APPLICATION NOTE

Purification of Oligonucleotides via Anion Exchange Chromatography

Abstract

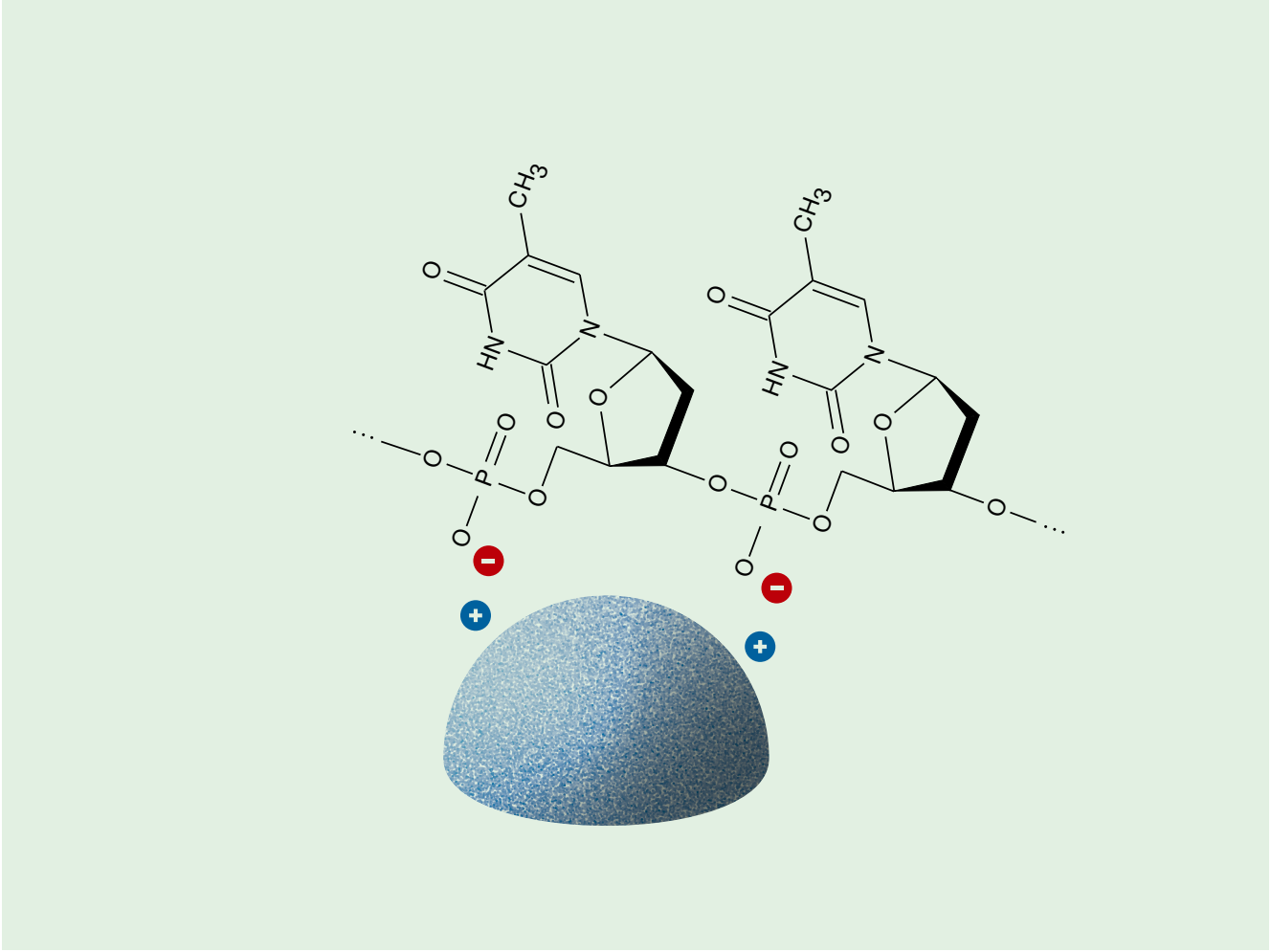
Oligonucleotides are highly important biopharmaceuticals with strongly increasing relevance. Due to their physico-chemical properties, oligonucleotides can be easily separated and purified using ion exchange chromatography (IEX). However, they often differ in both length and possible modifications. Therefore, IEX resins must meet certain requirements to successfully separate different types of oligonucleotides.

