



## Oligonucleotide Separation on SEC, IEX and RP Columns

### Highlighted FACTS:

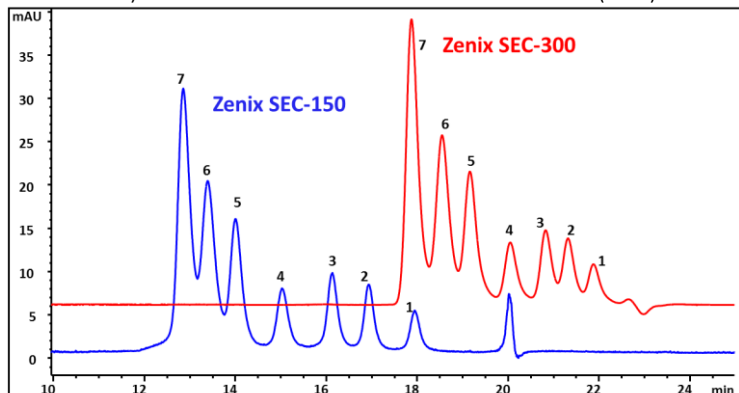
- Zenix / SRT and Zenix-C / SRT-C are suitable for protein/peptide oligonucleotide conjugates separation.
- Zenix 150 Å and 300 Å provides baseline separation for smaller oligonucleotides with 5 base difference. SRT have large pore size columns available 500 Å, 1000 Å and Å for larger oligonucleotides.
- 300 Å gives better resolution for longer oligonucleotides (>30 nt), 150 Å separates poly dAs with baseline resolution for shorter oligos (<35 nt)
- Separation resolution is improved with reduced flow rate. Need to use reduced flow rate when test columns in tandem to avoid too high backpressure.
- Proteomix SAX separates varieties of oligonucleotides such as single stranded DNA fragments and small interfering RNAs.
- High separation resolution on Proteomix SAX is achieved between full length oligonucleotides and their degradation products.
- Polymer based reversed phase Proteomix RP is suitable for oligonucleotide separation under denaturing separation conditions.

### Order Information

213300-7830	Zenix SEC-300, 3µm, 300 A 7.8 x 300 mm
213150-7830	Zenix SEC-150, 3µm, 150 A 7.8 x 300 mm
233150-7830	Zenix-C SEC-150, 3µm, 150 A 7.8 x 300 mm
469300-4615	Proteomix RP-300, 10µm, 300 A 4.6x150 mm
403NP5-4615	Proteomix SAX-NP5, 5µm, NP 4.6x150 mm
403NP5-4625	Proteomix SAX-NP5, 5µm, NP 4.6x250 mm

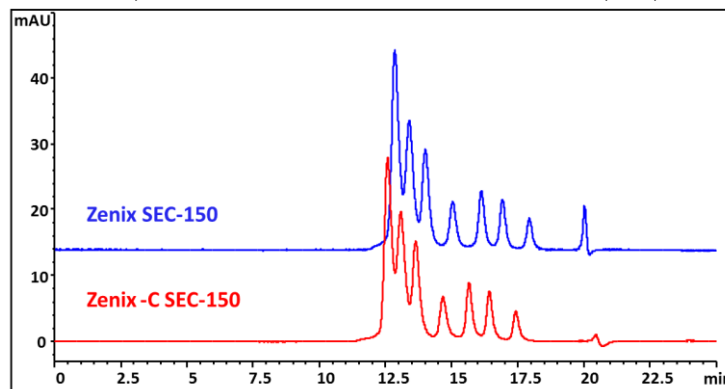
### Zenix SEC-150 and Zenix SEC-300 comparison

Column: Zenix SEC- 300, Zenix SEC-150, 7.8 x 300 mm  
 Mobile phase : 150 mM Phosphate buffer, pH 7; Flow rate: 0.5 mL/min;  
 Detector: UV 260 nm, Column temperature: 25 °C;  
 Injection volume: 30 µL; Pressure: 41 bar;  
 Sample: 1) dA10, 2) dA15, 3) dA20, 5) dA40, 6) dA50, 7) dA60, 0.1 µM each in water  
 4) 5'-ATATCTACACGGCTACCCGTACCAATGCTGCTCC-3' (35 nt)



