

Sepax Unix™ 1.8 µm UHPLC SEC Column

Ultra High Efficiency and Resolution Size Exclusion Chromatography

General Description

Utilizing proprietary surface technologies, Unix™ SEC-300 phase is made of uniform, hydrophilic, and neutral nanometer thick films chemically bonded to high purity and mechanically stabilized silica with the particle size of 1.8 µm. The combination of small particle size and large pore volume of Unix™ SEC-300 renders the highest separation efficiency and resolution of analytes. The well-controlled surface chemistry results in excellent lot-to-lot reproducibility. Our unique bonding chemistry, coupled with the maximized bonding density, allows Unix™ SEC-300 to provide high stability and negligible non-specific interactions. Typical applications for Unix™ SEC-300 columns include separation and analysis of biological molecules and water-soluble polymers.

Featured Characteristics

- Particle size of 1.8 µm with 300 Å pore
- Ultra high separation efficiency, capacity, and resolution
- High stability over low and high salt concentration
- Lot-to-lot reproducibility
- Excellent biomolecule separation while maintaining its original conformation due to low pressure resin property (up to 50% lower than competitor)
- >50% faster separation than traditional SEC columns without compromising resolution for high throughput screening
- Ideal for separation and analysis of biological molecules such as mAb, ADC, proteins, protein conjugates, and oligonucleotides
- Ideal for separation and analysis of natural and synthetic polymers and nanomaterials

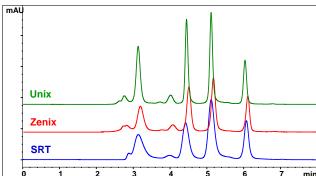
High Separation Efficiency

The advantages of small particle size are higher efficiency and higher resolution. Column efficiency nearly doubles upon decreasing the particle size from 5 μ m to 1.8 μ m (Figure 1 and 2).

Technical Specifications

Phase	Unix™ SEC-300
Material	Neutral, hydrophilic film bonded to silica
Average particle size	1.8 μm
Pore size	300 Å
Protein MW range (native)	5,000 – 1,250,000 Da
pH Stability	2.0 – 8.5 (pH 8.5-9.5 can be tolerated temporarily)
Back pressure for 4.6 x 150 mm (0.35 mL/min)	~1700 psi
Maximum back pressure	~3500 psi
Salt concentration range	20 mM - 2.0 M
Maximum temperature	~ 80 °C
Mobile phase compatibility	Aqueous and organic

Figure 1. Comparison of Unix™ 1.8 μm, Zenix® 3 μm, and SRT® 5 μm SEC-300 on Protein Standards



Column: Unix[™] SEC-300 (1.8 μm, 300 Å, 4.6 x 150 mm)

Zenix® SEC-300 (3 μm, 300 Å, 4.6 x 150 mm) SRT® SEC-300 (5 μm, 300 Å, 4.6 x 150 mm)

Mobile Phase: 150 mM sodium phosphate buffer

Injection: 3 µL
Flow Rate: 0.35 mL/min
Instrument UHPLC
Detection: UV 214 nm
Temperature 25 °C

Sample: 1. Thyroglobulin 670 kD; 2. BSA dimer 132 kD; 3. BSA 66

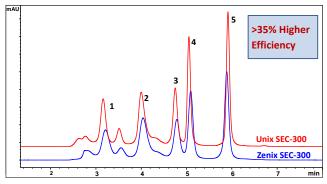
kD; 4. Ribonuclease A 14 kD; 5. Uracil 120 Da





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Figure 2. Comparison of Unix™ 1.8 μm and Zenix® 3 μm on Bio Rad Standards



Column: Unix™ SEC-300 (1.8 μm, 300 Å, 4.6 x 150 mm)

Zenix[®] SEC-300 (3 μm, 300 Å, 4.6 x 150 mm)

Mobile Phase: 150 mM sodium phosphate buffer

Injection: 1 µL

Flow Rate: 0.35 mL/min Instrument UHPLC Detection: UV 214 nm Temperature 25 °C

Sample: 1. Thyroglobulin 670 kD; 2. γ-globulin (bovine) 158 kD; 3.

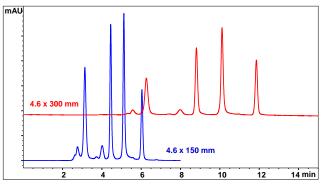
Ovalbumin (chicken) 44 kD; 4.Myoglobin (horse) 17 kD; 5.

Vitamin B12 1.35 kD

Column Dimension Impact

UnixTM SEC-300 column is available in 4.6 x 150, 4.6 x 250, and, $4.6 \times 300 \text{ mm}$ standard dimensions.