YMC's BioPro IEX SmartSep resins meet these requirements. They enable efficient oligonucleotide purifications – even single nucleotide differences can be separated. The different particle sizes of 10, 20 and 30 µm allow full flexibility and high resolution. This simplifies method development enormously. BioPro IEX SmartSep is the first choice for successful oligonucleotide purification.

Separation Principle

Oligonucleotides exhibit a strong negative charge due to their backbone structure. These high charge properties allow these molecules to be separated and purified by anion exchange chromatography (AEX).

The oligonucleotide molecules bind to the positively charged surface of the AEX resin and can subsequently be eluted by increasing the eluent salt concentration.



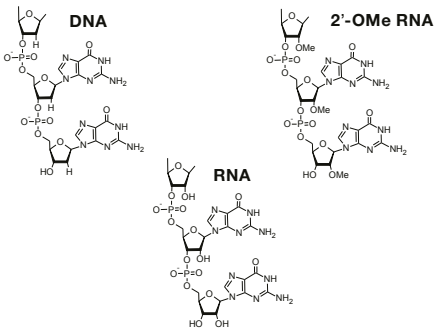
High Resolution Separation of Highly Similar Oligonucleotides with BioPro IEX

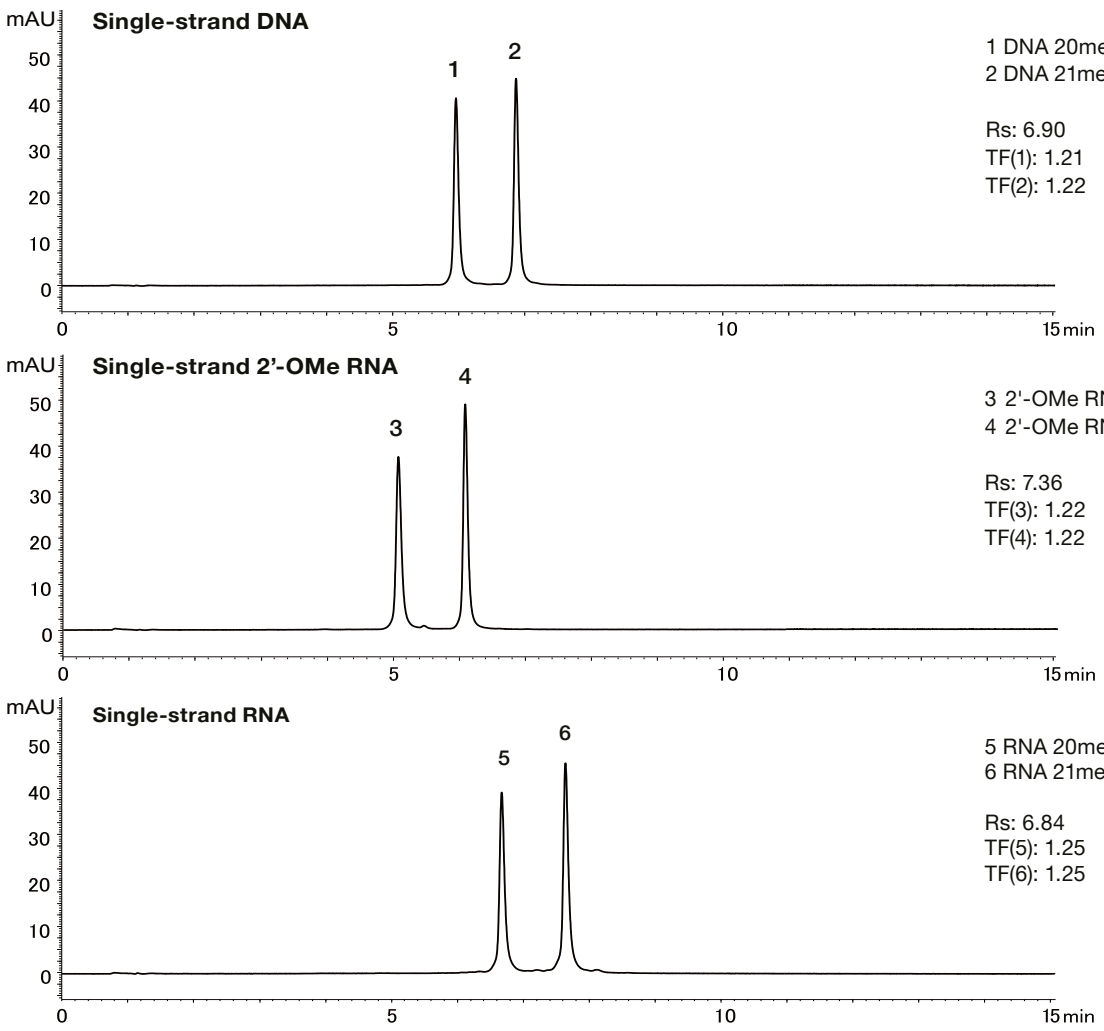
The separation of oligonucleotides of different length is one of the biggest challenges in purification. These shortmers (N-1) and longmers (N+1), which differ by one nucleotide unit, are typical impurities that must be removed within a purification process. BioPro IEX easily achieves the separation for different types of oligonucleotides, like DNA, RNA and 2'OMe-RNA with single nucleotide differences.

