns: 7.8 x 300 mm (10 μm)
Mobile phase: 150 mM phosphate buffer, pH 7.0
Flow rate: 1.0 mL/min
Detection: UV 214 nm
Temperature: Ambient (23 °C)
Injection volume: 5.0 μL
Sample: 1) Thyroglobulin (1.0 mg/mL), 670 kD;
2) BSA dimer, 132 kD; 3) BSA (1.0 mg/mL), 66 kD;
4) Ribonuclease A (1.0 mg/mL), 13.7 kD;
5) Uracil (2.5 μg/mL), 120 D.

Column Length Impact on Resolution

The separation resolution can be greatly enhanced by increasing the column length. Figure 2 shows the protein separation by a 60 cm long SRT-10 SEC-300 column vs a 30 cm SRT-10 SEC-300 column. The efficiency with 60 cm column is almost doubled as that of 30 cm column.

Figure 2. The column length impact on separation efficiency



Columns: SRT-10 SEC-300 (10 µm, 300 Å)
Mobile phase: 150 mM phosphate buffer, pH 7.0
Flow rate: 1.0 mL/min
Detection: UV 214 nm
Temperature: Ambient (23 °C)
Injection volume: 5.0 µL
Sample: 1) Thyroglobulin (670 kD); 2) BSA dimmer (132 kD); 3) BSA (66 kD); 4) Ribonuclease A (13.7 kD); 5) Uracil (120 D).

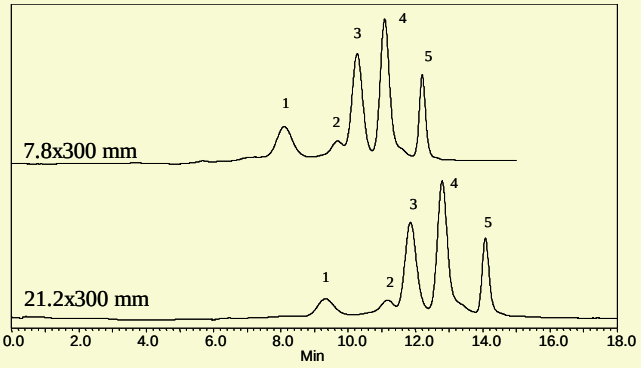
Table 1. Plate counts for SRT-10 SEC-300 columns with different lengths

Peak	Proteins	7.8 x 300 mm	7.8 x 600 mm
1	Thyroglobulin	691	1482
2	BSA dimer	2318	3452
3	BSA	3040	5827
4	Ribonuclease A	5272	10151
5	Uracil	14632	26821

Column ID Impact on Separation Resolution

Well packed SRT semi-preparative and preparative columns also increase the separation efficiency and resolution in comparison to the analytical column with the same length. Figure 3 shows the direct comparison of a 21.2 mm ID semi-prep column and a 7.8 mm ID analytical column, indicating that the semi-prep column increases the efficiency by 15%. The plate number of BSA is 4930 and 4338 for 21.2 x 300 mm and 7.8 x 300 mm columns respectively. For ribonuclease A, the plate number is 8098 and 6984 for 21.2 x 300 mm and 7.8 x 300 mm columns respectively.

Figure 3. The column ID impact on separation efficiency

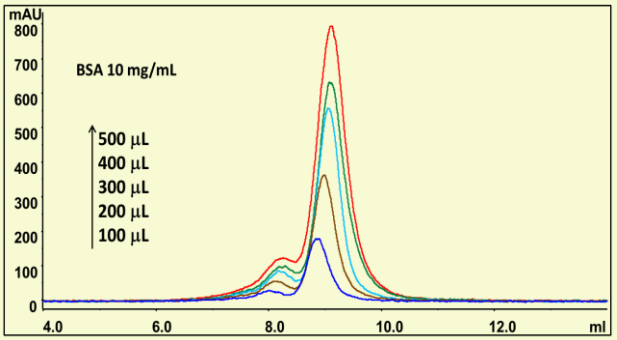


Columns: SRT-10 SEC-500 (10 µm, 500 Å)
Mobile phase: 150 mM phosphate buffer, pH 7.0
Flow rate: 1.0 mL/min for 7.8 x 300 mm
7.0 mL/min for 21.2 x 300 mm
Detection: UV 214 nm
Temperature: Ambient (23 °C)
Injection volume: 5.0 and 20 µL for 7.8 and 21.2 mm ID
Sample: 1) Thyroglobulin (1.0 mg/mL), 670 kD; 2) BSA dimer, 132 kD; 3) BSA (1.0 mg/mL), 66 kD; 4) Ribonuclease A (1.0 mg/mL), 13.7 kD; 5) Uracil (2.5 µg/mL), 120 D.

