

Sepax Technologies, Inc. **Preparative Size Exclusion Chromatography**

SRT®-10/10C (10 µm)

Fast Protein Purification For FPLC, HPLC and Prep LC

Better Surface Chemistry for Better Separation



SRT[®]-10/10C Size Exclusion Columns

FAST Speed, High Resolution and High Loading Semi-prep and Preparative Size Exclusion Separation For FPLC, HPLC and Prep LC

General Description

Utilizing proprietary innovative surface technologies, SRT-10/10C SEC phases are made of uniform, hydrophilic, and neutral nanometer thick films chemically bonded on high purity and mechanically stabilized 10 μ m silica. The combination of the two different types of SEC coating chemistries, SRT-10 with stand-up monolayer bonded on porous silica and SRT-10C with lay-down monolayer on porous silica (Figure 1), provides a powerful total solution for robust, reproducible and high resolution size based separation of biological molecules in the market.

Our unique bonding chemistry, coupled with the maximized bonding density, allows SRT-10/10C phases to provide high stability and negligible non-specific interactions. SRT-10/10C packings have large pore volumes, resulting in high separation resolution. The columns can tolerate high backpressure, which allows for higher flow rate/faster speed to save the total run time, thus increasing purification efficiency at the laboratory preparative scale. The well-controlled surface chemistry results in excellent lot-to-lot reproducibility.

Typical applications for SRT-10/10C columns include separation and detection of biological molecules like antibodies, antibody drug conjugates, proteins, peptides, oligonucleotides and virus like particles, as well as watersoluble polymers in aqueous buffers.

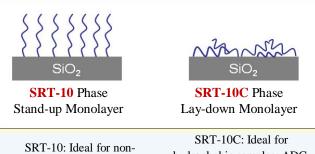
Featured Characteristics

- Particle size: 10 µm
- Pore size selection: 100, 300, 500, and 1000 Å
- pH stability 2-8.5
- High capacity and loadability
- Fast speed and high 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: antibody, proteins, peptides, oligonucleotides, peptides and virus
- Ideal for separation of natural polymers, e.g.

polysaccharides, water soluble synthetic polymers, and nanomaterials, e.g. nanoparticles

Stationary Phase Structure

Figure 1. Phase structure difference: a monolayer stands up on the silica surface for SRT-10, and a monolayer lays down on the silica surface for SRT-10C.



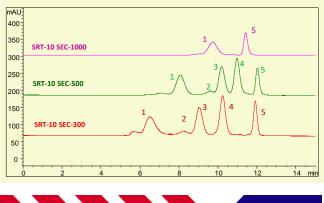
SRT-10: Ideal for nonhydrophobic samples: various types of non-hydrophobic protein including MAb and etc. SRT-10C: Ideal for hydrophobic samples: ADC, Derivatized MAb, Pegylated Protein, Membrane Protein, "Super sticky protein"

Pore size vs. MW exclusion limit

The narrowly dispersed, spherical silica particles of the SRT-10/10C SEC have nominal pore sizes at 100, 300, 500, and 1000 Å, respectively. Figure 2 and 3 show the separation profiles of protein mixture on the SRT-10 SEC columns with different pore sizes (100, 300, 500, 1000 Å).

Phases (10 μm)	Pore Size	Protein MW Exclusion Limit (Da)
SRT-10/10C SEC-100	100 Å	100,000
SRT-10/10C SEC-300	300 Å	1,250,000
SRT-10/10C SEC-500	500 Å	5,000,000
SRT-10/10C SEC-1000	1000 Å	7,500,000

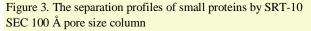
Figure 2. Comparison of the separation profiles of a protein mixture by SRT-10 SEC columns with different pore sizes: 300, 500 and 1000 Å, respectively

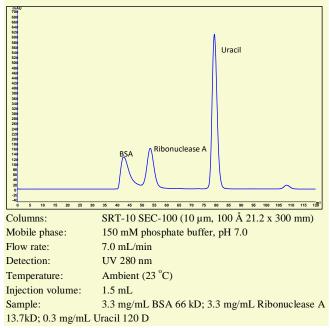


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Columns:	SRT-10 SEC (10 µm, 7.8 x 300 mm)			
Mobile phase:	150 mM phosphate buffer, pH 7.0			
Flow rate:	1.0 mL/min			
Detection:	UV 214 nm			
Temperature:	Ambient (23 °C)			
Injection volume:	3.0 µL			
Sample:	1) Thyroglobulin 670 kD; 2) BSA dimer 132 kD; 3)			
BSA 66 kD; 4) Ribonuclease A 13.7 kD; 5) Uracil 120 D.				