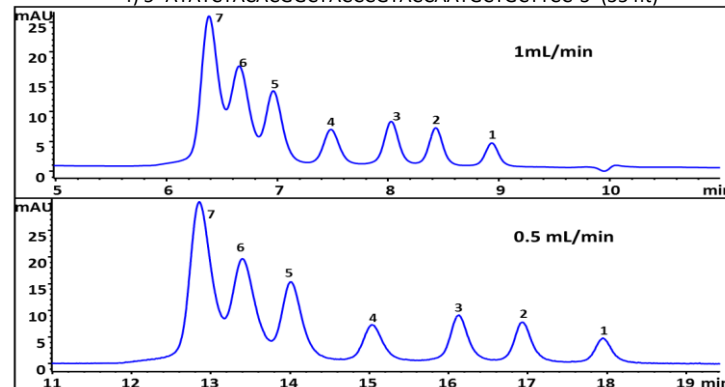
### Zenix and Zenix-C SEC-150 comparison

Column: Zenix SEC- 150, Zenix-C SEC-150, 7.8 x 300 mm  
 Mobile phase : 150 mM Phosphate buffer, pH 7; Flow rate: 0.5 mL/min;  
 Detector: UV 260 nm, Column temperature: 25 °C;  
 Injection volume: 30 µL; Pressure: 41 bar;  
 Sample: 1) dA10, 2) dA15, 3) dA20, 5) dA40, 6) dA50, 7) dA60, 0.1 µM each in water  
 4) 5'-ATATCTACACGGCTACCCGTACCAATGCTGCTCC-3' (35 nt)



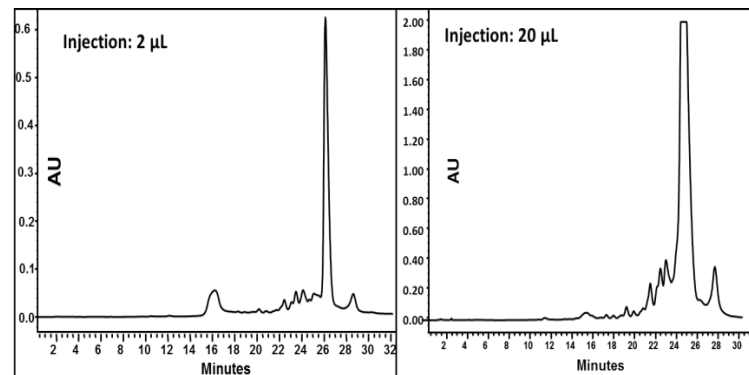
### Zenix SEC-150 with different flow rates

Column: Zenix SEC- 150, 7.8 x 300 mm  
 Mobile phase : 150 mM Phosphate buffer, pH 7; Flow rate: 0.5 mL/min, 1 mL/min  
 Detector: UV 260 nm, Column temperature: 25 °C;  
 Injection volume: 30 µL; Pressure: 41 bar;  
 Sample: 1) dA10, 2) dA15, 3) dA20, 5) dA40, 6) dA50, 7) dA60, 0.1 µM each in water  
 4) 5'-ATATCTACACGGCTACCCGTACCAATGCTGCTCC-3' (35 nt)



### Separation of DNA by Proteomix RP Column

Column: Proteomix RP-300 (10µm, 300A, 4.6x150mm)  
 Mobile phase: A, 0.1%TEAA, pH=7.0; B, ACN; Flow rate: 1.0 mL/min  
 Gradient: 0% - 12% - 30%B (0 - 30 - 50 min); Wavelength: 260 nm; Column temperature: RT; Sample: DNA (21 bp, MW ~ 6,000) (1.0 mg/mL)

