Oligonucleotide purification using anion exchange (AEX) and ion-pair reversed phase chromatography (IP-RP LC)

YMC

Oligonucleotides can be purified via anion exchange chromatography (AEX) and ion-pair reversed phase chromatography (IP-RP).
Depending on the impurity profile, both techniques have its specific strengths and advantages. The objectives for purifications in general are:

- **Efficient separation of very similar oligonucleotides**
- **High productive large-scale processes**
- **High resolution oligo purifications.**

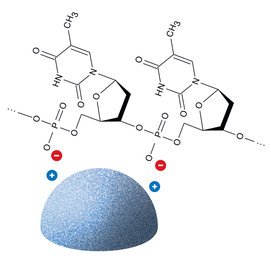
The main aspect is preparative purification regarding the following application focus:

- **Single nucleotide differences**
- **Separation of PS- and PO-oligos**
- **Method optimisation for large scale applications**

To select the ideal separation mode for the separation of oligos of interest, a comprehensive screening is the best approach.

AEX oligo purification

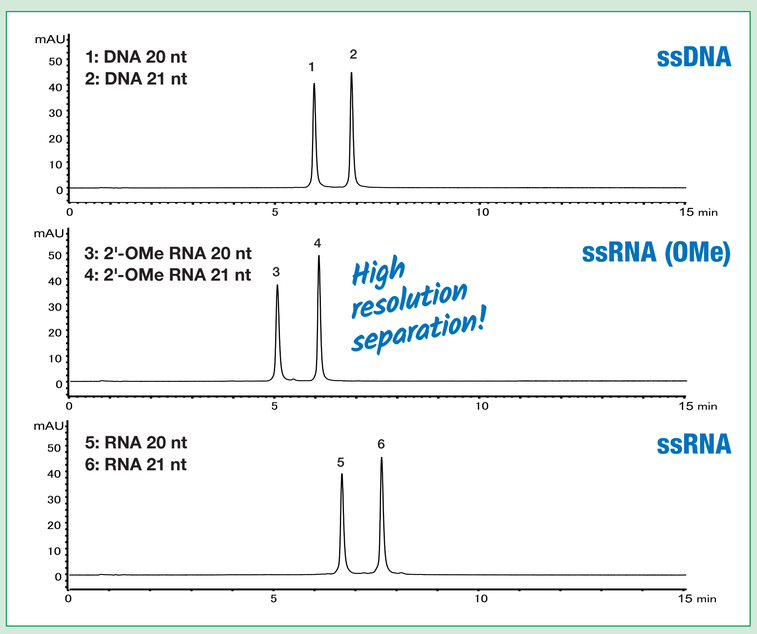
Anion Exchange Chromatography (AEX)



- **Charge-charge interaction between oligo backbone and AEX- resin**
- **Elution with increasing the ionic strength of eluent system**

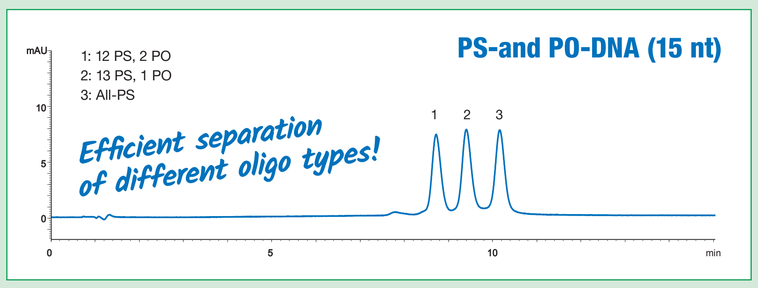
Separation of single nucleotide differences

Advantage: separation of single nucleotide differences are possible as shown in this example for DNA; RNA and OMe-RNA-oligos with 20 nt and 21 nt.



Separation of PS-and PO-oligos

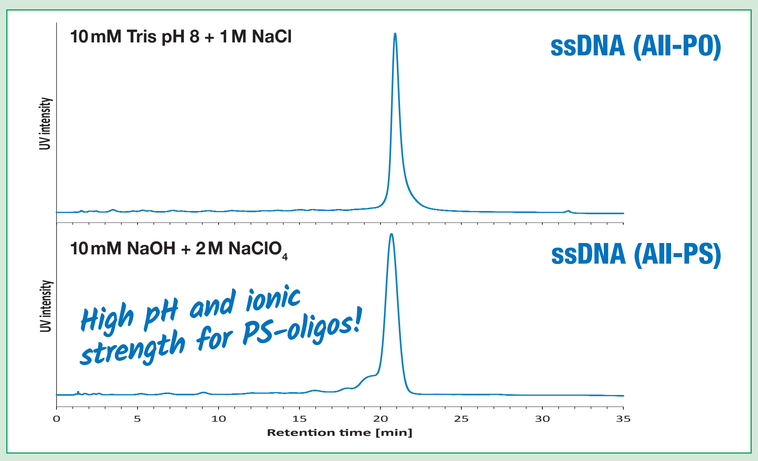
The separation of oligos with different degree of thiolation is an important issue of oligo purification. This example shows the separation of DNA 15 nt with different PO-/PS-linkages.



