



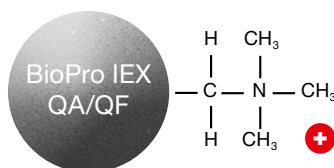
AEX



AEX – HPLC Selectivities

Features

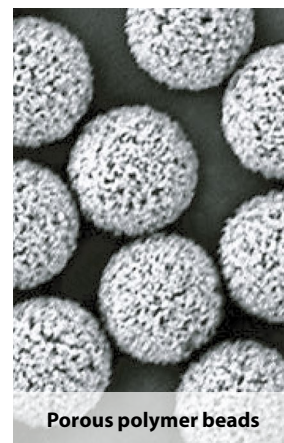
- Porous or non-porous hydrophilic polymers
- High recovery of oligonucleotides
- Very high resolution
- Low nonspecific adsorption
- Excellent reproducibility



strong anion
exchanger

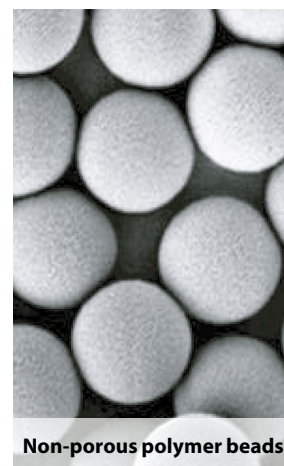
	BioPro IEX QA
Matrix	hydrophilic polymer (polymethacrylate)
Particle size / μm	5
Pore size / nm	100
Charged group	$-\text{CH}_2\text{N}^+(\text{CH}_3)_3$
Counter ion	Cl^-
Available pH range	2.0–12.0
Temperature range	4–60°C
Pressure limit	2.5–3.5 MPa (360–510 psi)
Column hardware	PEEK

Also available in 10, 20, 30 or 75 μm for preparative scale



Porous polymer beads

	BioPro IEX QF
Matrix	hydrophilic polymer (polymethacrylate)
Particle size / μm	3, 5
Pore size / nm	non-porous
Charged group	$-\text{CH}_2\text{N}^+(\text{CH}_3)_3$
Counter ion	Cl^-
Available pH range	2.0–12.0
Temperature range	4–60°C
Pressure limit	3 μm : 25 MPa (3,625 psi) 5 μm : 6–12 MPa (870–1,740 psi)
Column hardware	PEEK