Figure 3. Unix™ SEC-300 4.6 x 150 vs. 4.6x300 mm on Protein Standards



Column: Unix™ SEC-300 (1.8 µm, 300 Å, 4.6 x 150 mm) Unix™ SEC-300 (1.8 µm, 300 Å, 4.6 x 300 mm)

Mobile Phase: 150 mM sodium phosphate buffer

 $\begin{array}{lll} \mbox{Injection:} & 3 \ \mu \mbox{L} \\ \mbox{Flow Rate:} & 0.35 \ \mbox{mL/min} \\ \mbox{Instrument} & \mbox{UHPLC} \\ \mbox{Detection:} & \mbox{UV 214 nm} \\ \mbox{Temperature} & 25 \ \mbox{°C} \\ \end{array}$

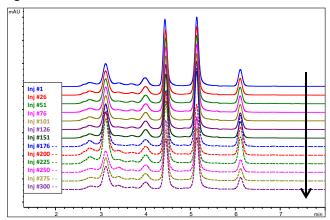
Sample: 1. Thyroglobulin 670 kD; 2. BSA dimer 132 kD; 3. BSA 66

kD; 4. Ribonuclease A 14 kD; 5. Uracil 120 Da

High Stability

Application of proprietary surface modification techniques to Unix™ SEC-300 resin produces a densely bonded silica surface, which greatly hinders the diffusion of molecules that would attack the bond between silica surface and tethered moieties, thus enabling high stability over a wide range of pH from 2.0 to 8.5.

Figure 4. Unix™ Lifetime Test - Protein Standards



Column: Unix[™] SEC-300 (1.8 μm, 300 Å, 4.6 x 150 mm)

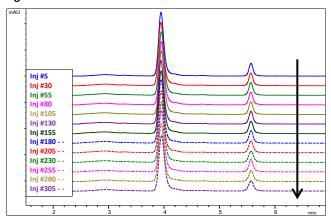
Mobile Phase: 150 mM sodium phosphate buffer

Injection: 3 µL
Flow Rate: 0.35 mL/min
Instrument UHPLC
Detection: UV 214 nm
Temperature 25 °C

Sample: 1. Thyroglobulin 670 kD; 2. BSA dimer 132 kD; 3. BSA 66

kD; 4. Ribonuclease A 14 kD; 5. Uracil 120 Da

Figure 5. Unix™ Lifetime Test - mAb



Column: Unix™ SEC-300 (1.8 μm, 300 Å, 4.6 x 150 mm)

Mobile Phase: 150 mM sodium phosphate buffer

 $\begin{array}{lll} & \text{Injection:} & \text{$1\,\mu$L} \\ & \text{Flow Rate:} & \text{$0.35\,\text{mL/min}$} \\ & \text{Instrument} & \text{$UHPLC$} \\ & \text{Detection:} & \text{$UV~214\,\text{nm}$} \\ & \text{Temperature} & \text{$25~^{\circ}\text{C}$} \\ \end{array}$

Sample: mAb 321 (5 mg/mL)



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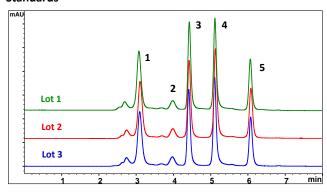
Mobile Phase Compatibility

Unix™ SEC-300 phase is compatible with most aqueous buffers, such as ammonium acetate, phosphate, trizma and so on. Unix™ SEC-300 phase can tolerate high concentration of salts. Furthermore, Unix™ SEC columns are stable in both organic solvents, such as methanol, ethanol, THF, DMF, DMSO, etc. as well as mixture of water and organic solvents.

Lot-to-Lot Reproducibility

The controlled surface chemistry used to synthesize Unix[™] SEC-300 phase makes the surface coating highly reproducible, leading to consistent column manufacturing. Separation variation from batch-to-batch is controlled to be within 5% for retention time. Figure 6 demonstrates the lot-to-lot variation of three different Unix[™] SEC-300 lots.

Figure 6. Unix™ Lot-to-Lot Reproducibility on Protein Standards



Column: Unix™ SEC-300 (1.8 μm, 300 Å, 4.6 x 150 mm)

Mobile Phase: 150 mM sodium phosphate buffer

Injection: $1\,\mu L$

Flow Rate: 0.35 mL/min Instrument UHPLC Detection: UV 214 nm Temperature 25 °C

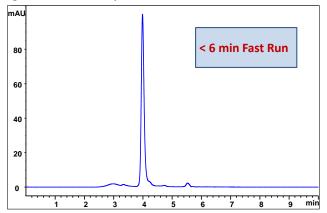
Sample: 1. Thyroglobulin 670 kD; 2. BSA dimer 132 kD; 3. BSA 66

kD; 4. Ribonuclease A 14 kD; 5. Uracil 120 Da

Applications

Unix™ SEC-300 columns are ideal for the identification of proteins, protein variants, peptide fragments, phosphorylated, sialylated, pegylated, and other derivatized proteins. They are well suited for studies such as molecular weight estimation and analysis of biological molecules.

