YMC

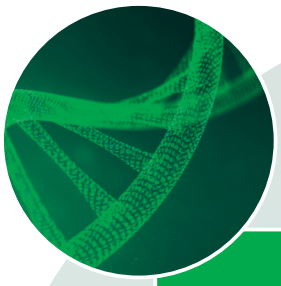
YMC Oligonucleotide Columns

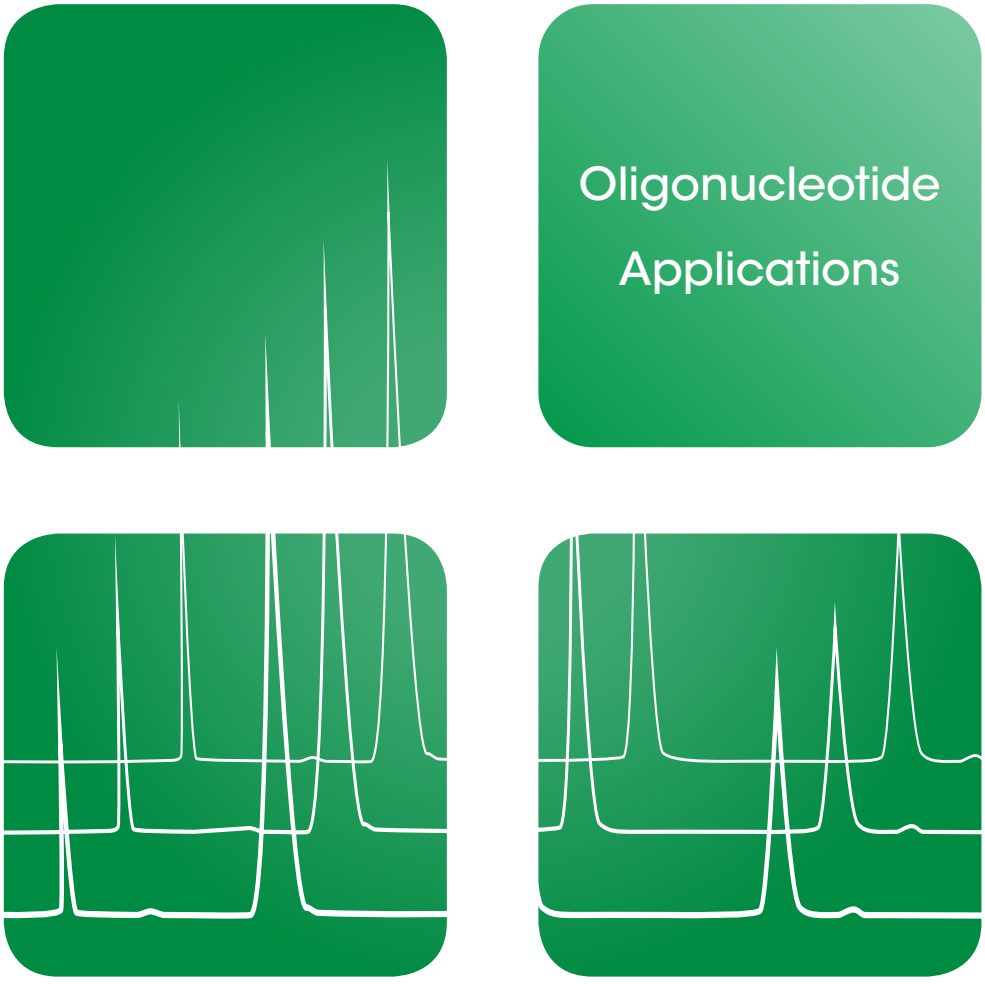
IP-RP

AEX

LC/MS

Content/Phase selection guide




Oligonucleotide
Applications

Oligonucleotide applications

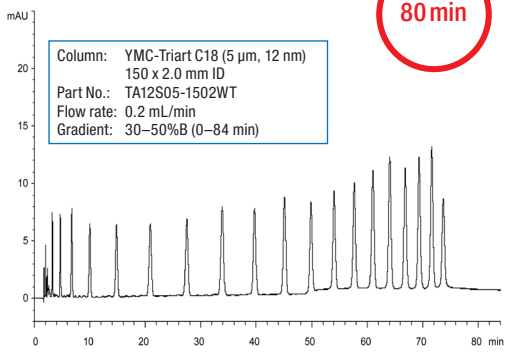
Oligonucleotides d(T)2-20 method transfer from HPLC to UHPLC

RP

Conventional LC method

80 min

Column: YMC-Triart C18 (5 µm, 12 nm) 150 x 2.0 mm ID
Part No.: TA12S05-1502WT
Flow rate: 0.2 mL/min
Gradient: 30–50%B (0–84 min)

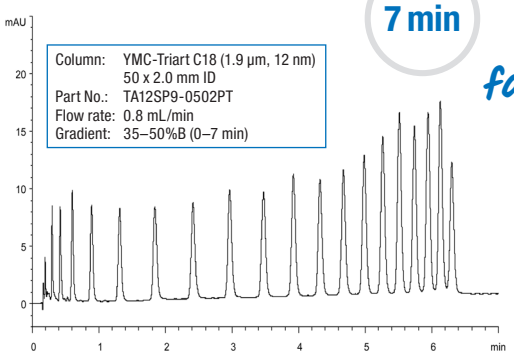


UHPLC method

7 min

11x faster

Column: YMC-Triart C18 (1.9 µm, 12 nm) 50 x 2.0 mm ID
Part No.: TA12SP9-0502PT
Flow rate: 0.8 mL/min
Gradient: 35–50%B (0–7 min)

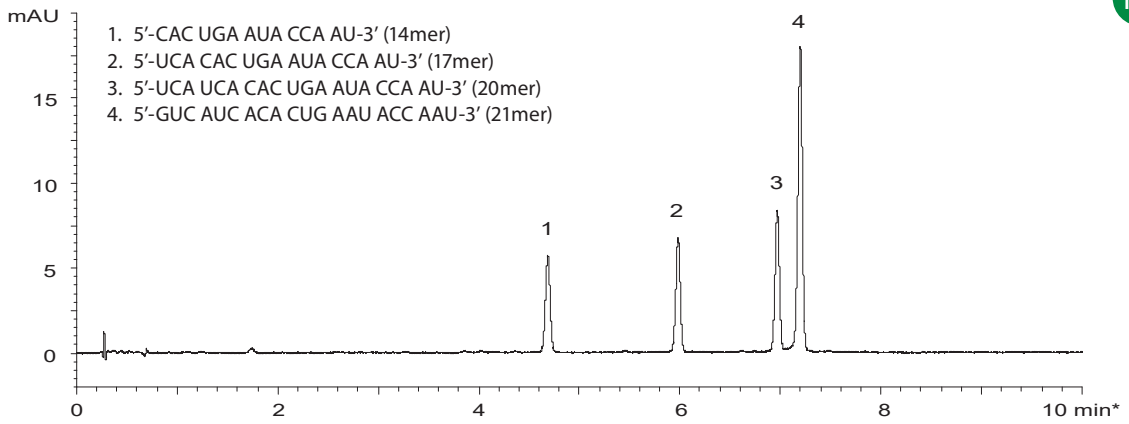


Eluent: A) 10 mM di-n-butylamine-acetic acid (pH 6.0)
B) methanol
Temperature: 37°C

Detection: UV at 269 nm
Injection: 1 µL (5 nmol/mL)

Synthetic oligonucleotides

RP



Column: YMC-Triart C18 (1.9 µm, 12 nm) 50 x 2.1 mm ID
Part No.: TA12SP9-05Q1PT
Eluent: A) 200 mM HFIP*-8 mM triethylamine
B) methanol
Gradient: 10–20%B (0–10 min)

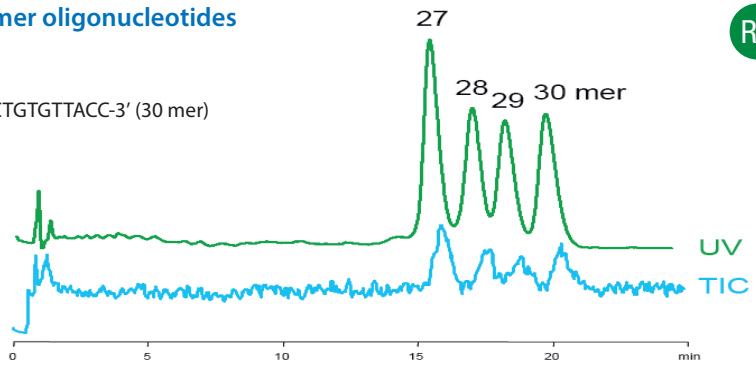
Flow rate: 0.42 mL/min
Temperature: 65°C
Detection: UV at 260 nm
Injection: 1 µL (2–4 nmol/mL)
Courtesy of M. Yamada, SHIMADZU CORPORATION (JP)

*hexafluoroisopropanol

LC-MS analysis of synthetic 27-30 mer oligonucleotides

RP

Sample: Primer of DNA sequencing
5′-CCGCTCGAGCTAAAAAAAGCCTGTGTTACC-3′ (30 mer)

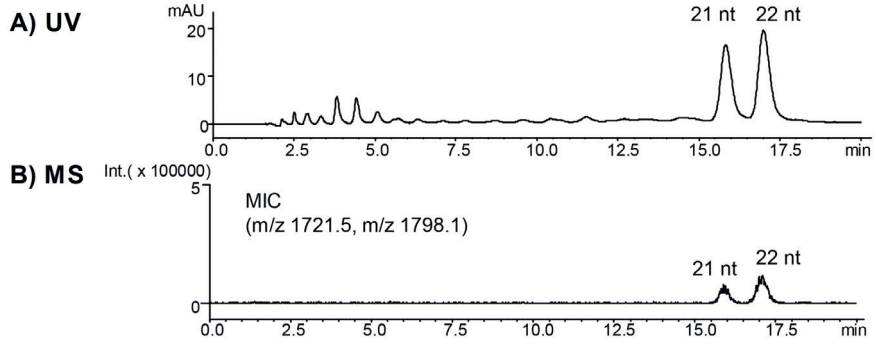


Column: Hydrosphere C18 (3 µm) 50 x 2.0 mm ID
Part No.: HS12S03-0502WT
Eluent: A) 10 mM DBAA (pH 6.0)
B) Mobile phase A / acetonitrile (50/50)
Gradient: 58%–62%B (0–20 min), 62%B (20–25 min)

Flow rate: 0.2 mL/min
Temperature: 35°C
Detection: UV at 269 nm and ESI negative-mode
Injection: 1 µL (10 pmol/component)

LC/MS analysis of miRNA

RP



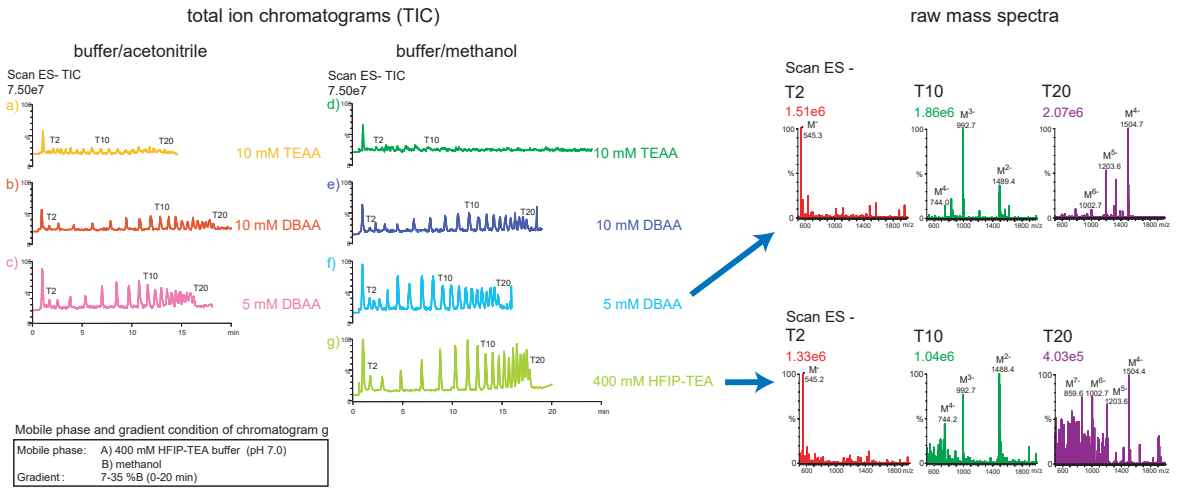
Courtesy of M. Yamada, SHIMADZU CORPORATION