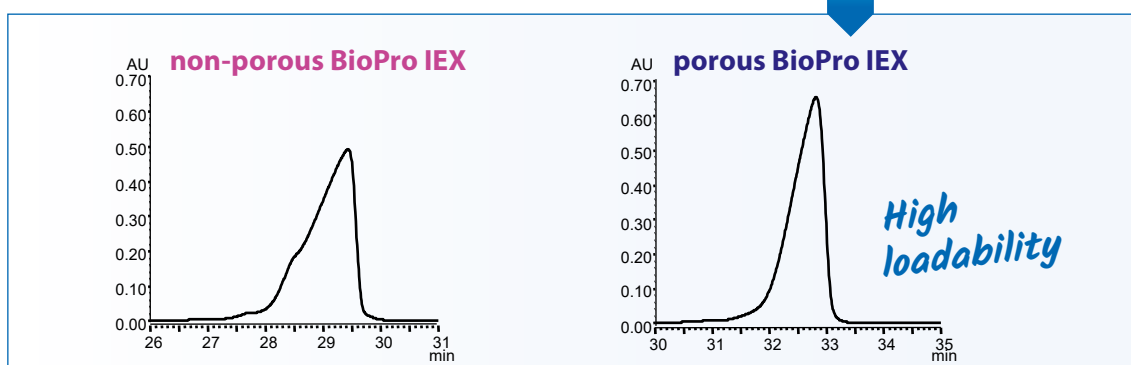
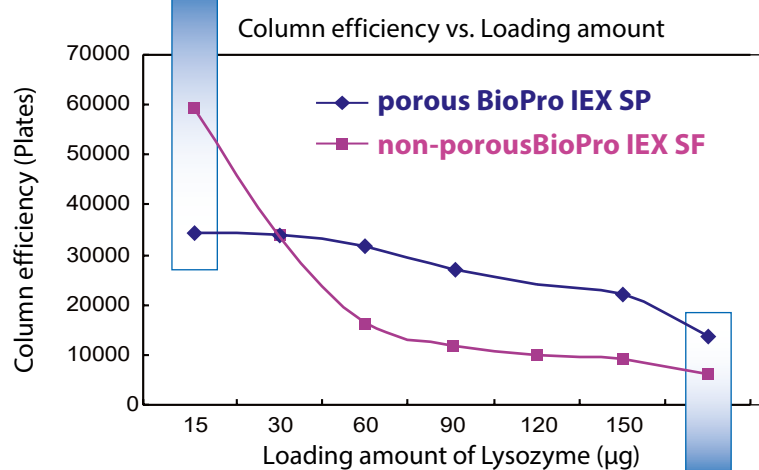
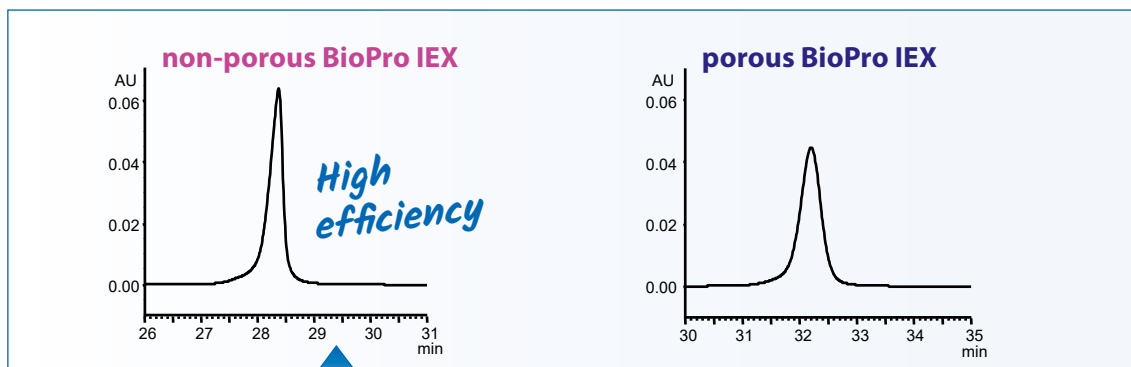
Non-porous polymer beads

YMC's anion exchanger (AEX) columns of the BioPro IEX series are available with strong exchanger modification, based on 5 μm porous (QA columns) and on 3 or 5 μm non-porous (QF columns) hydrophilic polymer beads.

The porous materials offer excellent binding capacity with exceptionally high efficiency and low operating pressure, whilst the non-porous particles offer high efficiency, very high resolution and low operating pressures.

Column efficiency and loadability

When to use porous and non-porous BioPro IEX



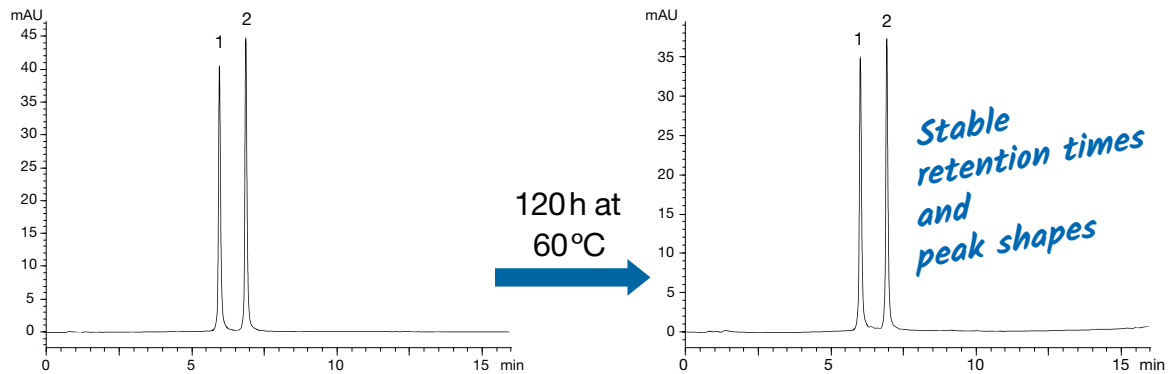
Column: BioPro IEX SF/SP
 Eluent: A) 20 mM NaH_2PO_4 - Na_2HPO_4 (pH 6.8)
 B) 20 mM NaH_2PO_4 - Na_2HPO_4 (pH 6.8) containing 0.5 M NaCl
 Gradient: 0–100%B (0–60 min)
 Flow rate: 0.5 mL/min
 Temperature: 25°C