Samples

1	Single-strand DNA	5'-TCATCACACTGAATACCAAT-3' (DNA 20mer)
2		5'-GTCATCACACTGAATACCAAT-3' (DNA 21mer)
3	Single-strand RNA	5'-U(M)C(M)A(M)U(M)C(M)A(M)C(M)A(M)C(M)U(M)G(M)A(M)A(M)U(M)A(M)C(M)C(M)A(M)A(M)U(M)-3' (2'-OMe RNA 20mer)
4		5'-G(M)U(M)C(M)A(M)U(M)C(M)A(M)C(M)A(M)C(M)U(M)G(M)A(M)A(M)U(M)A(M)C(M)C(M)C(M)A(M)A(M)U(M)-3' (2'-OMe RNA 21mer)
5		s5'-UCAUCACACUGAAUACCAAU-3' (RNA 20mer)
6		5'-GUCAUCACACUGAAUACCAAU-3' (RNA 21mer)

N(M)=2'-OMe RNA





Column:	BioPro IEX QF (5 µm) 100 x 4.6 mm ID	Flow rate:	1.0 mL/min
Part No.:	QF00S05-1046WP	Temperature:	25 °C
Eluent:	A) 10 mM NaOH	Detection:	UV at 260 nm
	B) 10 mM NaOH containing 1.0 M $NaClO_4$	Injection:	4 µL (5 nmol/mL each)
Gradient:	25–55%B (0–15 min), 100%B (15–20 min)		

High Resolution Separation of PS and PO-Modified Oligonucleotides

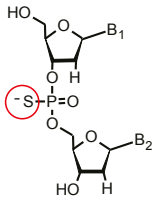
But it is not just the length of the oligonucleotides and the associated diverse modifications that pose a challenge. Another demanding task in oligonucleotide purification is the separation of thiolated oligonucleotides with a phosphorothioate bond (PS) from phosphodiester oligonucleotides (PO).

Thiolation is a common modification to increase the stability of the molecule and preparative separation from the phosphodiester species is extremely important. With BioPro IEX the separation of three oligos with different degrees of thiolation is possible.