High Loading Capacity

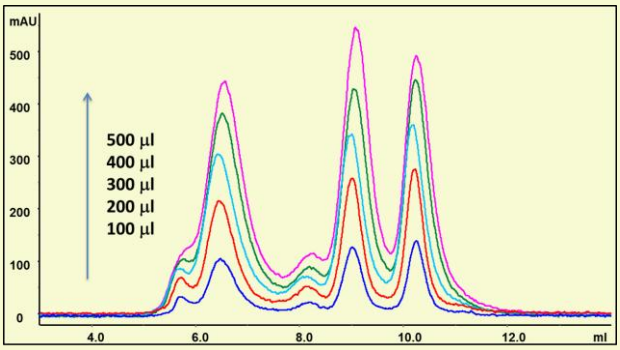
Loading capacity is critical for size exclusion separation and purification. The following figures show high protein loading capacity. For example, more than 1 mg BSA can be loaded onto a SRT-10 SEC-300 7.8 x 300 mm analytical column. (The instrument for the following applications is AKTA Explorer FPLC)

Figure 4. BSA (10 mg/mL) loading on SRT-10 SEC-300



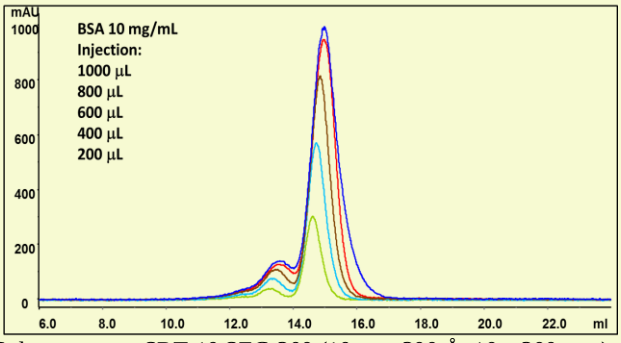
Column: SRT-10 SEC-300, (10 µm, 300 Å, 7.8 x 300 mm)
Mobile phase: 150 mM PB, pH 7.0
Flow rate: 1 mL/min
Detector: UV 280 nm

Figure 5. QC protein standards loading on SRT-10 SEC-300



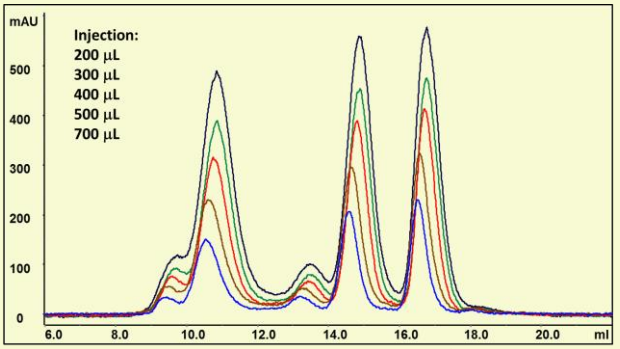
Column: SRT-10 SEC-300 (10 µm, 300 Å, 7.8 x 300 mm)
Mobile phase: 150 mM PB, pH 7.0
Flow rate: 1 mL/min
Detector: UV 280 nm
Sample: 5.5 mg/mL Thyroglobulin, 6.3 mg/mL BSA, 5.9 mg/mL Ribonuclease A