5′-pUGG AGU GUG ACA AUG GUG UUG-3′	(21 nt, MW 6,890.1)
5′-pUGG AGU GUG ACA AUG GUG UUG U-3′	(22 nt, MW 7,196.3)

Column:	YMC-Triart C18 (3 µm, 12 nm) 150 x 2.0 mm ID	Temperature:	30°C
Part No.:	TA12S03-1502WT	Detection:	A) UV at 260 nm
Eluent:	A) 10 mM di-n-butylamine-acetic acid (pH 7.5)		B) ESI-negative mode
	B) 10 mM di-n-butylamine-acetic acid (pH 7.5)/acetonitrile (50/50)	Injection:	4 µL (5 nmol/mL)
Gradient:	62–72%B (0–20 min)	System:	LC) Shimadzu Prominence
Flow rate:	0.2 mL/min		MS) Shimadzu LCMS2020

Influences of mobile phase conditions on intensity of ESI-MS

RP



Mobile phase and gradient condition of chromatogram g

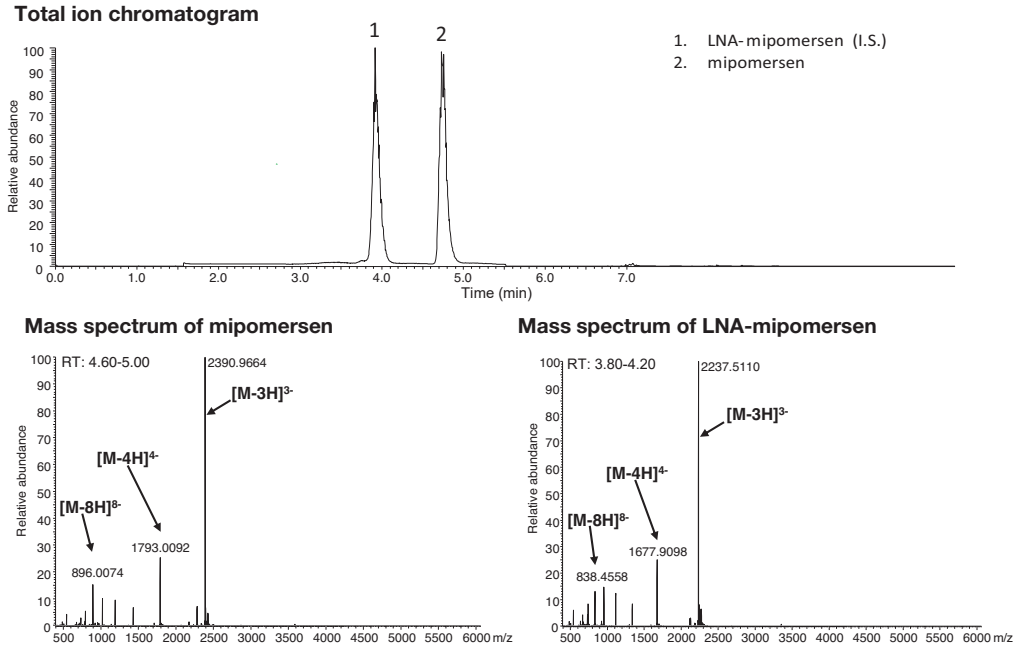
Mobile phase:	A) 400 mM HFIP-TEA buffer (pH 7.0)
	B) methanol
Gradient:	7-35 %B (0-20 min)

Column:	Hydrosphere C18 (3 µm) 50 x 2.0 mm	d)	Eluent: A) 10 mM TEAA buffer (pH 6.0)
Part No.:	HS12S03-0502WT		B) eluent A/methanol (50/50)
Flow rate:	0.2 mL/min		Gradient: 44–50%B (0–25 min)
Temperature:	35°C	e/f)	Eluent: A) 10/5 mM DBAA buffer (pH 6.0)
Detection:	ESI negative mode		B) eluent A/methanol (20/80)
Injection:	5 µL (25 pmol/component)		Gradient: 42–70%B (0–20 min)
a)	Eluent: A) 10 mM TEAA buffer (pH 6.0)	g)	Eluent: A) 400 mM HFIP-TEA buffer (pH 7.0)
	B) eluent A/acetonitrile (80/20)		B) methanol
	Gradient: 50–65%B (0–20 min)		Gradient: 7–35%B (0–20 min)
b/c)	Eluent: A) 10/5 mM DBAA buffer (pH 6.0)	Sample:	Oligodeoxythymidylic acid [d(pT)$_{2\text{-}20}$]
	B) eluent A/acetonitrile (50/50)		
	Gradient: 30–75%B (0–20 min)		

Oligonucleotide applications

LC-HRMS analysis of the antisense oligonucleotide Mipomersen (Kynamro®)

RP



Column:	YMC-Triart C8 **metal-free** (1.9 µm, 12 nm)*1 100 x 2.1 mm ID
Part No.:	TO12SP9-10Q1PTP
Eluent:	A) water/triethylamine/HFIP*2 (100/0.4/2; triethylamine 28.0 mM, HFIP 135.8 mM)
	B) methanol/triethylamine/HFIP (100/0.4/2)
Gradient:	[Sample separation step]
	10–40%B (0–5.0 min)
	[Column wash steps]
	40–70%B (5.0–5.1 min), 70%B (5.1–7.0 min), 70–10%B (7.0–7.1 min), 10%B (7.1–8.0 min), 10–90%B (8.0–8.1 min), 90%B (8.1–9.0 min), 90–10%B (9.0–9.1 min), 10%B (9.1–10.0 min), 10–90%B (10.0–10.1 min), 90 %B (10.1–11.0 min), 90–10 %B (11.0–11.1 min)
Flow rate:	0.3 mL/min
Temperature:	50°C
Injection:	10 µL (1000 ng/mL)
System :	LC) Vanquish Binary Pump H system
	HRMS) Orbitrap HRMS Q Exactive Plus

*1 Prewash the column prior to the first use with water/methanol/phosphoric acid (70/30/0.1) for 1 hour
*2 1,1,1,3,3,3-hexafluoro-2-propanol

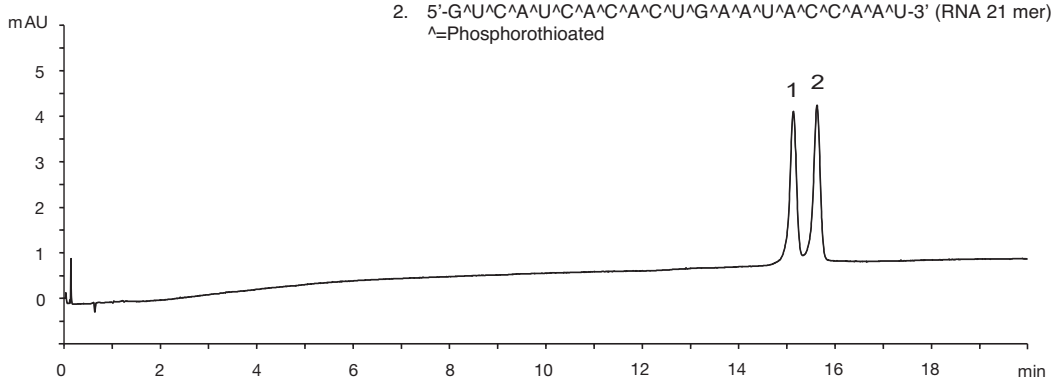
Reference: Y. Sun et al, Development of a bioanalytical method for an antisense therapeutic using high-resolution mass spectrometry, Bioanalysis, 2020 NOV 26, doi: 10.4155/bio-2020-0225.

Challenging phosphorothioate oligonucleotides

RP

1. 5'-U^C^A^U^C^A^C^A^C^U^G^A^A^U^A^C^C^A^A^U-3' (RNA 20 mer)
2. 5'-G^U^C^A^U^C^A^C^A^C^U^G^A^A^U^A^C^C^A^A^U-3' (RNA 21 mer)

^=Phosphorothioated

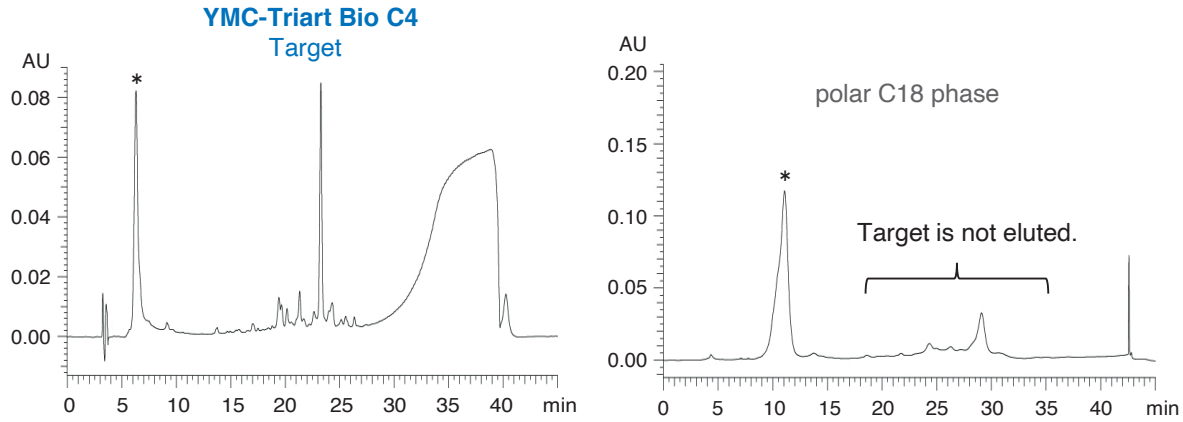


Column:	YMC-Triart C8 metal-free (1.9 µm, 12 nm) 100 x 2.1 mm ID
Part No.:	TO12SP9-10Q1PTP
Eluent:	A) 15 mM triethylamine-400 mM HFIP*
	B) methanol
Gradient:	10–20%B (0–20 min)
Flow rate:	0.42 mL/min
Temperature:	70°C
Detection:	UV at 260 nm
Injection:	1 µL (each 1.25 nmol/mL)

*1,1,1,3,3,3-hexafluoro-2-propanol

Separation of oligonucleotides modified with disulfides

RP



*phosphorothioate oligonucleotides without disulfide-unit

Column: 5 µm, 250 x 4.6 mm ID
Product code: TB30S05-2546PTH
HS12S05-2546WT
Eluent: A) 50 mM TEAA* (pH 7.0)/acetonitrile (95/5)
B) acetonitrile
Gradient: 5–95%B (0-30 min), 95%B (30–35 min), 95–5%B (35–35.1 min), 5%B (35.1–45 min)

Flow rate: 1 mL/min
Temperature: 50°C
Detection: UV at 260 nm
Sample: Crude reaction mixture
By courtesy of Saki Kawaguchi, Chemistry Department, Nagoya University, Japan