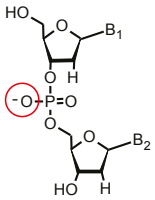
Samples

7	Single-stranded DNA	5'-T^T^T^T^T^T^T^T^T^T^T^T^TTT-3' (DNA 15mer 12PS, 2PO)
8		5'-T^T^T^T^T^T^T^T^T^T^T^T^TT-3' (DNA 15mer 13PS, 1PO)
9		5'-T^T^T^T^T^T^T^T^T^T^T^T^T-3' (DNA 15mer All PS)

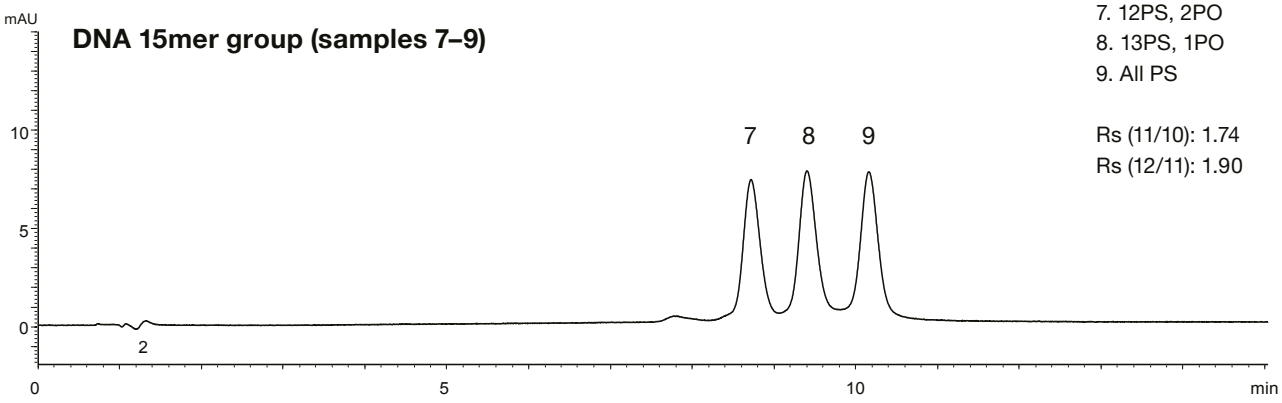
^=Phosphorothioated



Phosphorothioate Linkage (PS)



Phosphodiester Linkage (PO)