Efficient separation of various oligo types and lengths using AEX!

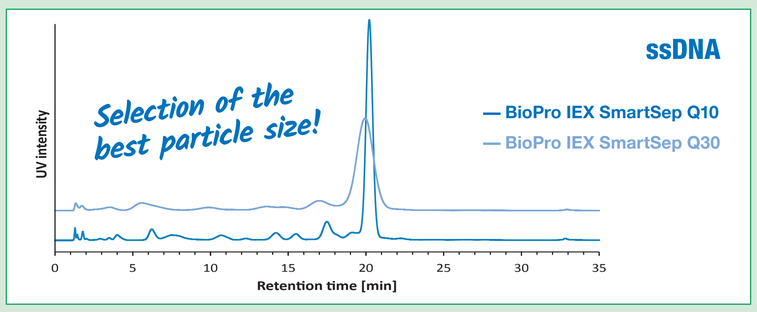
Optimised methods for PO-/PS-oligos

The purification of PS-oligos is highly challenging and may require high ionic strength as well as high pH-buffers for efficient elution. This example shows the optimised methods for PO- and PS-oligos purification via AEX.



Consideration for process scale

For efficient oligo purification, the selection of the most suitable particle size is important. The availability of small resin particle sizes allows high resolution, whereas larger particles provide lower backpressures. Therefore early method development should consider resins with several particle sizes available.



Process scale resins for oligo purification

Key Performance Indicators for process scale resins are

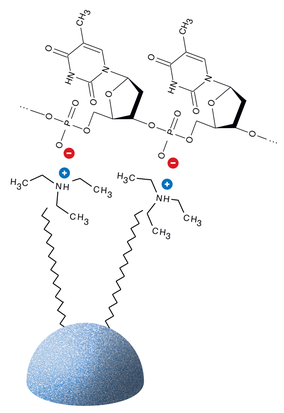
- Availability of different particle sizes
- High dynamic binding capacities
- Low backpressures

BioPro IEX resins are the perfect choice as modern process resin for highly efficient oligo purifications!

BioPro IEX Series	BioPro IEX SmartSep Q30	BioPro IEX SmartSep Q20	BioPro IEX SmartSep Q10
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	30 µm	20 µm	10 µm
Pressure resistance	2 MPa (Max. 3 MPa)		3 MPa (Max. 4 MPa)
Typical flow rate	200 – 1,000 cm / hr (Max. 2,000 cm / hr)		
Ion exchange capacity	0.08 meq/mL Resin		
Dynamic binding capacity	min. 100 mg/mL Resin (BSA)		

IP-RP oligo purification

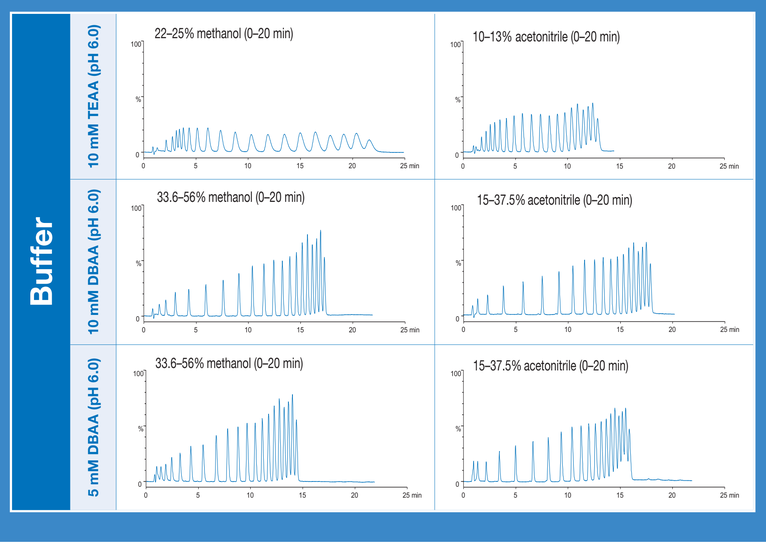
Ion-Pair Reversed-Phase Chromatography (IP-RP)



Hydrophobic RP phases and ion-pair containing eluents as mediator for the interaction between oligonucleotides and stationary phase.

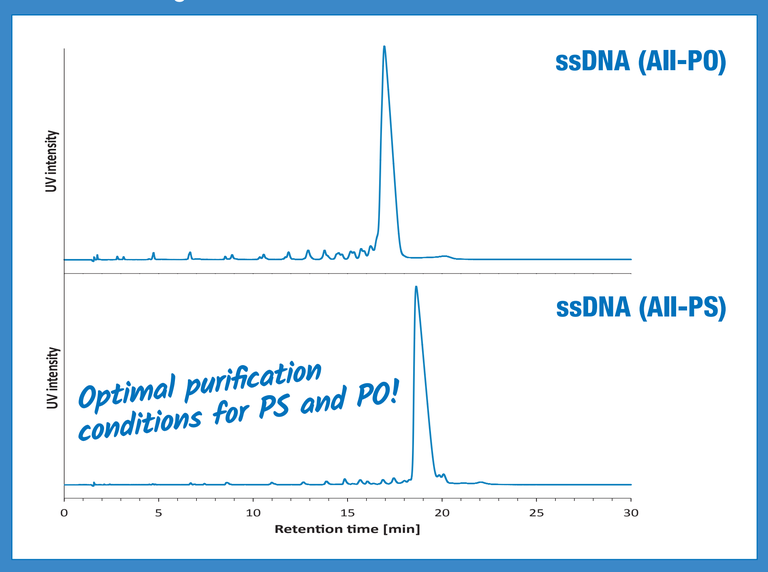
Optimised mobile phase conditions for high resolution