Figure 6. BSA (10 mg/mL) loading on SRT-10 SEC-300



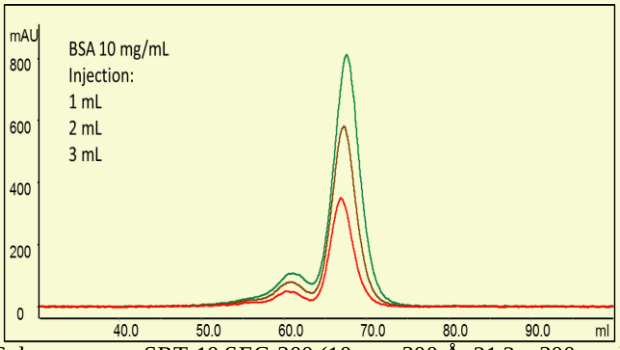
Column: SRT-10 SEC-300 (10 µm, 300 Å, 10 x 300 mm)
Mobile phase: 150 mM PB, pH 7.0
Flow rate: 1-1.5 mL/min
Detector: UV 280 nm

Figure 7. QC protein standards loading on SRT-10 SEC-300



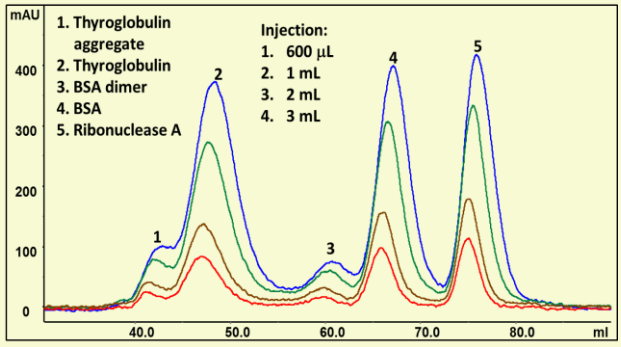
Column: SRT-10 SEC-300 (10 µm, 300 Å, 10 x 300 mm)
Mobile phase: 150 mM PB, pH 7.0
Flow rate: 1-1.5 mL/min
Detector: UV 280 nm
Sample: Thyroglobulin 5.1 mg/mL, BSA 5.3 mg/mL, Ribonuclease A 5.2 mg/mL

Figure 8. High loading with 10 mg/mL BSA separation SRT-10 SEC-300



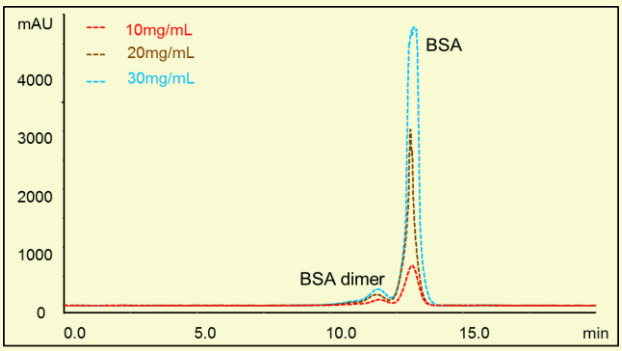
Column: SRT-10 SEC-300 (10 µm, 300 Å, 21.2 x 300 mm)
Mobile phase: 150 mM PB, pH 7.0
Flow rate: 7 mL/min
Detector: UV 280 nm

Figure 9. QC protein standards loading on SRT-10 SEC-300



Column: SRT-10 SEC-300 (10 µm, 300 Å, 21.2 x 300 mm)
Mobile phase: 150 mM PB, pH 7.0
Flow rate: 7 mL/min
Detector: UV 280 nm
Sample: 5 mg/mL Thyroglobulin, 5.4 mg/mL BSA, 5.22 mg/mL Ribonuclease A

Figure 10. SEC prep column- BSA loading study



Column: SRT-10 SEC-300 (10 µm, 300Å, 21.2 x 400 mm)
Mobile phase: phosphate buffer, 150mM, pH7.0
Flow rate: 7 mL/min, 17 bar
Detector: UV 280 nm
Column temperature: 23 °C
Samples: 3 mL BSA (10, 20, or 30 mg/mL)

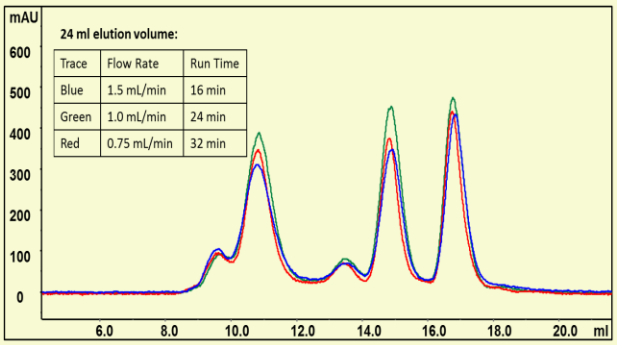
Table 2. SEC prep column-BSA loading (See above chromatogram)