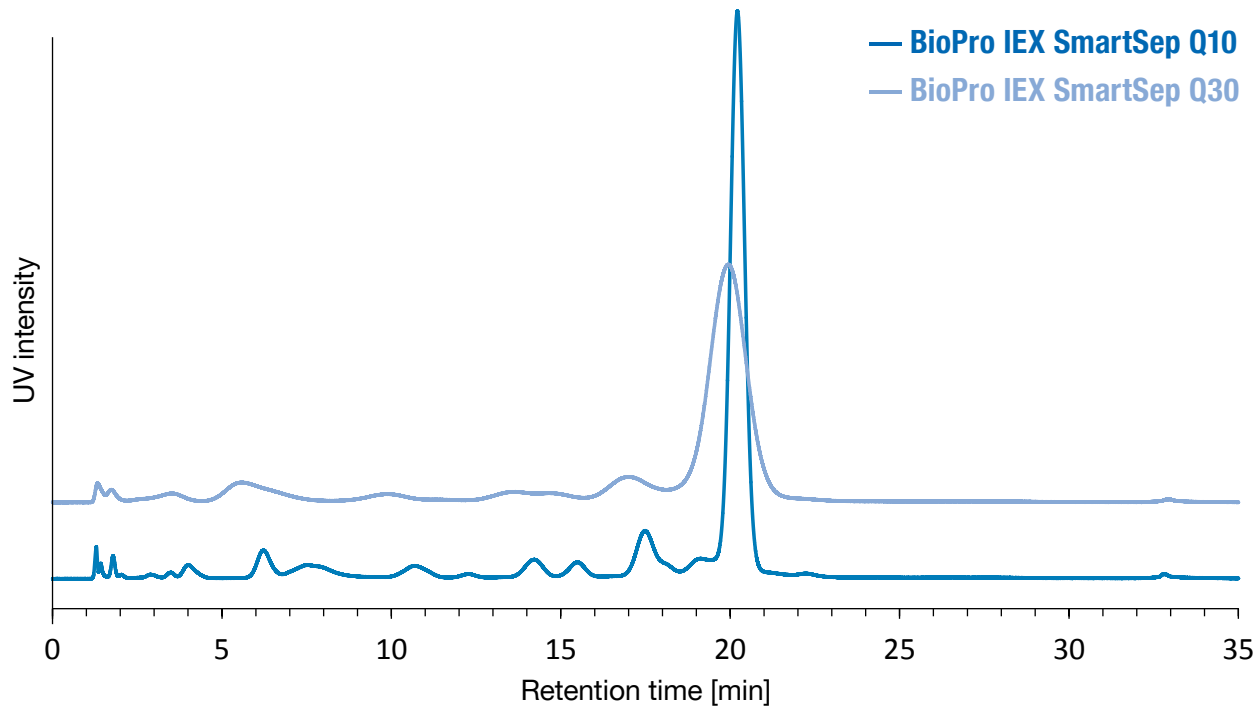


Column:	BioPro IEX QF 5 µm, 100 x 4.6 mm ID
Eluent:	A) 10 mM NaOH
	B) 10 mM NaOH containing 1.0 M NaClO4
Gradient:	40–70%B (0–15 min), 100%B (15–20 min)
Flow rate:	1.0 mL/min
Temperature:	25 °C
Detection:	UV at 260 nm
Injection:	6 µL (each 3.3 nmol/mL)

Different Particle Sizes for Efficient Oligonucleotide Purification

For the efficient purification of oligonucleotides, the optimal particle size of the IEX resin is a key factor. Smaller particles such as 10 µm provide results with higher resolution and therefore highly efficient separations. For larger scale purifications, larger resin beads such as 20 µm or even 30 µm are a better choice as they result in lower backpressures. With BioPro IEX SmartSep, the optimal bead size can be determined for every purification process. The different resins are fully scalable so analytical separation can easily be transferred to the larger particle sizes and vice versa, without changing the retention profile.

This is demonstrated by the example of purifying a 20mer DNA-oligo with two different BioPro IEX SmartSep resins (10 µm and 30 µm). The resolution differs for both bead sizes, but the retention profile is completely compatible. Therefore, the most suitable particle size can be easily selected by testing the different BioPro IEX SmartSep resins.



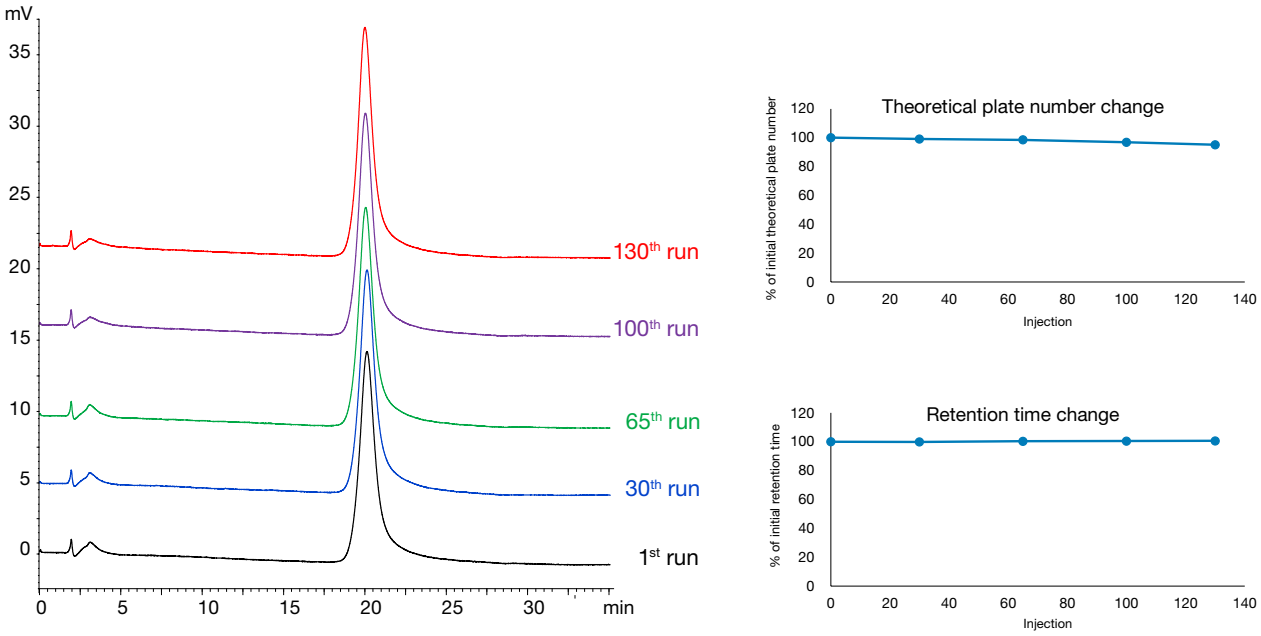
Column Dimension:	100 × 4.6 mm ID
Resin Part Nos.:	BioPro IEX SmartSep Q10 (10 µm): QSA0S10
	BioPro IEX SmartSep Q30 (30 µm): QSA0S30
Eluent:	A) 10 mM NaOH
	B) 10 mM NaOH + 1 M $NaClO_4$
Gradient:	25–40%B (0–30 min), 40–100%B (30–35 min)
Flow rate:	1 mL/min
Temperature:	25 °C
Detection:	UV at 260 nm
Injection:	5 µL
Sample:	Trityl-OFF 5'-ATA CCG ATT AAG CGA AGT TT-3' (DNA 20mer)