*Triethylammonium acetate

Easy purification of oligonucleotides with YMC-Actus semi prep columns

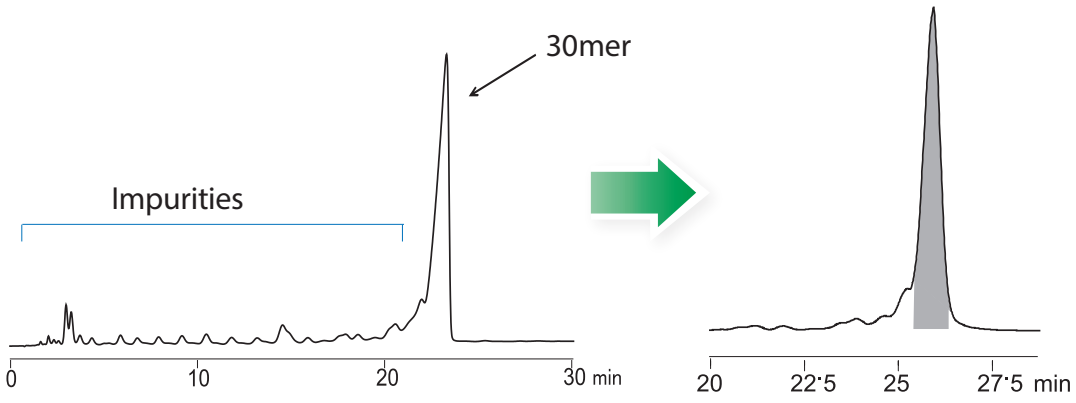
RP

Purification of synthetic 30mer oligonucleotide

Analysis 1.0 mL/min, 5 µL injection
Hydrosphere C18
50 x 4.6 mm ID, 5 µm

Purification 19 mL/min, 100 µL injection
YMC-Actus Hydrosphere C18
50 x 20 mm ID, 5 µm

Recovery 56%



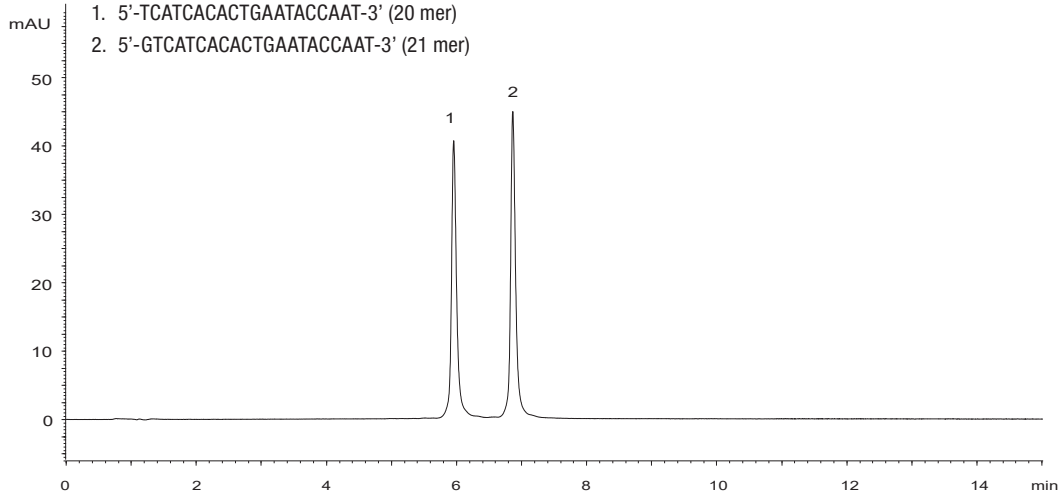
Part Nos.: HS12S05-0546WT
HS12S05-0520WX
Eluent: A) 10 mM DBA-acetic acid (pH 6.0) / methanol (60/40)
B) 10 mM DBA-acetic acid (pH 6.0) / methanol (20/80)
Gradient: 10%–35%B (0–30 min.)
Temperature: ambient
Detection: UV at 269 nm
Sample: synthetic oligonucleotide (100 µM)

purity>99%

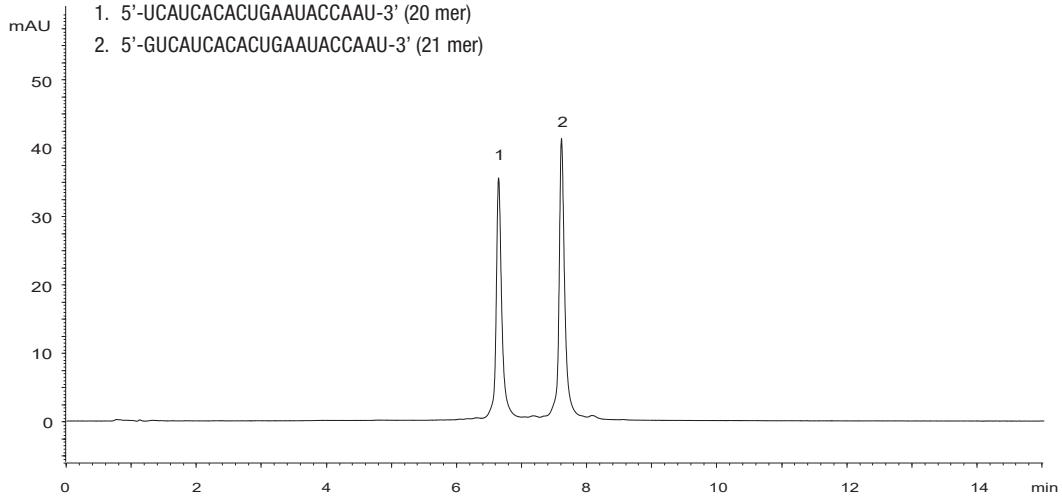
Oligonucleotide applications

Separation of synthetic oligonucleotides (single-strand DNA)

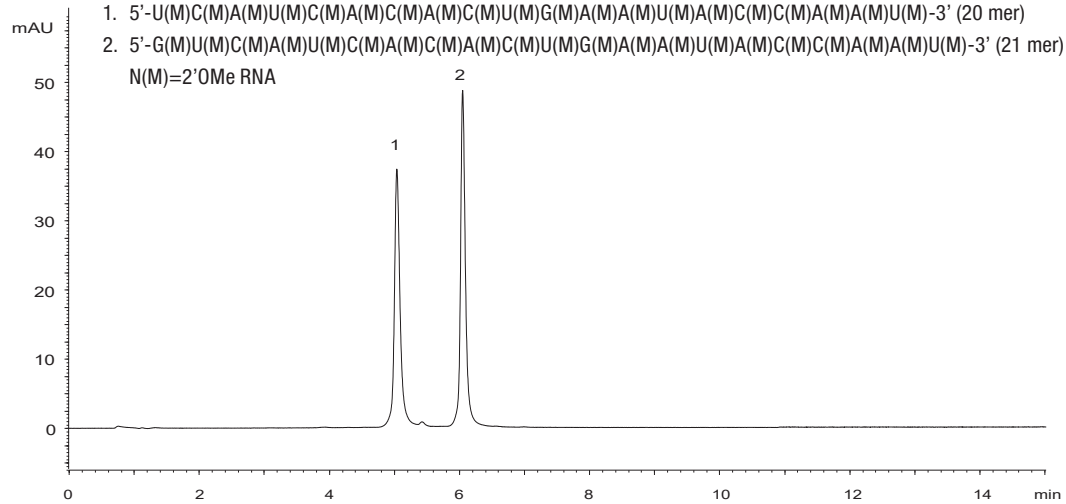
AEX



Separation of synthetic oligonucleotides (single-strand RNA)

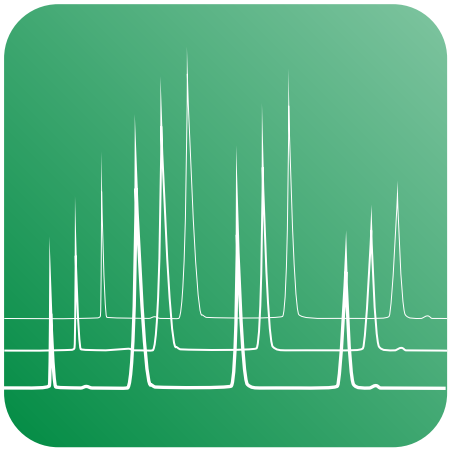


Separation of synthetic 2'OMe oligonucleotides (single-strand RNA)



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/L)
Gradient:	25–55%B (0–15 min), 100%B (15–20 min)		

*1,1,1,3,3,3-hexafluoro-2-propanol



IP-RP

IP-RP – UHPLC/HPLC Selectivities

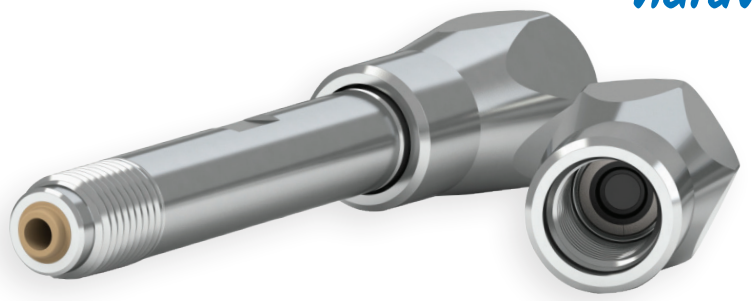
Features

- **Selection of C18, C8 and C4 columns**
- **For ion-pairing (IP-RP) UHPLC and HPLC**
- **pH- and temperature stable phases**
- **Superior reproducibility**
- **Optional bioinert hardware**

Selectivities for oligonucleotides

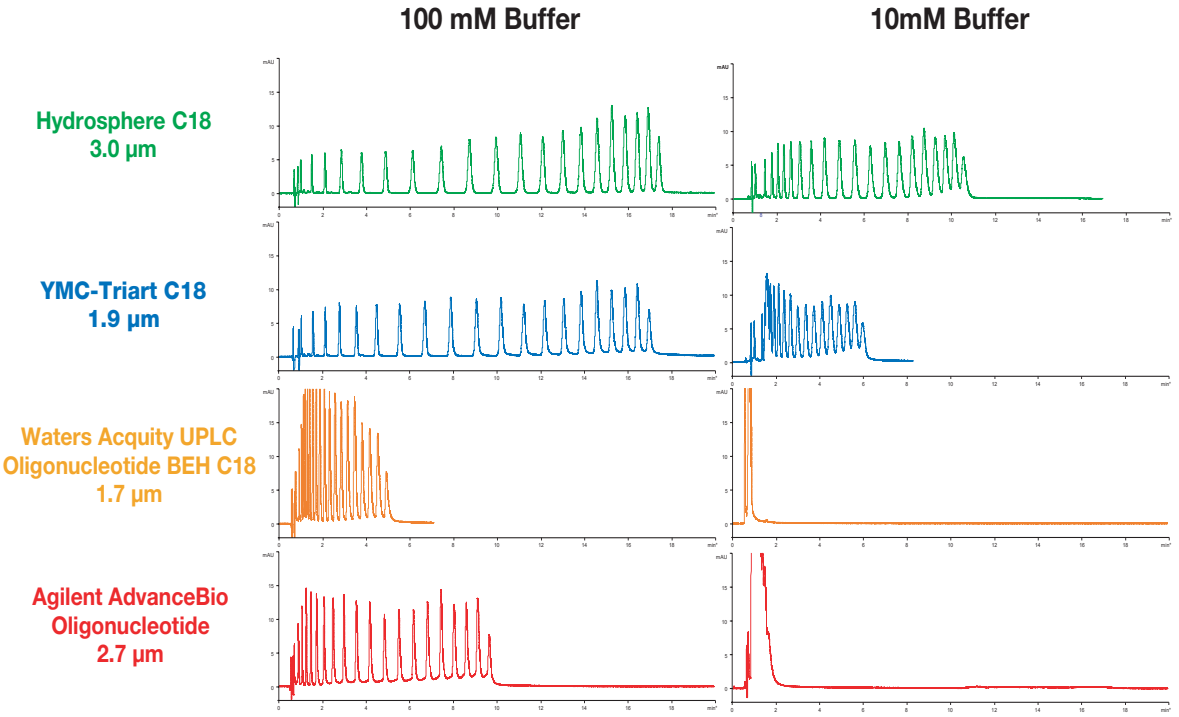
	YMC-Triart C18	YMC-Triart Bio C18	Hydrosphere C18	YMC-Triart C8	YMC-Triart Bio C4
Modification	C18 (USP L1)	C18 (USP L1)	C18 (USP L1)	C8 (USP L7)	C4 (USP L26)
Particle Size / µm	1.9, 3, 5	1.9, 3, 5	2, 3, 5	1.9, 3, 5	1.9, 3, 5
Pore Size / nm	12	30	12	12	30
pH range	1.0–12.0	1.0–12.0	2.0–8.0	1.0–12.0	1.0–10.0
Temperature range	pH < 7: 90°C pH > 7: 50°C	pH < 7: 90°C pH > 7: 50°C	50°C	pH < 7: 90°C pH > 7: 50°C	pH < 7: 90°C pH > 7: 50°C

Biocompatible hardware available!