BSA30mg							
Peak	RT(min)	Area (mAU*min)	Height (mAU)	W 1/2 (min)	Rs	Plates/meter (N/m)	Asymmetry
BSA dimer	11.85	101.6082	102.906	0.8		3069	0.34
BSA	13.07	417.1044	690.324	0.55	1.06	7720	0.88
BSA60mg							
Peak	RT(min)	Area (mAU*min)	Height (mAU)	W 1/2 (min)	Rs	Plates/meter (N/m)	Asymmetry
BSA dimer	11.76	195.445	190.721	0.87		2535	0.34
BSA	13.02	1163.3833	3033.566	0.26	1.31	35524	0.86
BSA90mg							
Peak	RT(min)	Area (mAU*min)	Height (mAU)	W 1/2 (min)	Rs	Plates/meter (N/m)	Asymmetry
BSA dimer	11.82	28.065	20.617	0.86		2640	0.33
BSA	13.19	2629.0897	4781.054	0.47	1.22	11005	0.64

Column: SRT-10 SEC-300 (10 µm, 300 Å, 21.2 x 400 mm)
Mobile phase: phosphate buffer, 150 mM, pH 7.0
Flow rate: 7 mL/min, 17 bar
Detector: UV 280 nm
Column temperature: 23 °C
Samples: 3 mL BSA (10, 20, or 30 mg/mL)

Flow Rate

Figure 11. Sepax SRT-10 SEC-300 10 x 300 mm at different flow rate

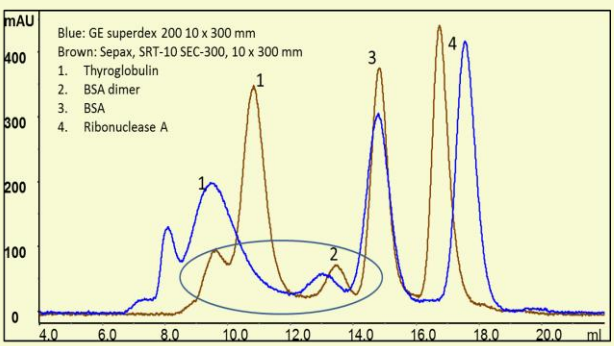


Sepax SRT-10 SEC-300 (10 µm, 300Å, 10 x 300 mm), flow rate 0.75, 1, 1.5 mL/min, 500 µL mixture of Thyroglobulin 5.1 mg/mL, BSA 5.3 mg/mL, Ribonuclease A 5.2 mg/mL

Sepax SRT-10 SEC-300 can be run under faster flow rate than GE column. With 0.75, 1 and 1.5 mL/min flow rate, Sepax column shows minimal resolution change.

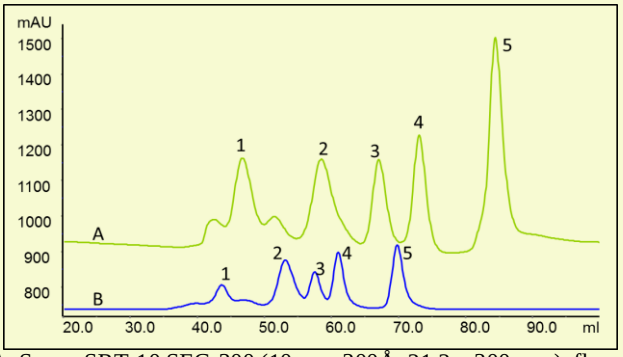
Comparison

Figure 12. GE and Sepax 10 x 300 mm comparison



Sepax column has better separation between Thyroglobulin and BSA (circled area). If lower molecular weight protein separation (between BSA and Ribonuclease A region, peak 3 and peak 4) is of interest, smaller pore size (150 Å) SEC column is recommended.