Detection: UV at 280 nm
 Injection: 100 μL
 Sample: 1. Ribonuclease A
 2. Cytochrome c
 3. Lysozyme

Non-porous BioPro IEX columns offer outstanding column efficiency for small sample loading amounts. These columns are especially suitable for microscale analysis, which requires higher resolution. Porous BioPro IEX columns maintain good peak shape even when the loading amount increases. These high-capacity columns are useful for high-load analytical separations and laboratory-scale purification.

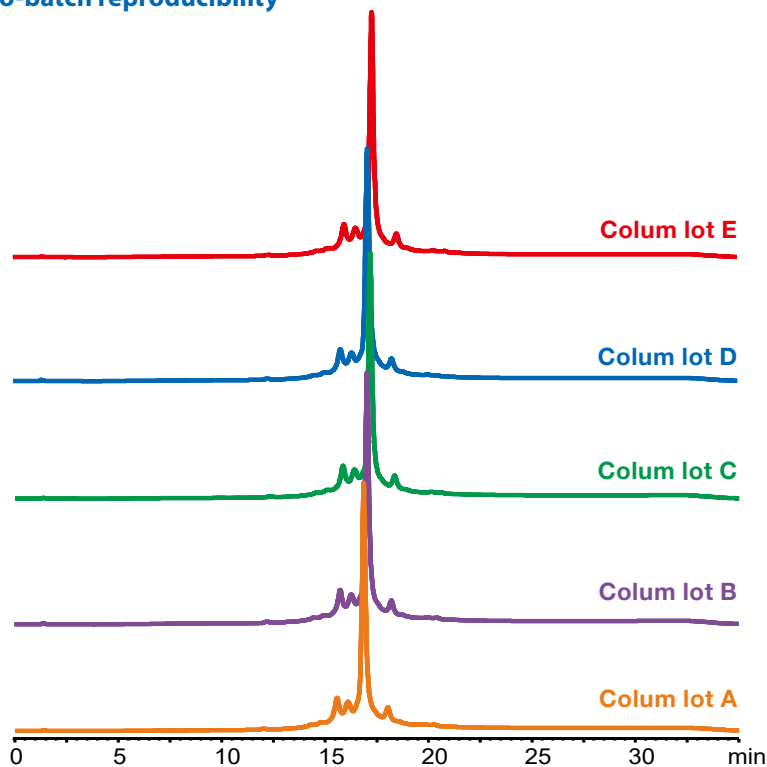
AEX – Stability and Reproducibility

High temperature stability of BioPro IEX columns



Column:	BioPro IEX QF (5 μ m) 100 x 4.6 mm ID	Temperature:	25 $^{\circ}$ C
Part No.:	QF00S05-1046WP	Detection:	UV at 260 nm
Eluent:	A) 10 mM NaOH	Injection:	4 μ l (each 5 nmol/ml)
	B) 10 mM NaOH containing 1.0 M NaClO ₄	Sample:	1) 5'-TCATCACA...GAATACCAAT-3' (DNA 20mer)
Gradient:	25–55%B (0–15 min), 100%B (15–20 min)		2) 5'-GTCATCACA...GAATACCAAT-3' (DNA 21mer)
Flow rate:	1.0 ml/min		

Excellent batch-to-batch reproducibility



Column:	BioPro IEX SF (5 μ m) 100 x 4.6 mm ID	Flow rate:	0.5 mL/min (180cm/hr)
Part No.:	SF00S05-1046WP	Temperature:	25 $^{\circ}$ C
Eluent:	A) 20 mM NaH ₂ PO ₄ -Na ₂ HPO ₄ (pH 6.5)	Detection:	UV at 215 nm
	B) 20 mM NaH ₂ PO ₄ -Na ₂ HPO ₄ (pH 6.5) containing 0.2 M NaCl	Injection:	10 μ L
Gradient:	0–50%B (0.5–30min)	Sample:	monoclonal antibody (IgG1)

BioPro IEX columns exhibit excellent batch-to-batch reproducibility. All gel batches are inspected by rigorous quality control tests, and must meet the required criteria before release. BioPro IEX columns are the best choice for the quality control of biopharmaceuticals such as oligonucleotides or mAbs as in this example.