YMC-Triart **metal-free** columns are available for improved sensitivity and peak shape of coordinating compounds such as nucleotides or oligonucleotides.

Enhanced retention and resolution even at low buffer concentrations

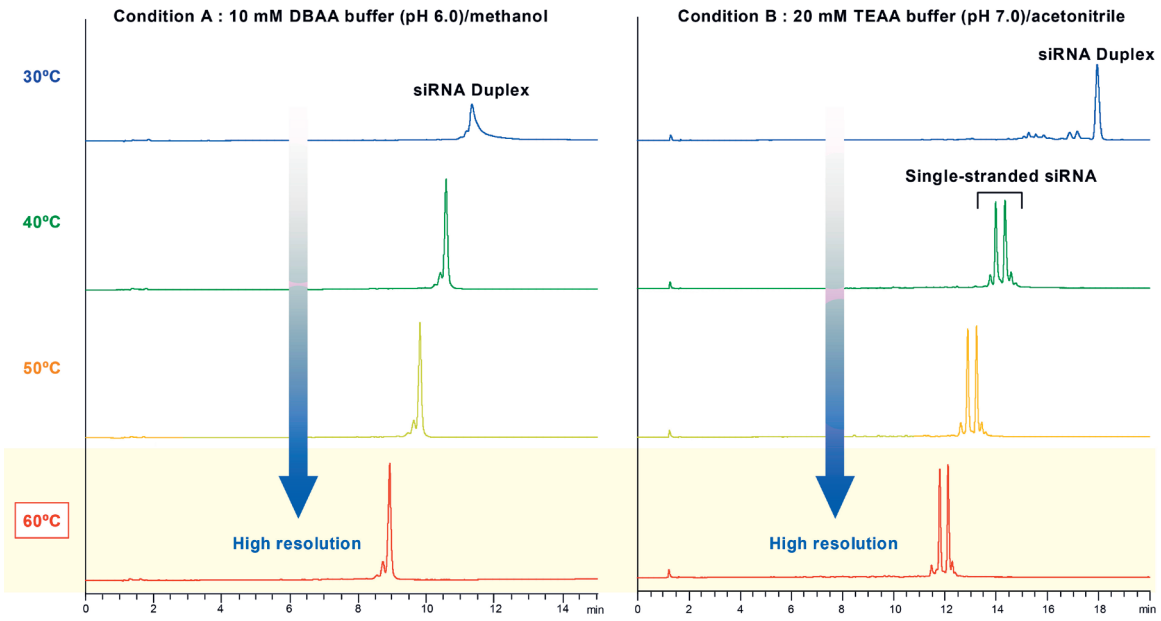


Column:	50 x 2.1 mm ID
Eluent:	A) 100 mM or 10 mM triethylamine-acetic acid (pH 6) B) A/acetonitrile (80/20)
Gradient:	50–65%B (0–20 min)
Flow rate:	0.21 mL/min
Temperature:	35°C
Detection:	UV at 260 nm
Injection:	2 µL (5 nmol/mL)
Sample:	Oligodeoxythymidylic acid [d(pT)2-20]

The separation of oligo(deoxythymidylic acids), d(pT)2-20, was compared using 100 mM or 10 mM triethylammonium acetate (TEAA) buffer, under the same gradient conditions. Both Hydrosphere C18 and YMC-Triart C18 showed enhanced retention and resolution compared to other commercially available C18 phases designed for oligonucleotide analysis, even at the low ion-pairing buffer concentration such as 10 mM.
The higher concentration provides stronger retention and superior resolution of oligonucleotides, although a lower concentration has the advantages of increasing the signal intensity and reducing system contamination in HPLC-MS analysis.

IP-RP – Temperature and mobile phase effects

Effect of mobile phase and column temperature on separation of siRNA duplex

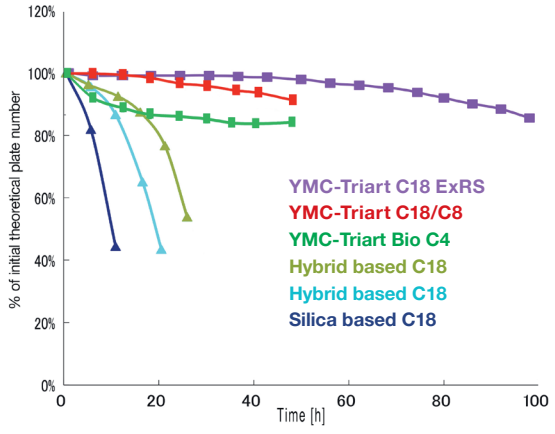


Crude synthetic siRNA duplex (19 bp): 5'-CGU ACG CGG AAU ACU UCG AdTdT-3'
3'-dTdTGCA UGC GCC UUA UGA AGC U-5'

Column:	YMC-Triart C18 (1.9 µm, 12 nm) 100 x 2.0 mm ID	**Condition A** Eluent:	A) 10 mM di-n-butylamine-acetic acid (pH 6.0)
Part No.:	TA12SP9-1002PT		B) methanol
Flow rate:	0.2 mL/min	Gradient:	35–60%B (0–15 min)
Detection:	UV at 269 nm	**Condition B** Eluent:	A) 20 mM triethylamine-acetic acid (pH 7.0)
Injection:	1 µL (5 nmol/mL)		B) acetonitrile
System:	Agilent 1290	Gradient:	5–12%B (0–20 min)

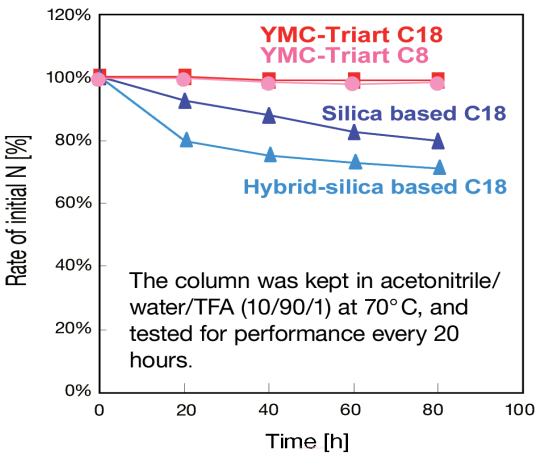
Versatile wide pH stability

Phosphate buffer (pH 11.5, 40° C)



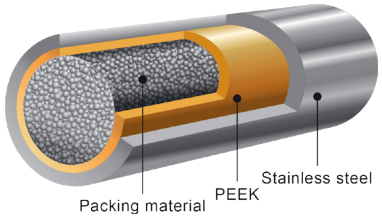
Column:	YMC-Triart (5 µm, 12 nm) 150 x 4.6 mm ID
Part No.:	TA12S05-1546PTH
Eluent:	50 mM K_2HPO_4-K_3PO_4 (pH 11.5)/methanol (90/10)
Flow rate:	1.0 mL/min
Temperature:	40°C
Sample:	benzyl alcohol

1% TFA (pH 1, 70°C)



Column:	YMC-Triart C18 (5 µm, 12 nm) 50 x 2.0 mm ID
Part No.:	TA12S05-0502WT
Eluent:	acetonitrile/water (60/40)
Flow rate:	0.2 mL/min
Temperature:	37°C
Sample:	butyl benzoate

Metal-free column hardware ideal for oligonucleotide analysis

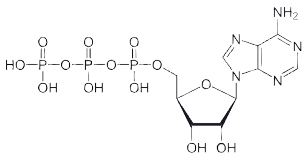


Specifications	
YMC-Triart Phases	C18, Bio C18, C8, Bio C4
Particle Size	1.9, 3, 5 µm
Inner layer	PEEK
Outer layer	Stainless steel
Frit	PEEK
Pressure limit	1.9 µm: 100 MPa (15,000 psi) 3/5 µm: 45 MPa (6,525 psi)

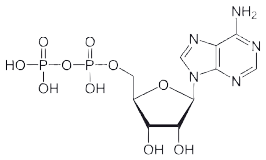
Special column connectors required.
See ordering information recommendations.

Improved sensitivity for coordination compounds

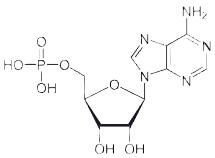
1. ATP



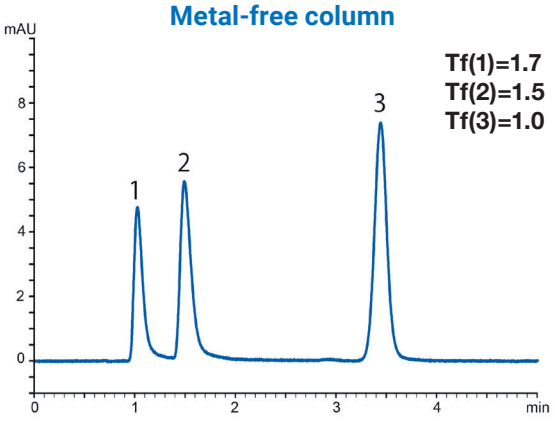
2. ADP



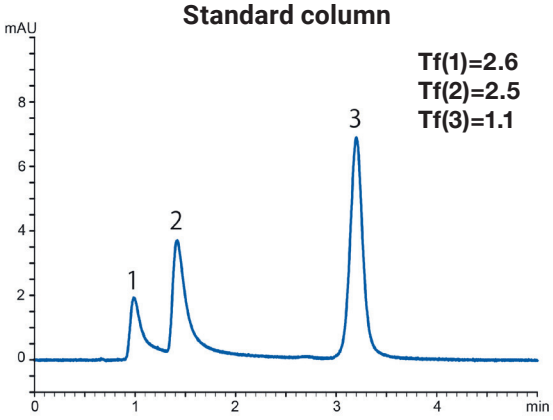
3. AMP



Metal-free column



Standard column



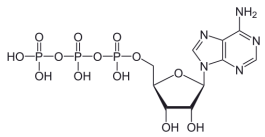
Column: YMC-Triart C18 (3 µm) 50 x 2.1 mm ID
Part Nos.: TA12S03-05Q1PTP (metal-free) or
TA12S03-05Q1PTH (regular hardware)
Eluent: 5 mM $HCOONH_4$
Flow rate: 0.21 mL/min
Temperature: 25°C
Detection: UV at 265 nm
Injection: 1 µL (10 mg/mL)
System: bioinert/"non-metal" HPLC system

Metal coordinating compounds, which have a phosphate group in their structure, tend to show poor peak shape due to interactions with metals, such as the stainless steel in column bodies and frits. By using the metal-free column hardware, better peak shapes can be expected. Nucleotides with phosphate groups show better peak shapes when compared to the regular column hardware. The metal-free column hardware is very suitable for highly sensitive analyses using LC/MS.