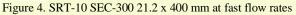


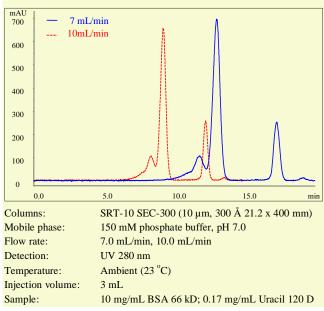
Fast Purification with High Flow Rate

Sepax SRT-10/10C preparative size SEC columns can run under a faster flow rate than the GE Superdex columns, thus greatly saving the purification run time and increasing the purification efficiency while still maintaining good separation with minimal resolution change.

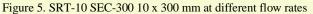
Figure 4 shows one example of Sepax SRT-10/10C columns in 21.2 x 400 mm size that can run under very fast

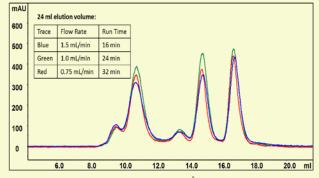
flow rate at 7 mL/min and 10 mL/min which shortens the purification time to below 20 minutes while still achieving high resolution with minimal change.





With 0.75, 1 and 1.5 mL/min flow rate, Sepax SRT-10/10C 10 x 300 mm size columns show minimal resolution change.





 Column:
 SRT-10 SEC-300 (10 μm, 300Å, 10 x 300 mm),

 Flow rate:
 0.75, 1, 1.5 mL/min,

 Sample:
 500 μL mixture of Thyroglobulin 5.1 mg/mL, BSA 5.3

mg/mL, Ribonuclease A 5.2 mg/mL

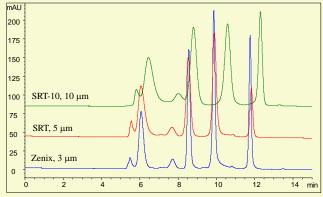
ID	4.6 x 300 mm	7.8 x 300 mm	10 x 300 mm	21.2 x 300 mm	30 x 300 mm	50 x 250 mm
Column Volume	5 mL	14 mL	24 mL	105 mL	212 mL	490 mL
V-injection	5-100 μL	15-250 μL	25-500 μL	0.1-4.2 mL	0.2-8.5 mL	0.5-20 mL
Column Loading Guideline (BSA)		≤ 1.5 mg	≤ 2.5 mg	≤ 42 mg	≤ 85 mg	≤ 235 mg
Standard Flow Rate (Maximum)	0.35 mL/min	1.0 mL/min	1.65 mL/min (2.0 mL/min)	7.5 mL/min (10 mL/min)	15 ml/min (25 ml/min)	41 ml/min (60 ml/min)
Typical Run Time	< 20 min	< 20 min	< 20 min	< 20 min	< 20 min	< 20 min
Column Pressure (at standard flow rate)	< 400 psi < 30 bar < 3 MPa	< 700 psi < 50 bar < 5 MPa	< 900 psi < 60 bar < 6 MPa`	< 900 psi < 60 bar < 6 MPa	< 900 psi < 60 bar < 6 MPa	< 900 psi < 60 bar < 6 MPa

Resolution

Benefiting from the rigid silica particle with high pore volume and uniform hydrophilic coating, SRT-10/10C packings provide a seamless transition from analytical separation scale up to preparative purification with high efficiency and high resolution for biomolecules.