For purifications using smaller scale with high demands on resolution, 10 µm is the ideal particle size!
For purifications using larger process scale with lower pressure ratings, 20 µm or even 30 µm are the best option!

The Benefit of High Temperatures in Oligonucleotide Purification

Increasing temperature is a highly effective tool for optimising the purification of oligonucleotides. By increasing the temperature, for example from 25 to 60 °C narrower peaks and improved resolution can be obtained, especially for strong-binding oligonucleotides. This is only possible with resins that are long-term stable even at elevated temperatures. At 60 °C, the elution profile of an antisense oligonucleotide remains constant even after 130 runs.

BioPro IEX SmartSep Q20 provides reproducible retention time and column efficiency even at high temperatures.



Column:	BioPro IEX SmartSep Q20, 100 x 4.6 mm ID
Part No:	QSA0S20-1046PTC
Eluent:	20 mM Tris-HCl (pH 8.6)
	20 mM Tris-HCl (pH 8.6) containing 1 M NaCl
Gradient:	40–90%B (0–25 min), 90%B (25–35 min), 40%B (35.01–60 min)
Flow rate:	0.5 mL/min
Temperature:	60 °C
Detection:	UV at 260 nm
Injection:	12 µL
Sample:	Firefly Luciferase GL2, antisense strand (5 nmol/mL)
	5'-UCG AAG UAU UCC GCG UAC GdTdT-3

Read more about the temperature stability of BioPro IEX SmartSep:

Technical Note
High Temperature Stability of BioPro IEX SmartSep Resins

Conclusion

Purification of oligonucleotides requires high resolution separations that efficiently remove very similar impurities from the feed. Anion exchange chromatography is a well-established technique for meeting these high demands. YMC's BioPro IEX SmartSep resins are ideal for the highly productive purification of various types of oligonucleotides. The resins are available in different particle sizes for tailor-made solutions in method development. Small particle sizes, for example, enable very high-resolution purification of oligonucleotides. A great advantage is the high temperature stability of the BioPro resins, which often determines the success of the purification of oligonucleotides.

Specifications

AEX resins	BioPro IEX SmartSep Q10	BioPro IEX SmartSep Q20	BioPro IEX SmartSep Q30
Ion Exchange Type	strong anion exchanger		
Charged Group	-R-N-$(CH_3)_3$		
Matrix	hydrophilic polymer beads		
Pore Size	porous		
pH Range	2–12		
Particle Size	10 µm	20 µm	30 µm
Pressure Resistance	3 MPa max. 4 MPa	2 MPa max. 3 MPa	
Typical Flow Rate	200–1,000 cm/h max. 2,000 cm/h		
Ion Exchange Capacity	0.08 meq/mL resin		
Dynamic Binding Capacity	min. 100 mg/mL Resin (BSA)		
Temperature	4–60 °C		

Request your free sample here!