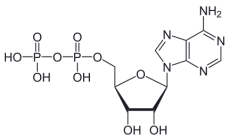
IP-RP – Expert Tips: System and Column hardware

Influence of system and column hardware on the analysis of nucleotides

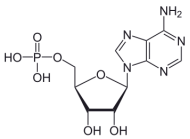
1 ATP



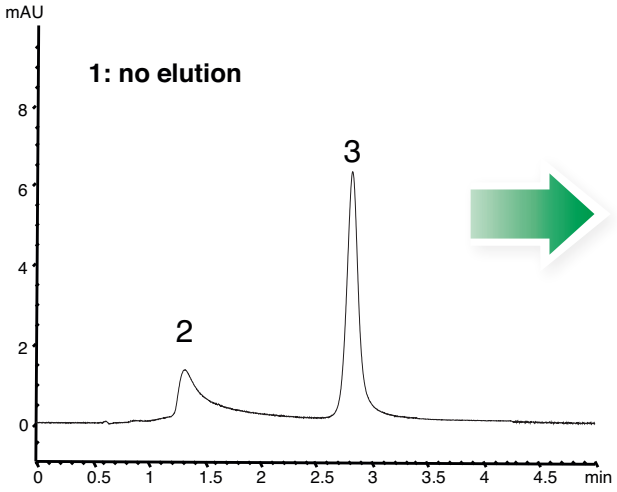
2 ADP



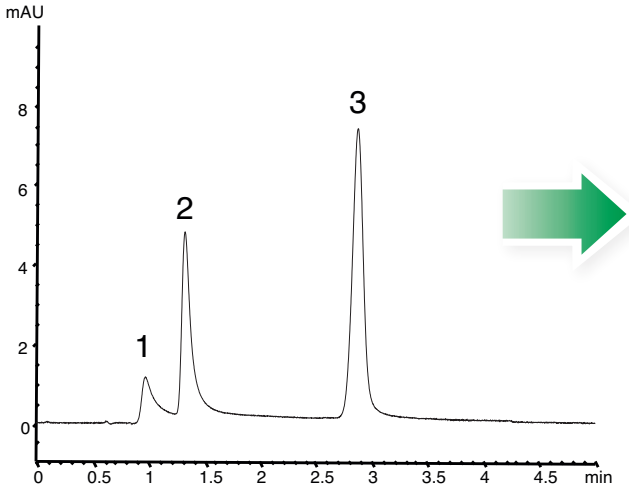
3 AMP



Ordinary HPLC system with standard column

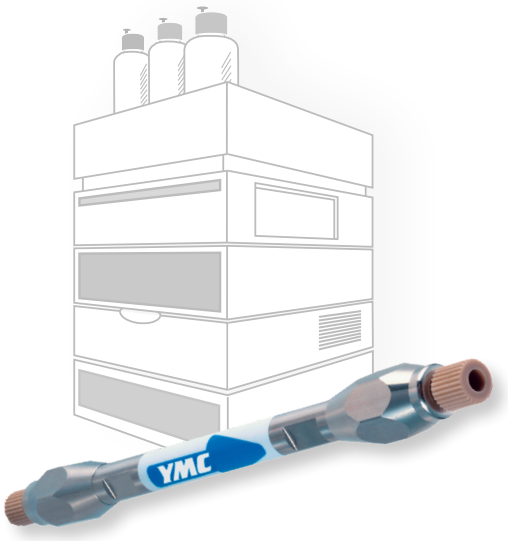


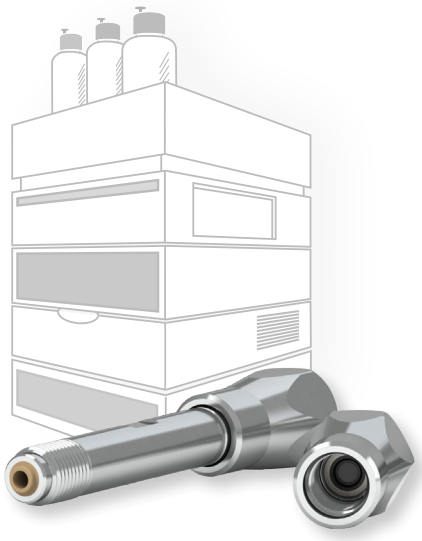
Ordinary HPLC system with metal-free column



Column:	YMC-Triart C18 (3 µm, 12 nm) 50 x 2.1 mm ID
Part Nos:	TA12S03-05Q1PT (standard hardware)
	TA12S03-05Q1PTP (**metal-free** hardware)
Eluent:	5 mM $HCOONH_4$
Flow rate:	0.21 mL/min
Temperature:	25°C
Detection:	UV at 265 nm
Injection:	1 µL (10 µg/mL)

*"Non-metal" HPLC system: PEEK sample loop, PEEK injector port, and PEEK tubing are used.

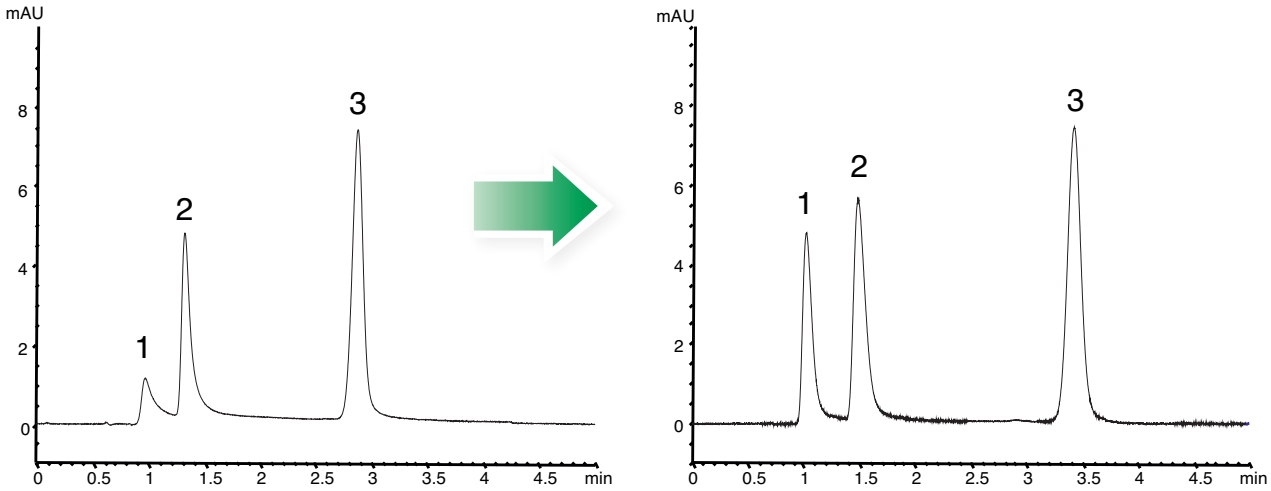


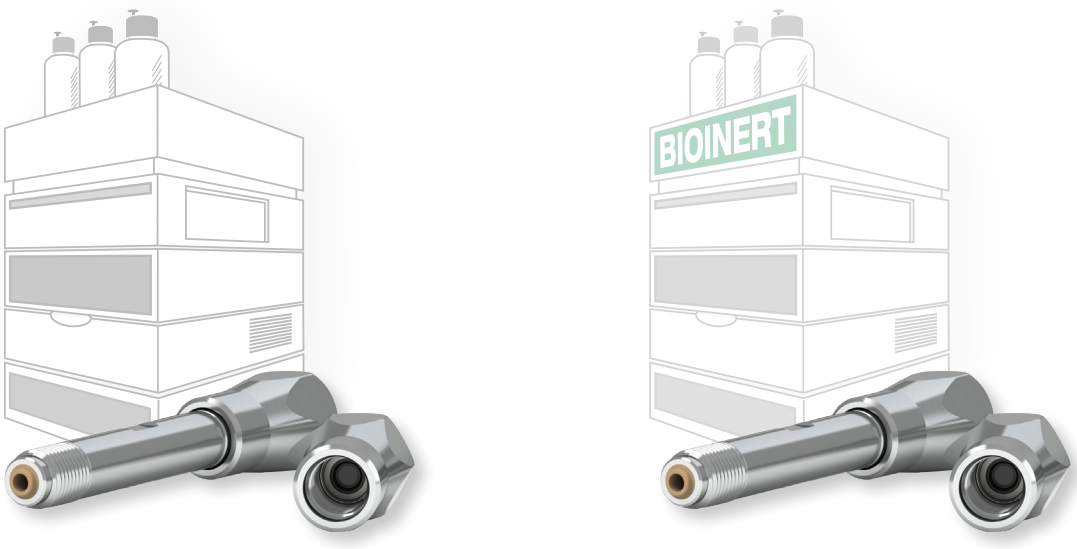


ATP peak is detected, and peak shape of ADP is improved as a result of using the metal-free column.

Ordinary HPLC system
with metal-free column

"Non-metal" HPLC system*
with metal-free column



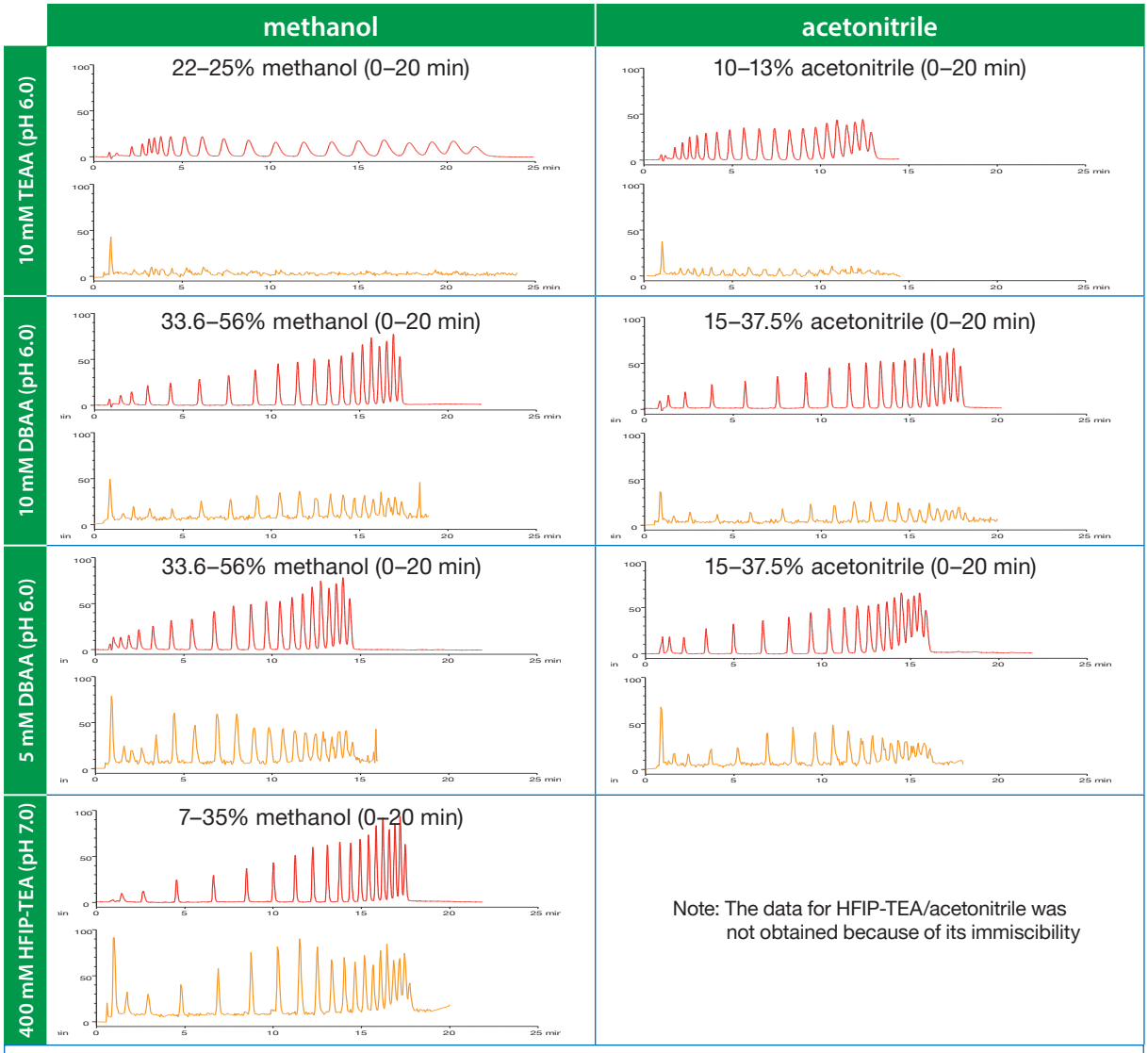


Peak shape is greatly improved as a result of using "non-metal" HPLC system

IP-RP – Expert Tips: Ion-pairing salts

Effect of composition and salt concentration of ion-pairing mobile phase on the separation and signal intensity