Optimisation of oligonucleotide separations on ion exchange chromatography

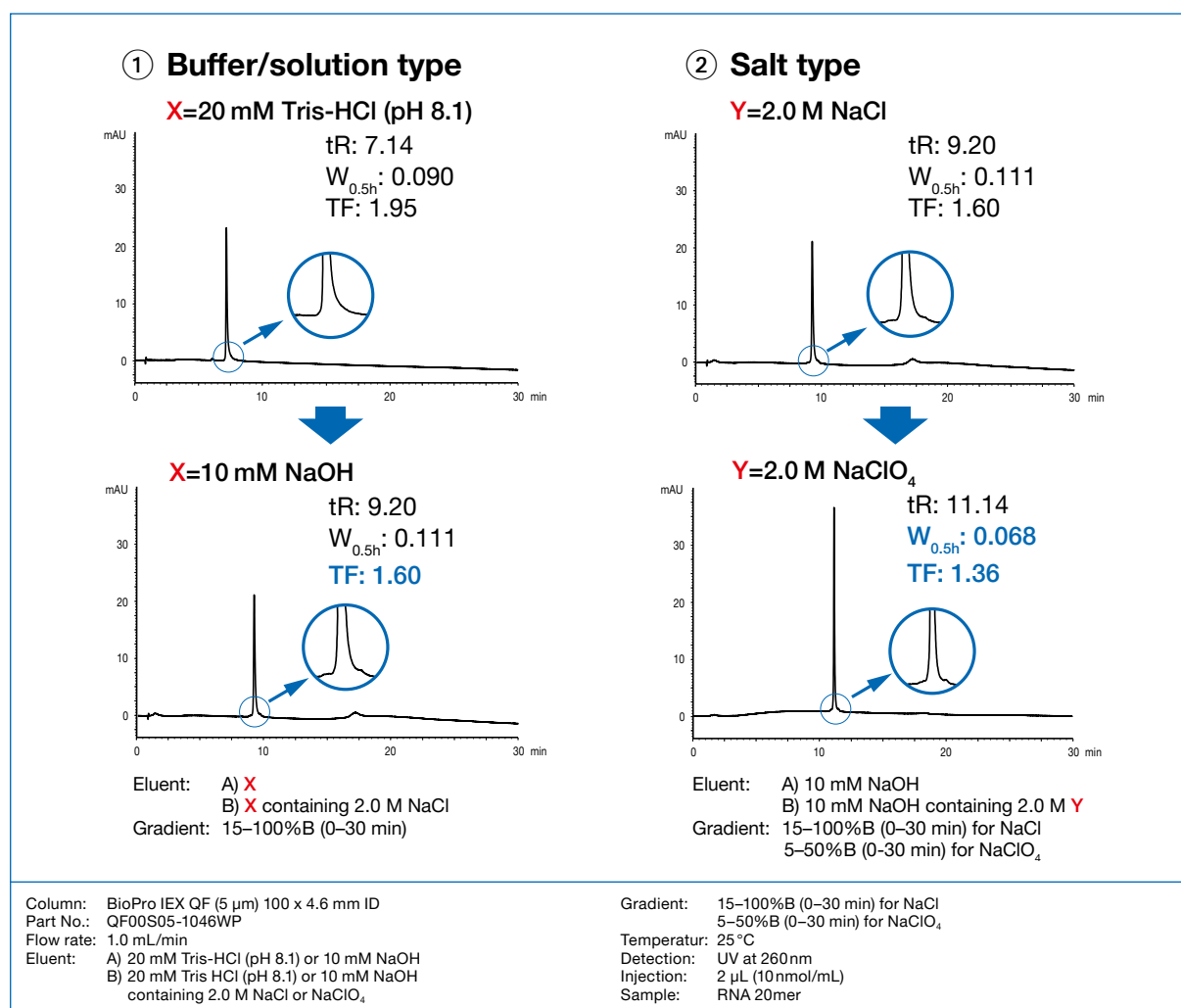
A non-porous anion exchange column is generally suitable for analysis of oligonucleotides. For optimisation of single-stranded DNA and RNA of about 20mer, some conditions, such as type of mobile phase and column temperature, can be changed.