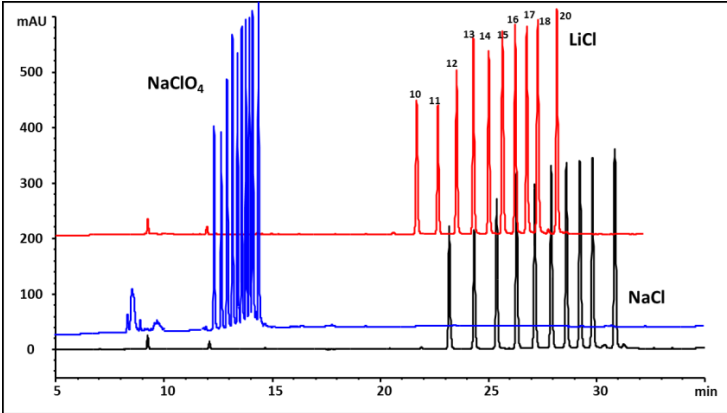




## Oligonucleotide Separation on SEC, IEX and RP Columns

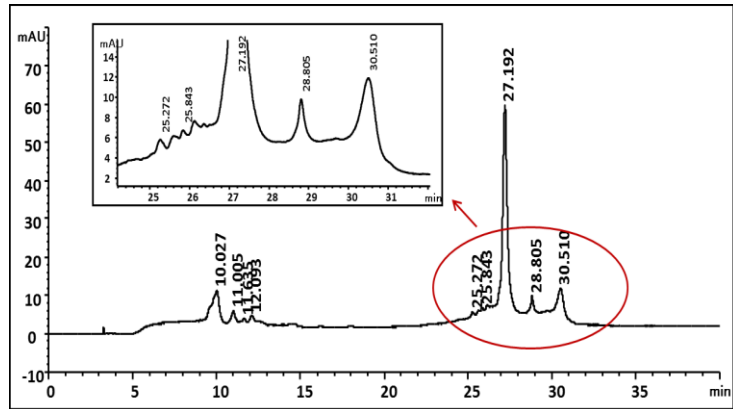
### Oligonucleotides Analysis on Proteomix SAX– salt effect

Column: Proteomix NP5 SAX ( 5 μm, 4.6 x 250 mm)  
 Mobile phase: A: 20 mM Tris, pH 8.0, B: A + 0.5 M NaCl/NaClO<sub>4</sub>/LiCl  
 Flow rate: 0.5 mL/min; Gradient: 0→100% B in 30 minutes;  
 Pressure: 73 bar; Detector: UV 260 nm; Column temperature: 25 °C; Samples:  
 Mixture of poly dA10-18, dA20, 10 μM each in water; Injection volume: 5 μL



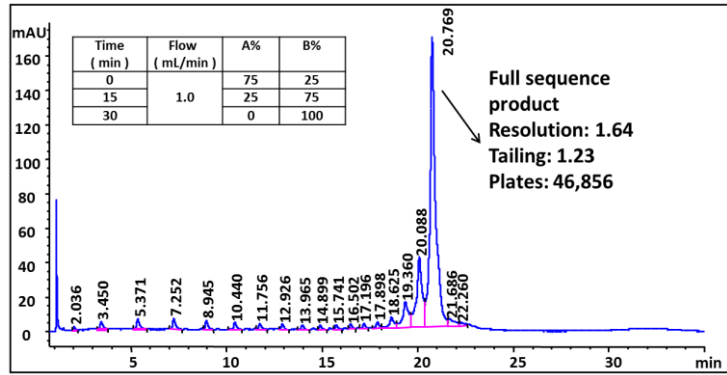
### ssDNA Analysis on Proteomix NP5 SAX ( 4625 )

Column: Proteomix NP5 SAX ( 5 μm, 4.6 x 250 mm)  
 Mobile phase: A: 20 mM Tris, pH 8.0 B: A + 1 M NaCl  
 Gradient: 0-100% B in 30 min; Flow rate: 0.5 mL/min;  
 Detector: UV 260 nm; Column temperature: 25 °C; Pressure: 93 bar;  
 Samples: ssDNA, diluted 100 times with water; Injection volume: 10 μL