Comparison of separation and ESI-MS signal intensity using different ion-pairing buffers and organic solvents



Column:	Hydrosphere C18 (3.0 µm, 12 nm) 50 x 2.0 mm ID
Part No.:	HS12S03-0502WT
Flow rate:	0.2 mL/min
Temperature:	35 °C
Detection:	UV at 269 nm (red), ESI negative mode (orange)
Injection:	5 µl (25 pmol/mL)
Sample:	Oligo(deoxythymidylic acid) [dp (dT) 2-20]

eluent abbreviations

TEAA:	triethylammonium acetate
DBAA:	di-n-butylammonium acetate
HFIP:	1,1,1,3,3,3-hexafluoro-2-propanol
TEA:	triethylamine

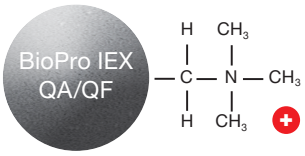
The mobile phase composition has different effects on the separation and signal intensity in electrospray ionisation mass spectrometry (ESI-MS) of oligonucleotides. Using different gradient conditions, acceptable retention and resolution can be achieved (upper UV chromatograms; red trace) for each separation by optimising the gradient slope of the organic solvent regardless of the type of mobile phase. The ESI-MS intensity is significantly influenced by the type and concentration of ion-pairing buffer as shown in the lower MS chromatograms (orange trace). HFIP-TEA buffer/methanol systems provide the maximum MS intensity. Enhanced retention and MS intensity are obtained using 10 mM DBAA buffer compared to 10 mM TEAA buffer, and the lower DBAA concentration results in approximately 1.5–3 times increase in the intensity without any change in the concentration of organic solvent.

AEX

AEX – BioPro IEX Series

Features

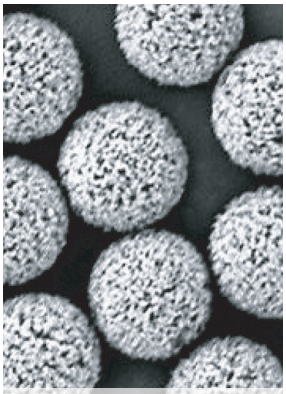
- **Porous or non-porous hydrophilic polymers**
- **High recovery of oligonucleotides**
- **Very high resolution**
- **Low nonspecific adsorption**
- **Excellent reproducibility**



strong anion exchanger

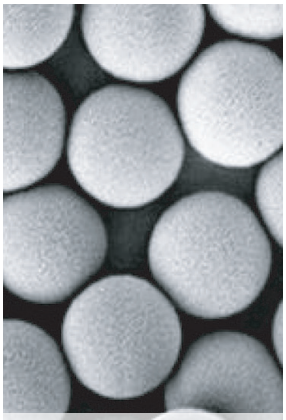
	BioPro IEX QA
Matrix	hydrophilic polymer (polymethacrylate)
Particle size / µm	5
Pore size / nm	100
Charged group	$-CH_2N^+(CH_3)_3$
Counter ion	Cl^-
Available pH range	2.0–12.0
Temperature range	4–60°C
Pressure limit	2.5–3.5 MPa (360–510 psi)
Column hardware	PEEK

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	$-CH_2N^+(CH_3)_3$
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



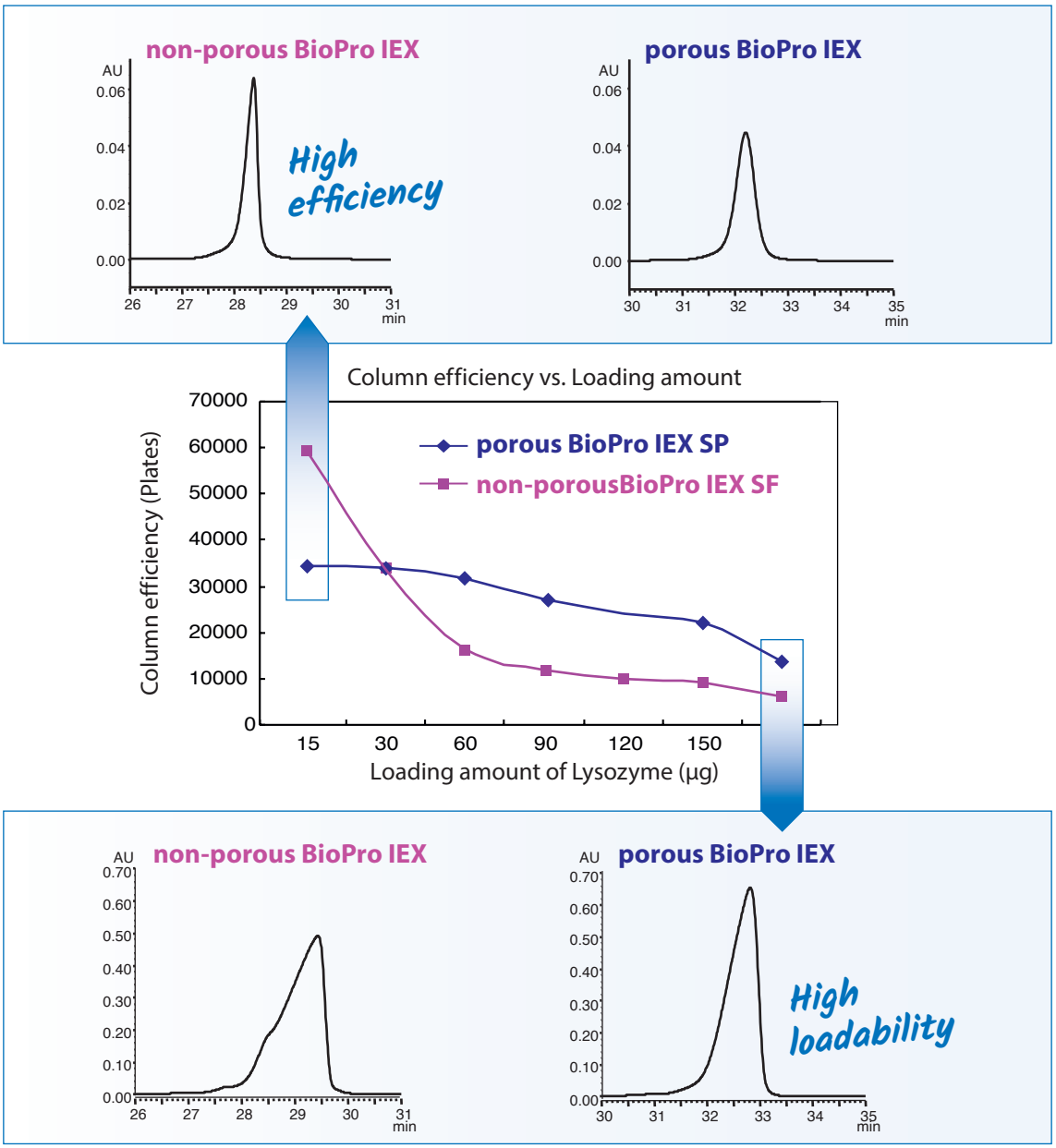
Non-porous polymer beads

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

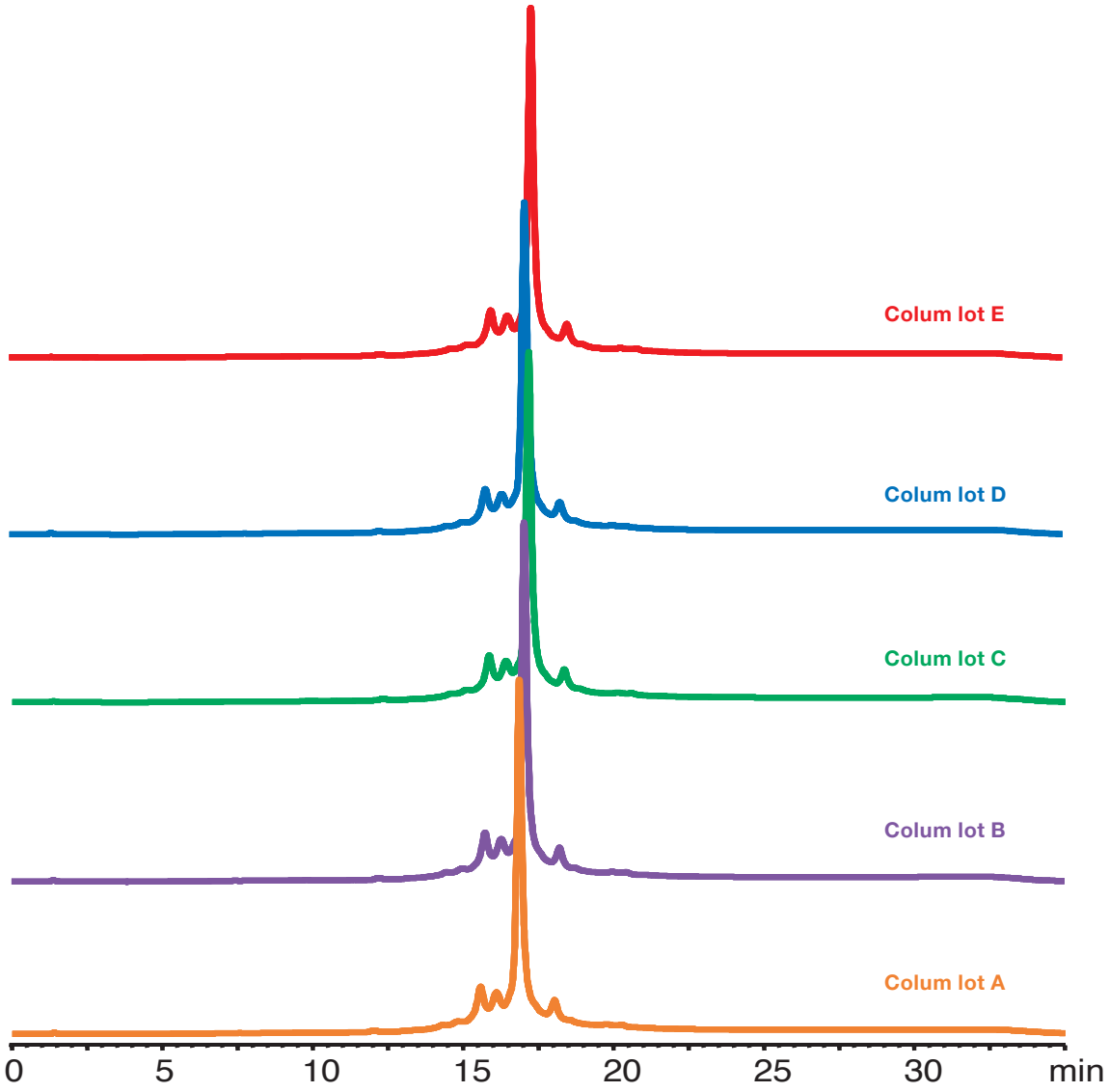


Column:	BioPro IEX SF/SP	Detection:	UV at 280 nm
Eluent:	A) 20 mM NaH_2PO_4-Na_2HPO_4 (pH 6.8)	Injection:	100 µL
	B) 20 mM NaH_2PO_4-Na_2HPO_4 (pH 6.8) containing 0.5 M NaCl	Sample:	1. Ribonuclease A
Gradient:	0–100%B (0–60 min)		2. Cytochrome c
Flow rate:	0.5 mL/min		3. Lysozyme
Temperature:	25°C		

Non-porous BioPro IEX offers outstanding column efficiency at small amount of sample loading. Non-porous type of BioPro IEX columns are especially suitable for microscale analysis which requires higher resolution. Porous BioPro IEX maintains the good peak shape even when the loading amount increases. Porous type BioPro IEX columns with high capacity are useful for high-load analytical separations and laboratory-scale purification.