During IP-RP purifications, the buffer system as well as the concentration needs to be optimised. This example shows the screening of different mobile phase conditions for the high-resolution separation of an oligo sample with multiple lengths.



Purification of PO- and PS-variants

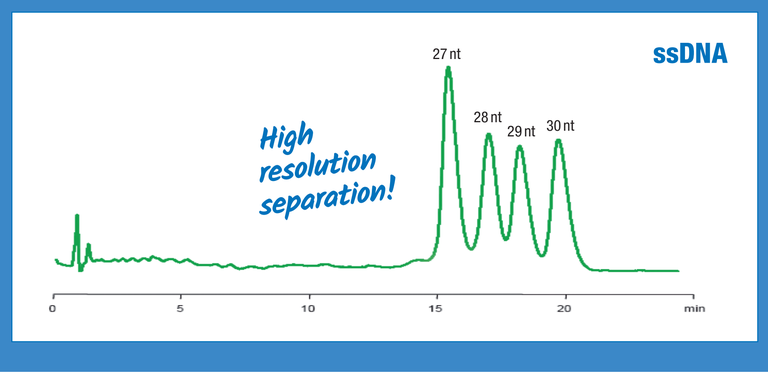
PO-oligos as well as PS-modified oligos can be purified via IP-RP with high resolution.



High resolution with IP-RP!

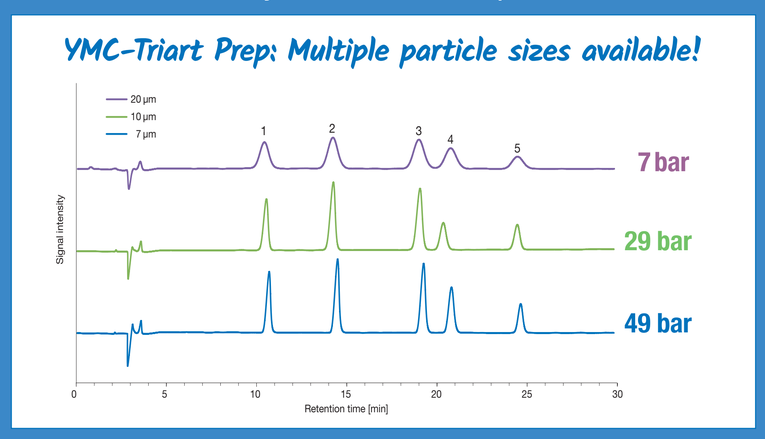
Separation of single nucleotide differences

IP-RP is very well suited for the separation of single nucleotide differences. The high resolution separation of DNA-molecules that differ only in length (27–30 nt) is easily accomplished. In this example YMC-Triart is employed.



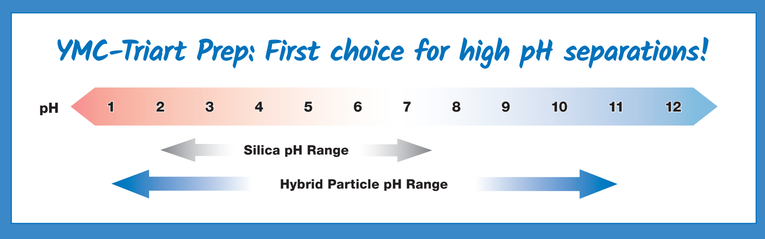
Optimal particle size for purification

Small particle sizes increase the resolution of oligo separations. The availability of RP phases with alternative particle sizes allows more flexibility in method development.



pH-stable phases for process scale

For oligo purifications, mobile phases with high pH can improve the elution of the target. Therefore pH-stable RP hybrid-silica resins as e. g. YMC-Triart Prep are an optimum choice.



	YMC-Triart Prep C18-S	YMC-Triart Prep C8-S	YMC-Triart Prep Bio200 C8	YMC-Triart Prep Phenyl-S
Base material	inorganic / organic hybrid silica			
Particle size [µm]	7, 10, 15, 20	10, 15, 20	10	10
Pore size [nm]	12	12	20	12
Specific surface area [m^2/g]	360	360	proprietary	360
Bonding	trifunctional C18	trifunctional C8	trifunctional C8	trifunctional Phenyl
End-capping	yes	yes	yes	yes
Flexible pH range	2.0 ~ 10.0	2.0 ~ 10.0	2.0 ~ 10.0	2.0 ~ 10.0
Column cleaning	common procedures up to pH 12	common procedures up to pH 12	common procedures up to pH 12	common procedures up to pH 12

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