1 Improvement of peak tailing

Sample Group 1 (Phosphodiester oligonucleotides; PO)

Single-stranded RNA (ssRNA) 5'-UCAUCACACUGAAUACCAAU-3' (RNA 20mer)

By changing the buffer from 20 mM Tris-HCl (pH 8.1) to 10 mM NaOH, the tailing factor for an oligonucleotide is reduced. Furthermore, the peak tailing is further suppressed when NaClO₄ was added to 10 mM NaOH instead of NaCl.



2 Improvement of carry-over

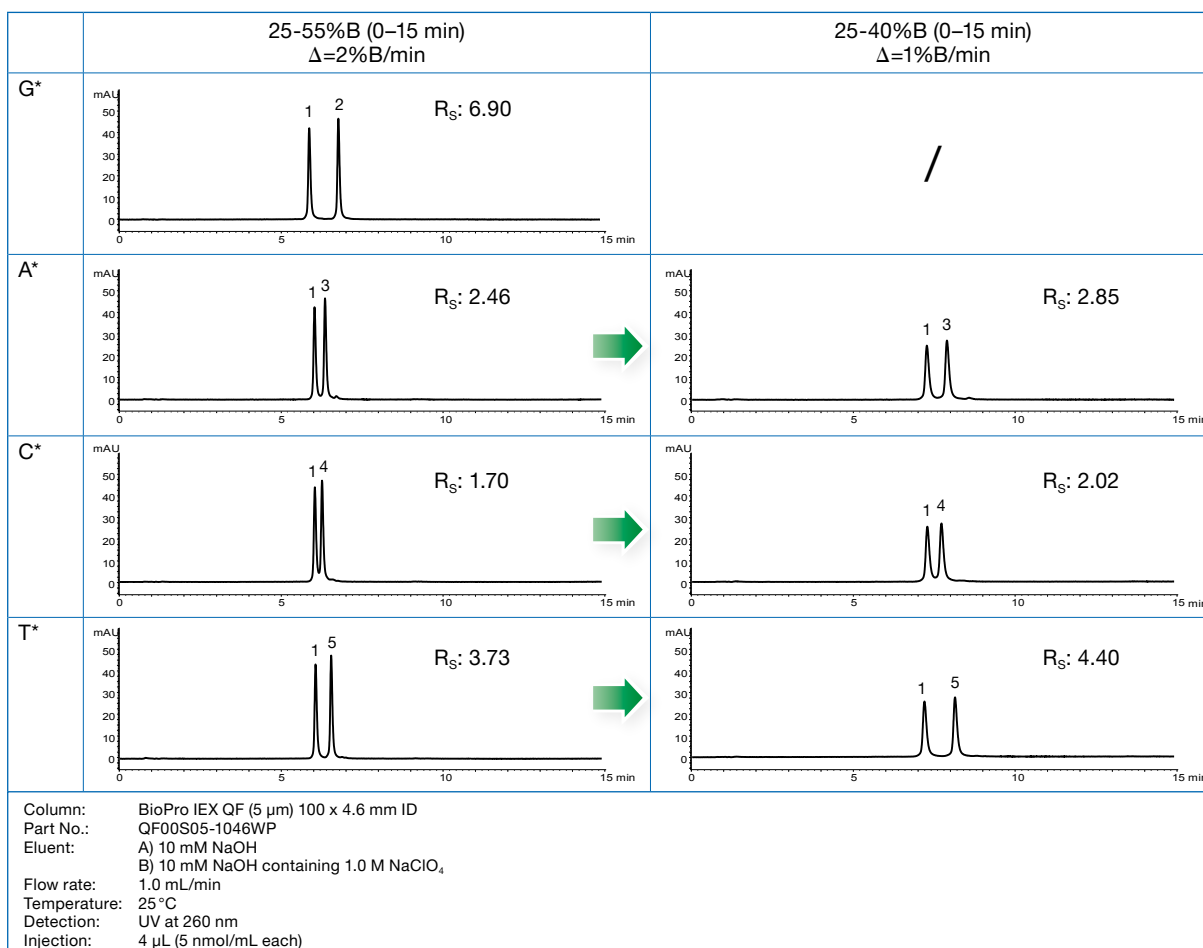
When the initial gradient concentration of NaCl is low (ex. 50 mM), carry-over is observed. By increasing the initial gradient concentration of NaCl up to 400 mM, carry-over can be avoided with good reproducibility.