Figure 7. mAb 321 Separation on Unix™ SEC-300



Column: Unix™ SEC-300 (1.8 μm, 300 Å, 4.6 x 150 mm)

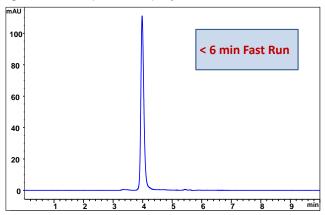
Mobile Phase: 150 mM sodium phosphate buffer

Injection: 1 µL

Flow Rate: 0.35 mL/min Instrument UHPLC Detection: UV 280 nm Temperature 25 °C

Sample: mAb 321 (5 mg/mL)

Figure 8. Erbitux (Cetuximab) Separation on Unix™ SEC-300



Column: Unix[™] SEC-300 (1.8 μm, 300 Å, 4.6 x 150 mm)

Mobile Phase: 150 mM sodium phosphate buffer

Injection: 2.5 μL
Flow Rate: 0.35 mL/min
Instrument UHPLC
Detection: UV 280 nm
Temperature 25 °C

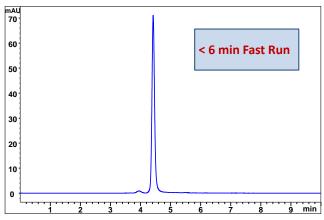
Sample: Erbitux (2 mg/mL)





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Figure 9. Transferrin Apo Separation on Unix™ SEC-300



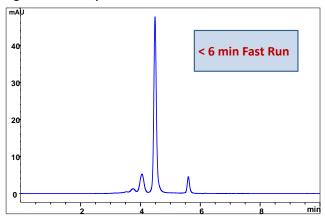
Column: Unix™ SEC-300 (1.8 μm, 300 Å, 4.6 x 150 mm)

Mobile Phase: 150 mM sodium phosphate buffer

 $\begin{array}{lll} \mbox{Injection:} & \mbox{1 μL} \\ \mbox{Flow Rate:} & \mbox{0.35 mL/min} \\ \mbox{Instrument} & \mbox{UHPLC} \\ \mbox{Detection:} & \mbox{UV 280 nm} \\ \mbox{Temperature} & \mbox{25 °C} \\ \end{array}$

Sample: Transferrin Apo (5 mg/mL), about 80 kD

Figure 10. BSA Separation on Unix™ SEC-300



Column: Unix[™] SEC-300 (1.8 μm, 300 Å, 4.6 x 150 mm)

Mobile Phase: 150 mM sodium phosphate buffer

 $\begin{array}{lll} \mbox{Injection:} & \mbox{1 \mu L$} \\ \mbox{Flow Rate:} & \mbox{0.35 mL/min} \\ \mbox{Instrument} & \mbox{$UHPLC$} \\ \mbox{Detection:} & \mbox{UV 280 nm$} \\ \mbox{Temperature} & \mbox{25 °C$} \\ \end{array}$

Sample: BSA (5 mg/mL), 66 kD

Order Information

PN#	Description
211300-4615	Unix™ SEC-300, 1.8 µm, 300 Å, 4.6 x 150 mm
211300-4625	Unix™ SEC-300, 1.8 μm, 300 Å, 4.6 x 250 mm
211300-4630	Unix™ SEC-300, 1.8 µm, 300 Å, 4.6 x 300 mm
102000-P346	Pre-column Filter with 0.5 um stainless steel frit
102001-P346	0.5 um stainless steel refill frits (for Precolumn Filter)

How to Order

It's fast and easy to order from the Sepax on-line store at

www.sepax-tech.com

Or, contact Sepax Sales Department by

Phone: (302) 366-1101 Fax: (302) 366-1151

E-mail: sales@sepax-tech.com

Mail

5 Innovation Way, Suite 100 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 Terms

Terms of payment are net 30 days. Mastercard $\rm ^{"}, Visa ^{"}, and American Express <math>\rm ^{"}$ are accepted.

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

Returns are accepted only with prior authorization. Call Sepax Technical Support to describe the problem that happened. Please provide us with the sales order number, product number, and quantity damaged. Sepax Technical Support will give you instructions for returns. All claims must be made within 15 business days after receipt of product. A 10% charge will be made on cancelled orders or customer order errors.

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.