Particle Size Impact on Resolution

Figure 6. The particle size impact on separation efficiency



SRT-10 10 µm, SRT 5 µm and Zenix 3 µm, SEC-300 Columns: (300 Å, 7.8 x 300 mm)

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

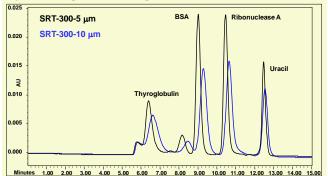
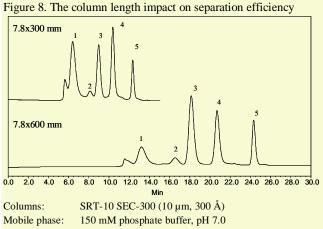


Figure 7. The particle size impact on resolution - 21.2 x 300 mm

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

Column Length Impact on Resolution

The separation resolution can be greatly enhanced by increasing the column length. Figure 8 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 the 60 cm column is almost doubled that of the 30 cm column.



moone phase.	150 milli phosphate baller, pri 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);
() DO ((((1)))	

3) BSA (66 kD); 4) Ribonuclease A (13.7 kD); 5) Uracil (120 D).

Plate counts for SRT-10 SEC-3	00 columns with different lengths
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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

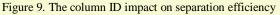
Column ID Impact on Separation Resolution

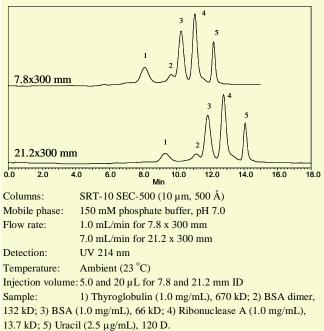
Well packed SRT-10/10C semi-preparative and preparative columns also increase the separation efficiency and resolution in comparison to the analytical column with the same length.

Figure 9 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.



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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)

Column Size: 7.8 x 300 mm

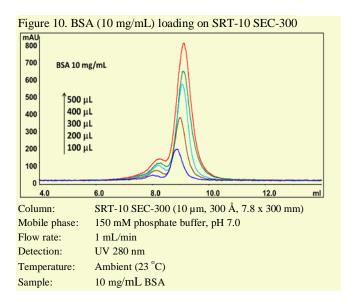
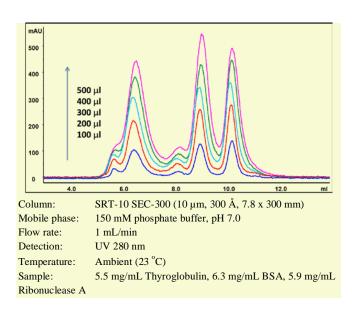
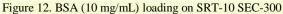
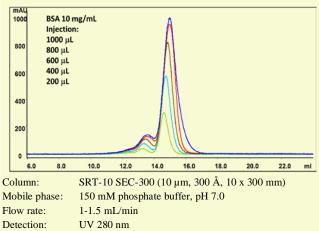


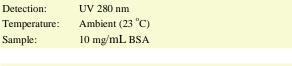
Figure 11. QC protein standards loading on SRT-10 SEC-300

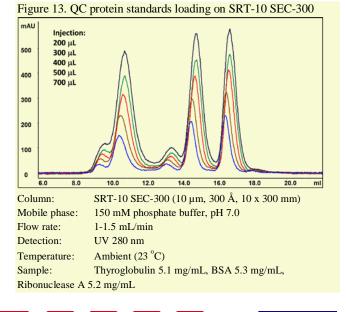


Column Size: 10 x 300 mm





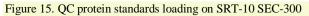


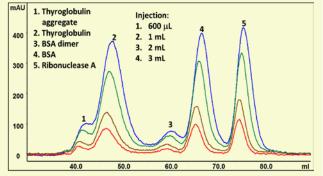


Column Size: 21.2 x 300 mm and 400mm

Figure 14. High loading with 10 mg/mL BSA separation

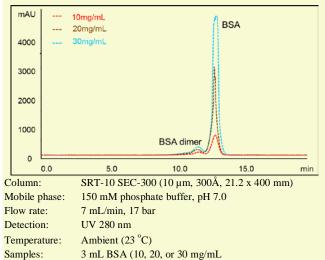
SRT-10 SEC-300 mAU BSA 10 mg/mL 800 Injection: 1 mL 600 2 mL 3 mL 400 200 0 40.0 50.0 60.0 70.0 80.0 90.0 ml SRT-10 SEC-300 (10 µm, 300 Å, 21.2 x 300 mm) Column: Mobile phase: 150 mM PB, pH 7.0 Flow rate: 7 mL/min Detection: UV 280 nm Ambient (23 °C) Temperature: Sample: 10 mg/mL BSA