AEX – Expert Tips: Oligonucleotides

3 Improvement of ssDNA separation with single-base differences (differing in the type of base at the 5' end of DNA 21mer)

When ssDNAs with single-base differences are analysed, the degree of separation varies depending on the type of base at the 5' end. If the separation is difficult, it can be improved by making the gradient steeper.

1	Single-stranded DNA	5'-TCATCACACTGAATACCAAT-3' (DNA 20mer)
2		5'-GTCATCACACTGAATACCAAT-3' (DNA 21mer)
3		5'-ATCATCACACTGAATACCAAT-3' (DNA 21mer)
4		5'-CTCATCACACTGAATACCAAT-3' (DNA 21mer)
5		5'-TTCATCACACTGAATACCAAT-3' (DNA 21mer)



*base of 5' end of DNA 21mer

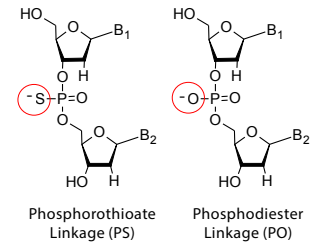
4 Improvement of the separation of phosphorothioate oligonucleotides with single-base differences in length

Since acidity of all PS is much higher than all PO, a higher salt concentration is required for elution. The peak of all PS is much broader because it is thought that all PS contains as many as 219 (524,288) stereoisomers. A steeper gradient curve, increasing column temperature and adding organic solvent can improve peak separation.

Sample Group 2 (Phosphorothioate oligonucleotides; PS)

1	Single-stranded RNA	5'-U~C~A~U~C~A~C~A~C~U~G~A~A~U~A~C~A~A~U-3' (RNA 20mer All PS)
2	RNA	5'-G~U~C~A~U~C~A~C~A~C~U~G~A~A~U~A~C~A~A~U-3' (RNA 21mer All PS)

~ = Phosphorothioated



X/Y=100/0
32–80%B (0–24 min)
= Δ20 mM NaClO₄/min
25°C



Step gradient curve
X/Y=100/0
32–80%B (0–8 min)
= Δ60 mM NaClO₄/min
25°C



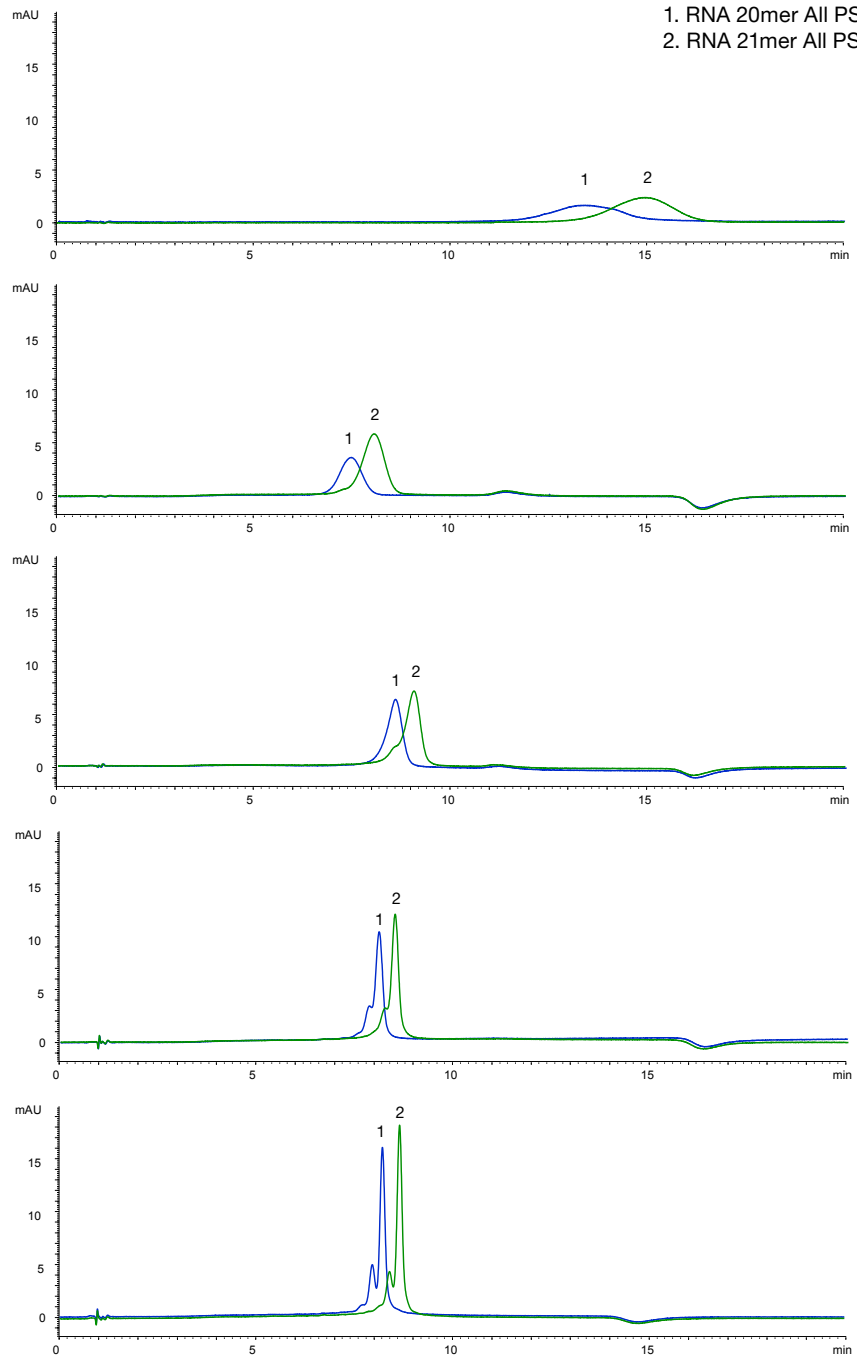
Raising column temperature
X/Y=100/0
32–80%B (0–8 min)
= Δ60 mM NaClO₄/min
60°C