Figure 13. SRT-10 SEC-300 and Vendor S SEC comparison

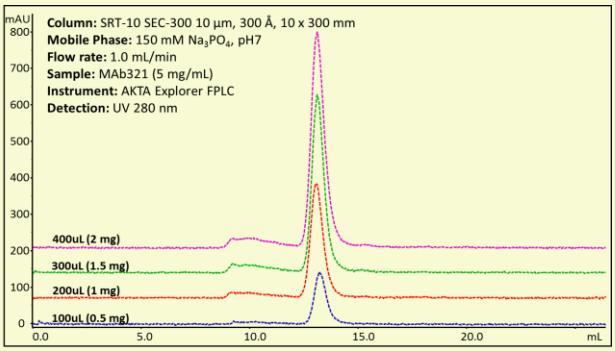


A: Sepax SRT-10 SEC-300 (10 µm, 300Å, 21.2 x 300 mm), flow rate 2 mL/min, 200 µL injection, UV280 nm
B: Vendor S SEC (5 µm, 20 x 300 mm), flow rate 1mL/min, 100 µL injection, UV280 nm
Mobile phase: 50 mM TrisHCl, pH 7.5, 150 mM NaCl, 10% glycerol

Sepax column performs better with higher loading, better resolution, and shorter run time.

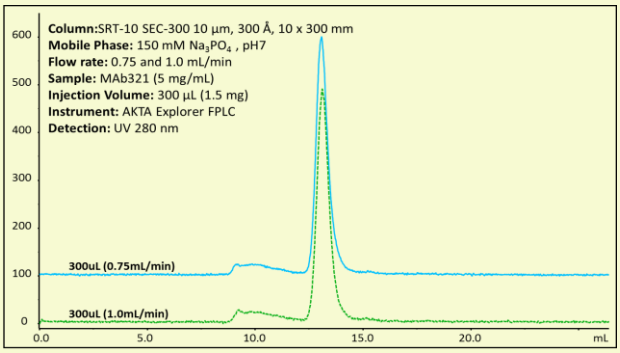
Monoclonal Antibody Separation Application

Figure 14. Monoclonal antibody separation at various loadings



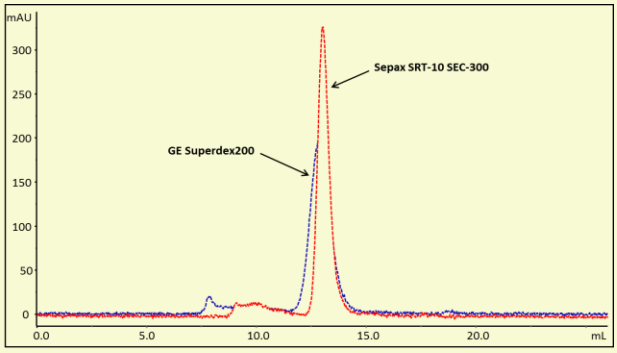
Separation resolution remains constant with increasing mAb loading.

Figure 15. Monoclonal antibody separation at different flow rates



Sepax's SRT-10 SEC-300 gives consistently good resolution at different flow rates and is able to be run at higher flows than the GE Superdex 200.

Figure 16. Comparison of SRT-10 and GE Superdex200



Sepax's SRT-10 SEC-300 gives much sharper and taller peaks than GE's Superdex 200, which displays much broader peaks.

High Robustness

SRT-10 packings have specially designed stationary phases that are densely bonded on the silica surface which enhances the stability of the column, resulting in high robustness at high flow rates.

High Stability

The proprietary stationary phases of SRT-10 packings utilize densely bonded chemistry on the silica surface, which greatly hinders the diffusion of the molecules, thus enabling high stability over a wide range of pH from 2 to 8.5. If use in higher pH, such as 9.0, equilibrate the column with 150 mM sodium phosphate at pH 7.0 and store the column in 150 mM sodium phosphate at pH 7.0.

Mobile Phase Compatibility

SRT-10 phases are compatible with most aqueous buffers, such as ammonium acetate, phosphate, trizma, etc. SRT-10 phases can tolerate high concentration of salts, such as 2.0 M. Furthermore, SRT-10 columns are stable in both organic solvents (such as methanol, ethanol, THF, DMF and DMSO), and the mixture of water and organic solvents.

High Protein Recovery

SRT-10 phases are hydrophilic and neutral. Proteins and other biological molecules have negligible nonspecific interactions with SRT-10 stationary phases. The protein adsorption to the silica surface is suppressed, leading to high recovery of intact proteins, maintaining the protein activity after separation. More than 95% recovery is achieved for BSA and lysozyme, the representatives for acidic and basic proteins, respectively.