Column:	SRT-10 SEC-300 (10 µm, 300 Å, 21.2 x 300 mm)
Mobile phase:	150 mM phosphate buffer, pH 7.0
Flow rate:	7 mL/min
Detection:	UV 280 nm
Temperature:	Ambient (23 °C)
Sample:	5 mg/mL Thyroglobulin, 5.4 mg/mL BSA, 5.22 mg/mL
Ribonuclease A	

Figure 16. SEC prep column- BSA loading study



SEC prep column-BSA loading (See above chromatogram)

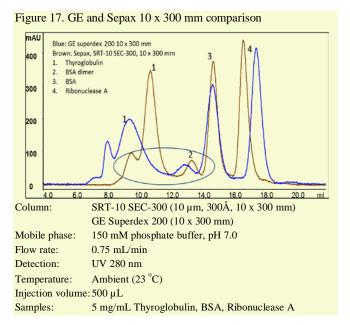
BSA30mg							
Peak	RT(min)	Area (mAU*min)	Height (mAU)	W 1/2 (min)	Rs	Plates/meter (N/m)	Asymmetry
BSA dimer	11.85	101.6082	102.906	0.8		3069	0.34
BSA	13.07	417.1044	690.324	0.55	1.06	7720	0.88
BSA60mg							
Peak	RT(min)	Area (mAU*min)	Height (mAU)	W 1/2 (min)	Rs	Plates/meter (N/m)	Asymmetry
BSA dimer	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

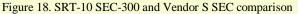
Column:SRT-10 SEC-300 (10 μm, 300 Å, 21.2 x 400 mm)Mobile phase:150 mM phosphate buffer, pH 7.0Flow rate:7 mL/min, 17 barDetection:UV 280 nmTemperature:Ambient (23 oC)Samples:3 mL BSA (10, 20, or 30 mg/mL)

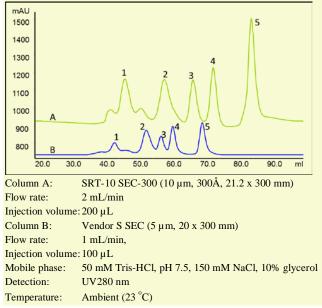
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Comparison

The Sepax SRT-10 SEC 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, a smaller pore size SEC column is recommended. Sepax columns perform better with higher loading, better resolution, and shorter run time.



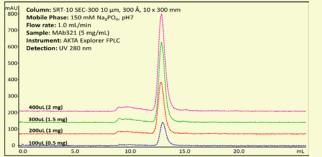




Monoclonal Antibody Separation

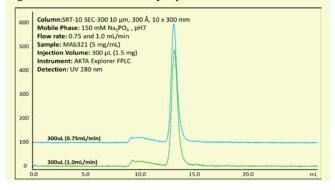
Figure 19 shows that separation resolution of monoclonal antibody remains constant with increasing mAb loading.

Figure 19. Monoc	lonal antibody of	anaration at a	vorious 1	ondinge
Figure 19. Wionoc	ional antibody se	sparation at	various i	Jaumgs

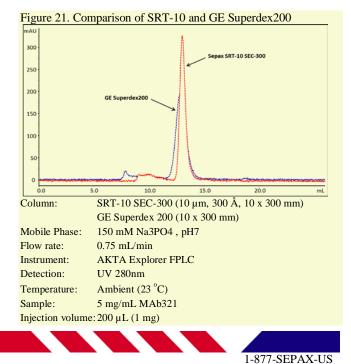


Sepax's SRT-10 SEC-300 gives consistently good resolution at different flow rates and is able to be run at higher flow rates than the GE Superdex 200.

Figure 20. Monoclonal antibody separation at different flow rates



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-10/10C 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-10/10C 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 a higher pH is being used, 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-10/10C phases are compatible with most aqueous buffers, such as ammonium acetate, phosphate, trizma, etc. SRT-10/10C phases can tolerate high concentration of salts, such as 2.0 M. Furthermore, SRT-10/10C 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-10/10C phases are hydrophilic and neutral. Proteins and other biological molecules have negligible nonspecific interactions with SRT-10/10C stationary phases. The protein adsorption to the silica surface is suppressed, leading to a high recovery of intact proteins, which maintains 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-10/10C 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.