AEX – BioPro IEX: Reproducibility

Excellent batch-to-batch reproducibility



Column:	BioPro IEX SF (5 µm) 100 x 4.6 mm ID
Part No.:	SF00S05-1046WP
Eluent:	A) 20 mM NaH_2PO_4-Na_2HPO_4 (pH 6.5)
	B) 20 mM NaH_2PO_4-Na_2HPO_4 (pH 6.5) containing 0.2 M NaCl
Gradient:	0–50%B (0.5–30 min)
Flow rate:	0.5 mL/min (180 cm/hr)
Temperature:	25°C
Detection:	UV at 215 nm
Injection:	10 µL
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

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

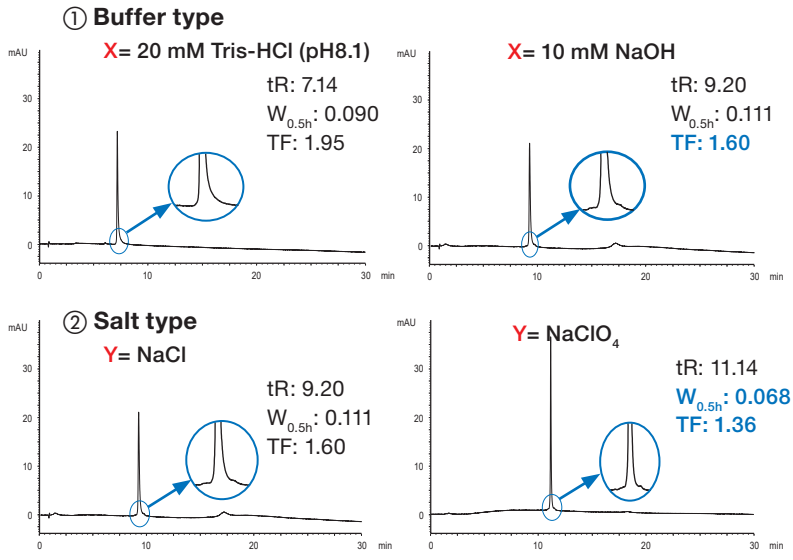
1 Improvement of peak tailing

Sample Group 1 (Phosphodiester oligonucleotides; PO)

1	Single-stranded DNA (ssDNA)	5'-TCATCACACTGAATACCAAT-3' (DNA 20 mer)
2		5'-GTCATCACACTGAATACCAAT-3' (DNA 21 mer)
3	Single-stranded RNA (ssRNA)	5'-UCAUCACACUGAAUACCAAU-3' (RNA 20 mer)
4		5'-GUCAUCACACUGAAUACCAAU-3' (RNA 21 mer)
5		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 20 mer)
6		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)A(M)A(M)U(M)-3' (2'-OMe RNA 21 mer)

N(M)=2'-OMe RNA

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 $NaClO_4$ was added to 10 mM NaOH instead of NaCl.



Column:	BioPro IEX QF (5 µm) 100 x 4.6 mm ID	Detection:	UV at 260 nm
Part No.:	QF00S05-1046WP	Injection:	2 µL (10 nmol/mL)
Flow rate:	1.0 mL/min	Sample:	RNA 20 mer
Temperatur:	25°C		

2 Improvement of carryover

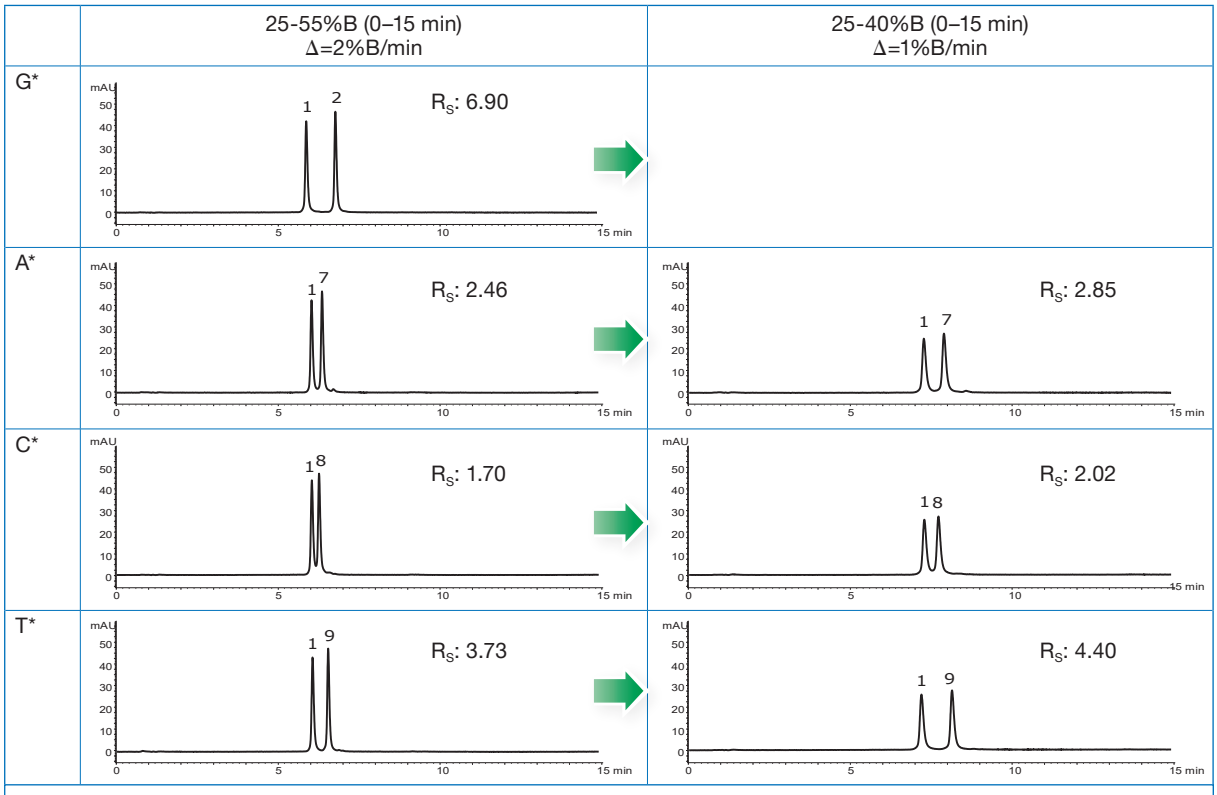
When the initial gradient concentration of NaCl is low (ex. 50 mM), carryover is observed. By increasing the initial gradient concentration of NaCl up to 400 carryover can be avoided with good reproducibility.

AEX Expert Tips

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

When ssDNAs with single-base differences (differing in the type of base of 5'end of DNA 21mer) is analysed under the described condition, all of peak separations got worse. By using a shallower gradient, better separations could be achieved.

1	Single-stranded DNA	5'-TCATCACACTGAATACCAAT-3' (DNA 20 mer)
2		5'-GTCATCACACTGAATACCAAT-3' (DNA 21 mer)
7		5'-ATCATCACACTGAATACCAAT-3' (DNA 21 mer)
8		5'-CTCATCACACTGAATACCAAT-3' (DNA 21 mer)
9		5'-TTCATCACACTGAATACCAAT-3' (DNA 21 mer)



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

*base of 5'end of DNA 21 mer

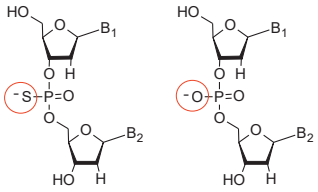
4 Improvement of the separation of phopsphorothioate oligonucleotides with single-base differences in length

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. However, increase of organic solvent ratio gave little improvement in peak separation.

Sample Group 2 (Phosphorothioate oligonucleotides; PS)

10	Single-stranded DNA	5′-T~T~T~T~T~T~T~T~T~T~T~T~TTT-3′ (DNA 15 mer 12PS, 2PO)
11		5′-T~T~T~T~T~T~T~T~T~T~T~T~TT-3′ (DNA 15 mer 13PS, 1PO)
12		5′-T~T~T~T~T~T~T~T~T~T~T~T~T-3′ (DNA 15 mer All PS)
13	Single-stranded RNA	5′-U~C~A~U~C~A~C~A~C~A~C~U~G~A~A~U~A~C~C~A~A~U-3′ (RNA 20 mer All PS)
14		5′-G~U~C~A~U~C~A~C~A~C~A~C~U~G~A~A~U~A~C~C~A~A~U-3′ (RNA 21 mer All PS)

~=Phosphorothioated



Phosphorothioate Linkage (PS) | Phosphodiester Linkage (PO)

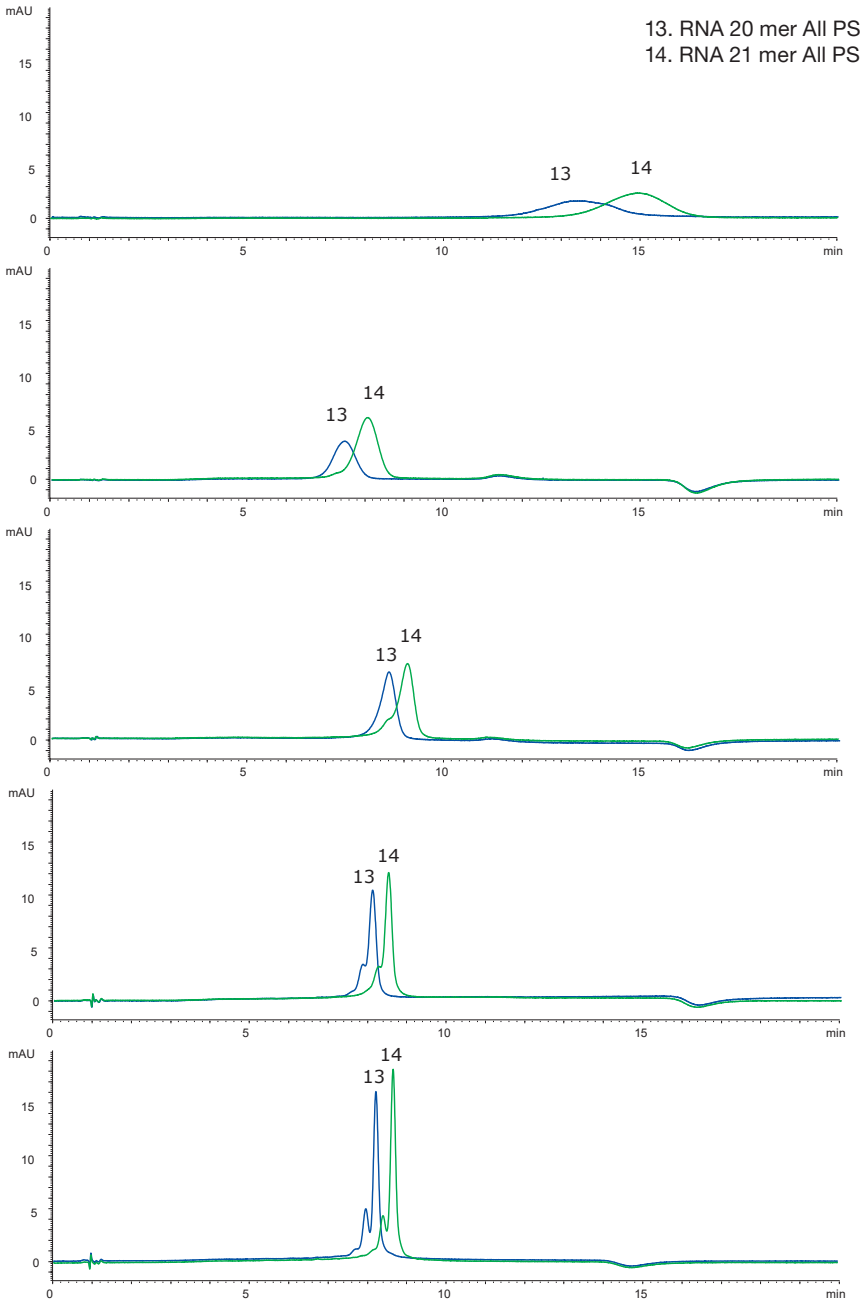
X/Y=100/0
32–80%B (0–24 min)
= Δ20 mM $NaClO_4$/min
25°C