Column Dimension Availability

SRT-10 SEC columns are available in dimensions 4.6, 7.8, 10, 21.2, 30 and 50 mm I.D., and 50, 100, 150, 250, 300 and 600 mm length. Sepax also offers custom-size columns.

Applications

SRT-10 bulk media and columns have wide applications in separation and purification, such as

- Proteins
- Monoclonal antibodies
- Cell lysates
- Nucleic acids
- Nucleotides
- Peptides
- Water-soluble polymers
- Nanoparticles
- Nanotube

SRT-10 Technical Specifications

Phase	SRT-10 SEC-300	SRT-10 SEC-500
Material	Neutral, hydrophilic film bonded silica	
Particle size	10 μm	10 μm
Pore size (Å)	~ 300	~ 500
Protein MW range (native)	*5,000 – 1,250,000*	*15,000 - 5,000,000*
pH stability	2 – 8.5 (pH 8.5-9.5 can be tolerated temporarily.)	
Backpressure (7.8x300 mm)	~ 300 psi (flow rate at 1.0 mL/min)	
Maximum backpressure (psi)	~ 3,500	~ 3,000
Salt concentration range	20 mM - 2.0 M	20 mM - 2.0 M
Maximum temperature (°C)	~ 80	~ 80
Mobile phase compatibility	Aqueous and organic	

Sample Loading Recommendation

ID	4.6 x 300 mm	7.8 x 300 mm	10 x 300mm	21.2 x 300mm	30 x 300mm	50 x 300mm
Type	Narrow-bore	Regular	Semi prep	Prep	Process	Process
V-injection	0.5-50 μL	1-150 μL	1-250 μL	0.01-4.2 mL	0.1-8.5 mL	0.5-23.5 mL
Column Loading Guideline (BSA)		≤ 1.5 mg	≤ 2.5 mg	≤ 42 mg	≤ 85 mg	≤ 235 mg
Standard Flow rate (Maximum)	0.35 mL/min	1.0 mL/min	1.65 mL/min (2.0 mL/min)	7.5 mL/min (10mL/min)	15 ml/min (25 ml/min)	41 ml/min (60 ml/min)
Sensitivity	Higher	High	N/A	N/A	N/A	N/A
Back pressure	~400psi	~700psi	700-900 psi	700-900 psi	700-900 psi	700-900psi
Instrument Type	Regular	Regular	Prep	Prep	Process	Process

Ordering Information

SRT-10 Column

SRT-10 300 (10 μm, 300 Å)

ID x Length (mm)	P/N
50x300	225300-50030
50x250	225300-50025
30x300	225300-30030
30x250	225300-30025
21.2x300	225300-21230
21.1x250	225300-21225
21.2x50 (Guard)	225300-21205
10x300	225300-10030
10x250	225300-10025
10x50 (Guard)	225300-10005
7.8x300	225300-7830
7.8x250	225300-7825
7.8x150	225300-7815
7.8x50 (Guard)	225300-7805
4.6x300	225300-4630
4.6x250	225300-4625
4.6x50 (Guard)	225300-4605

SRT-10 500 (10 μm, 500 Å)

ID x Length (mm)	P/N
50x300	225500-50030
50x250	225500-50025
30x300	225500-30030
30x250	225500-30025
21.2x300	225500-21230
21.1x250	225500-21225
21.2x50 (Guard)	225500-21205
10x300	225500-10030
10x250	225500-10025
10x50 (Guard)	225500-10005
7.8x300	225500-7830
7.8x250	225500-7825
7.8x150	225500-7815
7.8x50 (Guard)	225500-7805
4.6x300	225500-4630
4.6x250	225500-4625
4.6x50 (Guard)	225500-4605

SRT-10 Bulk Media

SRT-10 300 (10 μm, 300 Å)

Quantity	P/N
10 g	225300-0010
100 g	225300-0100
500 g	225300-0500
1 kg	225300-1000
5 kg	225300-5000

SRT-10 500 (10 μm, 500 Å)

Quantity	P/N
10 g	225500-0010
100 g	225500-0100
500 g	225500-0500
1 kg	225500-1000
5 kg	225500-5000

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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Delaware Technology Park Newark
Delaware 19711 USA

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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