SRT-10 Technical Specifications

Phase	SRT-10/10C SEC-100	SRT-10/10C SEC-300	SRT-10/10C SEC-500	SRT-10/10C SEC-1000		
Material		Neutral, hydrophilic film bonded silica				
Particle size	10 µm	10 µm	10 µm	10 µm		
Pore size (Å)	~100	~ 300	~ 500	~ 1000		
Protein MW range (native)	100-100,000	5,000 - 1,250,000	15,000 - 5,000,000	50,000-7,500,000		
pH stability		2 – 8.5 (pH 8.5-9.5 can be tolerated temporarily.)				
Salt concentration range	20 mM - 2.0 M	20 mM - 2.0 M	20 mM - 2.0 M	20 mM - 2.0 M		
Maximum temperature (°C)	~ 80	~ 80	~ 80	~ 80		
Mobile phase compatibility	Aqueous and organic					



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Ordering Information

SRT-10 SEC Column

	SRT-10 SEC-100	SRT-10 SEC-300	SRT-10 SEC-500	SRT-10 SEC-1000
	(10 µm, 100 Å)	(10 µm, 300 Å)	(10 µm, 500 Å)	(10 µm, 1000 Å)
ID x Length (mm)	P/N	P/N	P/N	P/N
50x250	225100-50025	225300-50025	225500-50025	225950-50025
30x300	225100-30030	225300-30030	225500-30030	225950-30030
30x250	225100-30025	225300-30025	225500-30025	225950-30025
21.2x300	225100-21230	225300-21230	225500-21230	225950-21230
21.1x250	225100-21225	225300-21225	225500-21225	225950-21225
21.2x50	225100-21205	225300-21205	225500-21205	225950-21205
10x300	225100-10030	225300-10030	225500-10030	225950-10030
10x250	225100-10025	225300-10025	225500-10025	225950-10025
10x50	225100-10005	225300-10005	225500-10005	225950-10005
7.8x300	225100-7830	225300-7830	225500-7830	225950-7830
4.6x300	225100-4630	225300-4630	225500-4630	225950-4630

SRT-10C SEC Column

	SRT-10C SEC-100	SRT-10C SEC-300	SRT-10C SEC-500	SRT-10C SEC-1000
	(10 µm, 100 Å)	(10 µm, 300 Å)	(10 µm, 500 Å)	(10 µm, 1000 Å)
ID x Length (mm)	P/N	P/N	P/N	P/N
50x250	239100-50025	239300-50025	239500-50025	239950-50025
30x300	239100-30030	239300-30030	239500-30030	233950-30030
30x250	239100-30025	239300-30025	239500-30025	239950-30025
21.2x300	239100-21230	239300-21230	239500-21230	233950-21230
21.2x250	239100-21225	239300-21225	239500-21225	233950-21225
21.2x50	239100-21205	239300-21205	239500-21205	233950-21205
10x300	239100-10030	239300-10030	239500-10030	233950-10030
10x250	239100-10025	239300-10025	239500-10025	233950-10025
10x50	239100-10005	239300-10005	239500-10005	233950-10005
7.8x300	239100-7830	239300-7830	239500-7830	233950-7830
4.6x300	239100-4630	239300-4630	239500-4630	233950-4630

SRT-10/10C SEC Bulk Media

	(10 µm, 100 Å)	(10 µm, 300 Å)	(10 µm, 500 Å)	(10 µm, 1000 Å)
SEC Phase	P/N	P/N	P/N	P/N
SRT-10 SEC	225100-0000	225300-0000	225500-0000	225950-0000
SRT-10C SEC	239100-0000	239300-0000	239500-0000	239950-0000

How to Order

Please contact Sepax Sales Department:

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

5 Innovation Way, Suite 100 Delaware Technology Park Newark, Delaware, 19711, USA

Discounts

Sepax Technologies offers the 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 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 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 is required for all returns. Please contact your local sales manager for prior authorization and Return Authorization Number.
- A 15% restocking charge will be made on all returns.
- Shipping costs are non-refundable. The customer pays for all of the shipping related costs including sending return product back to Sepax. A refund will only be processed upon receipt of the returned product.
- The return and refund will be made using 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 circumstances 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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