Addition of organic solvent
X/Y=80/20
40–100%B (0–8 min)
= Δ60 mM NaClO₄/min
60°C



Increasing ratio of organic solvent
X/Y=70/30
40–100%B (0–6.3 min)
= Δ60 mM NaClO₄/min
60°C

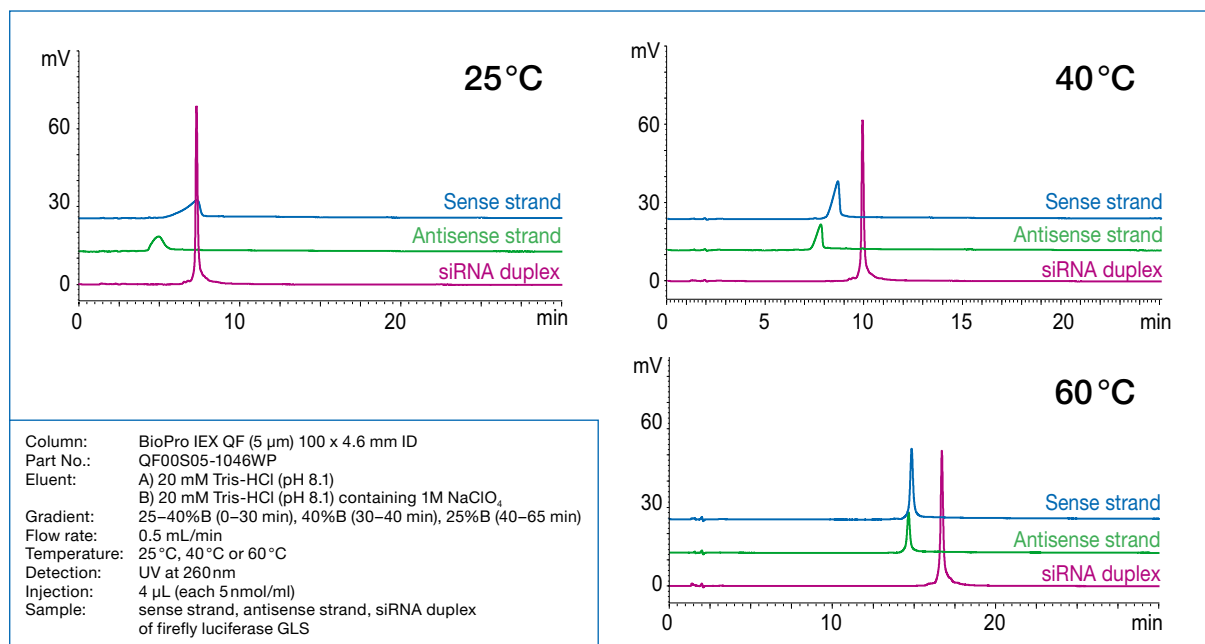


Column: BioPro IEX QF (5 μm) 100 x 4.6 mm ID
Part No.: QF00S05-1046WP
Eluent: A) 10 mM NaOH/methanol (X/Y)
B) 10 mM NaOH containing 1.0M NaClO₄/methanol (X/Y)

Flow rate: 1.0 mL/min
Detection: UV at 260 nm
Injection: 2 μL (10 nmol/mL)

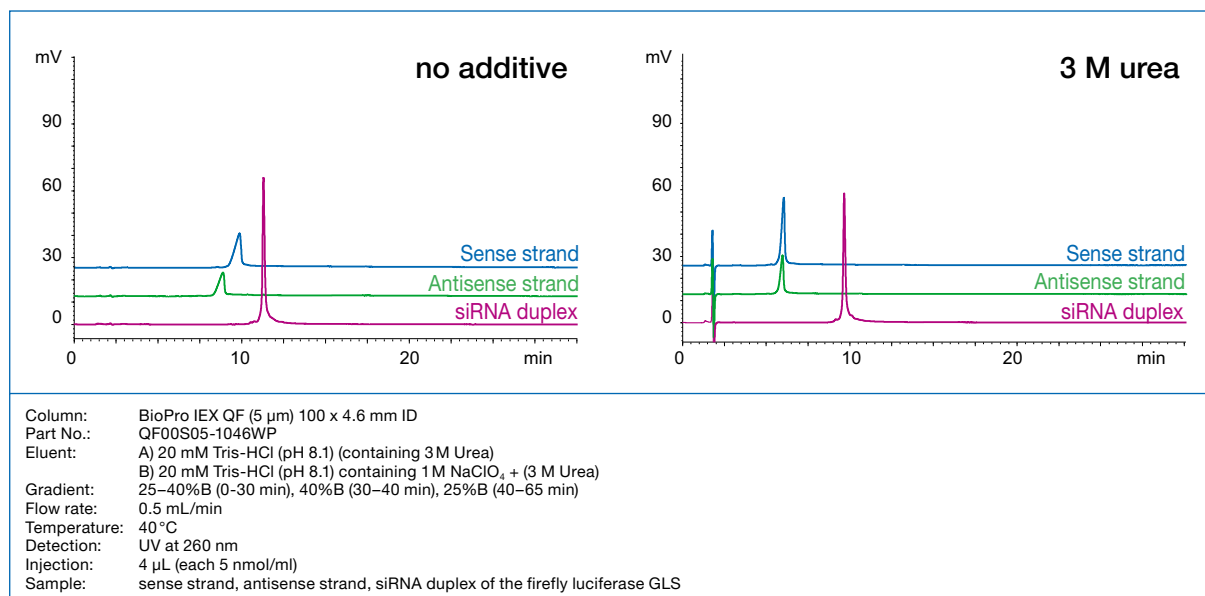
AEX – Expert Tips: Oligonucleotides

Influence of temperature on the analysis of a non-denaturated siRNA



A higher temperature tended to show improved peak shape. Slightly better peak shapes of the ssRNAs were observed at 40°C, while the dsRNA showed comparable and relatively good peak shape regardless of the temperature. An even higher temperature of 60°C provides better peak shape of the sense and antisense strands. However, peak height of the siRNA duplex decreases due to partial denaturation. It is considered that the higher order structure of ssRNAs is denatured when increasing temperature. The ssRNAs as well as dsRNA retain longer on the stationary phase, as the ion exchange group can access the phosphate groups more easily.

Influence of urea as additive on the analysis of a non-denaturated siRNA



Addition of 3 M urea to the mobile phase results in better peak shapes of both sense and antisense strands as well as the siRNA duplex. The retention time is reduced for all three analytes and an improvement in resolution of the single strands and the double stranded siRNA is also observed.