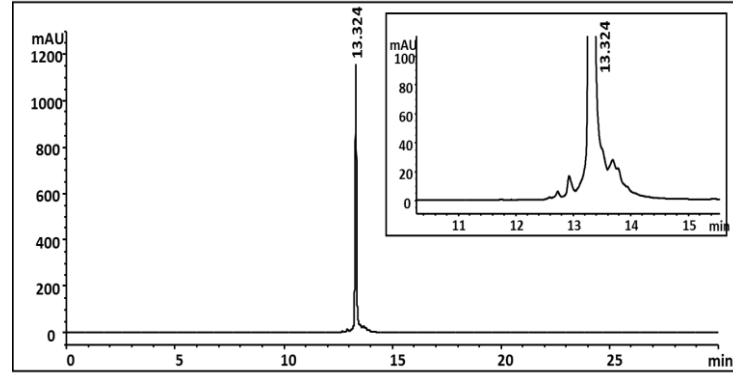
### Oligonucleotides Analysis on Proteomix NP5 SAX ( 4615 )

Column: Proteomix NP5 SAX ( 5 μm, 4.6 x 150 mm)  
 Mobile phase: A: 20 mM Tris, pH 8.0; B: A + 0.5 M NaCl  
 Flow rate: 1.0 mL/min;  
 Detector: UV 260 nm; Column temperature: 25 °C; Pressure: 81 bar;  
 Sample: 60 μg/mL Oligonucleotides in water (6,000 Da, at least one base difference, exact sequence unknown); Injection volume: 50 μL



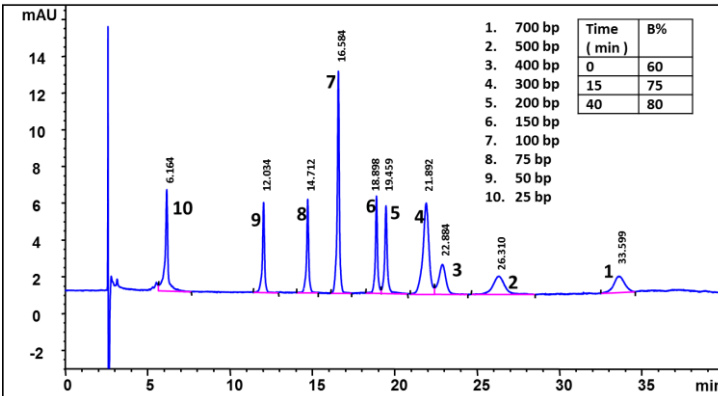
### siRNA Analysis on Proteomix NP5 SAX ( 4625 )

Column: Proteomix NP5 SAX ( 5 μm, 4.6 x 250 mm)  
 Mobile phase: A: 20 mM Tris, pH 8.0, B: A + 0.5 M NaClO<sub>4</sub>;  
 Gradient 3: 25-75% B in 30 min;  
 Flow rate: 0.5 mL/min; Detector: UV 260 nm, Injection volume: 2 μL;  
 Column temperature: 25 °C; Pressure: 61 bar;  
 Samples: 2 μL siRNA



### DNA Separation on Proteomix SAX

Column: Proteomix SAX-NP5 (5 μm, 4.6 x 250 mm)  
 Mobile phase: A: 20 mM Tris, pH 8.0, B: A + 1 M NaCl,  
 Flow rate: 0.5 mL/min, Detector: UV 260 nm, Column temperature: 25 °C,  
 Sample: DNA standard 0.5 mg/mL, Pressure: 135 bar



### Separation of Oligonucleotide

Column: Proteomix SAX-NP5 (5 μm, 4.6x150 mm)  
 Mobile Phase: A, 25mM Tris 1mM EDTA 10% ACN (8.0 pH); B, A+1.0M NaCl  
 Gradient: 0-75%B in 50min; Flow rate: 0.5mL/min; Detection: UV 280 nm;  
 Sample: Oligonucleotide (Mw 12196) and its degraded fragments