Steep gradient curve
X/Y=100/0
32–80%B (0–8 min)
= Δ60 mM $NaClO_4$/min
25°C

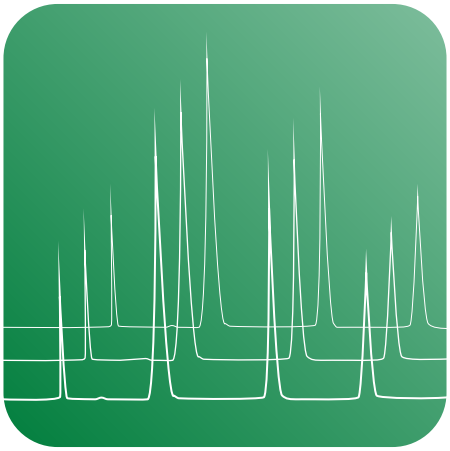
Raising column temperature
X/Y=100/0
32–80%B (0–8 min)
=Δ60 mM $NaClO_4$/min
60°C

Addition of organic solvent
X/Y=80/20
40–100%B (0–8 min)
= Δ60 mM $NaClO_4$/min
60°C

Increasing ratio of organic solvent
X/Y=70/30
40–100%B (0–6.3 min)
= Δ60 mM $NaClO_4$/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
Gradient: 1.0 M $NaClO_4$/methanol (X/Y)
Flow rate: 1.0 mL/min
Detection: UV at 260 nm
Injection: 2 µL (10 nmol/mL)



Ordering information

IP-RP – Ordering information

1.9 µm UHPLC columns

Phase	Column ID [mm]	Column length [mm]					Guard cartridges* with 5 mm length
		30	50	75	100	150	(pack of 3)
YMC-Triart C18	2.0	TA12SP9-0302PT	TA12SP9-0502PT	TA12SP9-L502PT	TA12SP9-1002PT	TA12SP9-1502PT	TA12SP9-E5Q1CC**
	2.1	TA12SP9-03Q1PT	TA12SP9-05Q1PT	TA12SP9-L5Q1PT	TA12SP9-10Q1PT	TA12SP9-15Q1PT	TA12SP9-E5Q1CC**
	3.0	—	TA12SP9-0503PT	TA12SP9-L503PT	TA12SP9-1003PT	TA12SP9-1503PT	TA12SP9-E503CC
YMC-Triart Bio C18	2.0	TA30SP9-0302PT	TA30SP9-0502PT	TA30SP9-L502PT	TA30SP9-1002PT	TA30SP9-1502PT	TA30SP9-E5Q1CC**
	2.1	TA30SP9-03Q1PT	TA30SP9-05Q1PT	TA30SP9-L5Q1PT	TA30SP9-10Q1PT	TA30SP9-15Q1PT	TA30SP9-E5Q1CC**
	3.0	—	TA30SP9-0503PT	TA30SP9-L503PT	TA30SP9-1003PT	TA30SP9-1503PT	TA30SP9-E503CC
YMC-Triart C8	2.0	TO12SP9-0302PT	TO12SP9-0502PT	TO12SP9-L502PT	TO12SP9-1002PT	TO12SP9-1502PT	TO12SP9-E5Q1CC**
	2.1	TO12SP9-03Q1PT	TO12SP9-05Q1PT	TO12SP9-L5Q1PT	TO12SP9-10Q1PT	TO12SP9-15Q1PT	TO12SP9-E5Q1CC**
	3.0	—	TO12SP9-0503PT	TO12SP9-L503PT	TO12SP9-1003PT	TO12SP9-1503PT	TO12SP9-E503CC
YMC-Triart Bio C4	2.0	TB30SP9-0302PT	TB30SP9-0502PT	TB30SP9-L502PT	TB30SP9-1002PT	TB30SP9-1502PT	TB30SP9-E5Q1CC**
	2.1	TB30SP9-03Q1PT	TB30SP9-05Q1PT	TB30SP9-L5Q1PT	TB30SP9-10Q1PT	TB30SP9-15Q1PT	TB30SP9-E5Q1CC**
	3.0	—	TB30SP9-0503PT	TB30SP9-L503PT	TB30SP9-1003PT	TB30SP9-1503PT	TB30SP9-E503CC

*Guard cartridge holder required, part no. XPCHUHP
**Guard cartridge: 2.1 mm ID

1.9 µm metal-free UHPLC columns

Phase	Column ID [mm]	Column length [mm]		
		50	100	150
YMC-Triart C18	2.1	TA12SP9-05Q1PTP	TA12SP9-10Q1PTP	TA12SP9-15Q1PTP
YMC-Triart Bio C18	2.1	TA30SP9-05Q1PTP	TA30SP9-10Q1PTP	TA30SP9-15Q1PTP
YMC-Triart C8	2.1	TO12SP9-05Q1PTP	TO12SP9-10Q1PTP	TO12SP9-15Q1PTP
YMC-Triart Bio C4	2.1	TB30SP9-05Q1PTP	TB30SP9-10Q1PTP	TB30SP9-15Q1PTP

Special column connectors required.

Column connectors for metal-free (U)HPLC columns

Recommendation	✓ ✓		✓	
Ferrule	no		replaceable	
Product	MarvelX™	MarvelXACT™	Handy connector 2	Hand-tight EXP® fitting
Manufacturer	IDEX Health & Science LLC	IDEX Health & Science LLC	YMC Co., Ltd.	Optimize Technologies, Inc.
				
Pressure rating	131 MPa / 1,310 bar	131 MPa / 1,310 bar	42 MPa / 420 bar	137 MPa / 1,370 bar
Product code	e.g. UPFP-6050250	e.g. UPFP-YM7050250	XRP0204	XRHTF-01

MarvelX (ACT) is a registered trademark of IDEX Health & Science LLC · EXP® is a registered trademark of Optimize Technologies, Inc.

3 µm HPLC columns

Phase	Column ID [mm]	Column length [mm]						Guard cartridges* with 10 mm length
		30/33	50	75	100	150	250	(pack of 5)
YMC-Triart C18	2.1	TA12S03-H3Q1PTH	TA12S03-05Q1PTH	TA12S03-L5Q1PTH	TA12S03-10Q1PTH	TA12S03-15Q1PTH	–	TA12S03-01Q1GC
	3.0	–	TA12S03-0503PTH	TA12S03-L503PTH	TA12S03-1003PTH	TA12S03-1503PTH	–	TA12S03-0103GC
	4.6	TA12S03-H346PTH	TA12S03-0546PTH	TA12S03-L546PTH	TA12S03-1046PTH	TA12S03-1546PTH	TA12S03-2546PTH	TA12S03-0104GC
YMC-Triart Bio C18	2.1	TA30S03-H3Q1PTH	TA30S03-05Q1PTH	TA30S03-L5Q1PTH	TA30S03-10Q1PTH	TA30S03-15Q1PTH	–	TA30S03-01Q1GC
	3.0	–	TA30S03-0503PTH	TA30S03-L503PTH	TA30S03-1003PTH	TA30S03-1503PTH	–	TA30S03-0103GC
	4.6	TA30S03-H346PTH	TA30S03-0546PTH	TA30S03-L546PTH	TA30S03-1046PTH	TA30S03-1546PTH	TA30S03-2546PTH	TA30S03-0104GC
YMC-Triart C8	2.1	TO12S03-H3Q1PTH	TO12S03-05Q1PTH	TO12S03-L5Q1PTH	TO12S03-10Q1PTH	TO12S03-15Q1PTH	–	TO12S03-01Q1GC
	3.0	–	TO12S03-0503PTH	TO12S03-L503PTH	TO12S03-1003PTH	TO12S03-1503PTH	–	TO12S03-0103GC
	4.6	TO12S03-H346PTH	TO12S03-0546PTH	TO12S03-L546PTH	TO12S03-1046PTH	TO12S03-1546PTH	TO12S03-2546PTH	TO12S03-0104GC
YMC-Triart Bio C4	2.1	TB30S03-03Q1PTH	TB30S03-05Q1PTH	TB30S03-L5Q1PTH	TB30S03-10Q1PTH	TB30S03-15Q1PTH	–	TB30S03-01Q1GC
	3.0	–	TB30S03-0503PTH	TB30S03-L503PTH	TB30S03-1003PTH	TB30S03-1503PTH	–	TB30S03-0103GC
	4.6	TB30S03-0346PTH	TB30S03-0546PTH	TB30S03-L546PTH	TB30S03-1046PTH	TB30S03-1546PTH	TB30S03-2546PTH	TB30S03-0104GC
Hydrosphere C18	2.1	HS12S03-03Q1WT	HS12S03-05Q1WT	HS12S03-L5Q1WT	HS12S03-10Q1WT	HS12S03-15Q1WT	HS12S03-25Q1WT	HS12S03-01Q1GC
	3.0	HS12S03-0303WT	HS12S03-0503WT	HS12S03-L503WT	HS12S03-1003WT	HS12S03-1503WT	HS12S03-2503WT	HS12S03-0103GC
	4.6	HS12S03-0346WT	HS12S03-0546WT	HS12S03-L546WT	HS12S03-1046WT	HS12S03-1546WT	HS12S03-2546WT	HS12S03-0104GC

*Guard cartridge holder required, part no. XPGCH-Q1

3 µm metal-free HPLC columns

Phase	Column ID [mm]	Column length [mm]		
		50	100	150
YMC-Triart C18	2.1	TA12S03-05Q1PTP	TA12S03-10Q1PTP	TA12S03-15Q1PTP
	4.6	TA12S03-0546PTP	TA12S03-1046PTP	TA12S03-1546PTP
YMC-Triart Bio C18	2.1	TA30S03-05Q1PTP	TA30S03-10Q1PTP	TA30S03-15Q1PTP
	4.6	TA30S03-0546PTP	TA30S03-1046PTP	TA30S03-1546PTP
YMC-Triart C8	2.1	TO12S03-05Q1PTP	TO12S03-10Q1PTP	TO12S03-15Q1PTP
	4.6	TO12S03-0546PTP	TO12S03-1046PTP	TO12S03-1546PTP
YMC-Triart Bio C4	2.1	TB30S03-05Q1PTP	TB30S03-10Q1PTP	TB30S03-15Q1PTP
	4.6	TB30S03-0546PTP	TB30S03-1046PTP	TB30S03-1546PTP

Special column connectors required.

IP-RP – Ordering information

5 µm HPLC columns

Phase	Column ID [mm]	Column length [mm]						Guard cartridges* with 10 mm length
		30/33	50	75	100	150	250	(pack of 5)
YMC-Triart C18	2.1	TA12S05-H3Q1PTH	TA12S05-05Q1PTH	TA12S05-L5Q1PTH	TA12S05-10Q1PTH	TA12S05-15Q1PTH	–	TA12S05-01Q1GC
	3.0	–	TA12S05-0503PTH	TA12S05-L503PTH	TA12S05-1003PTH	TA12S05-1503PTH	–	TA12S05-0103GC
	4.6	TA12S05-H346PTH	TA12S05-0546PTH	TA12S05-L546PTH	TA12S05-1046PTH	TA12S05-1546PTH	TA12S05-2546PTH	TA12S05-0104GC
YMC-Triart Bio C18	2.1	TA30S05-H3Q1PTH	TA30S05-05Q1PTH	TA30S05-L5Q1PTH	TA30S05-10Q1PTH	TA30S05-15Q1PTH	–	TA30S05-01Q1GC
	3.0	–	TA30S05-0503PTH	TA30S05-L503PTH	TA30S05-1003PTH	TA30S05-1503PTH	–	TA30S05-0103GC
	4.6	TA30S05-H346PTH	TA30S05-0546PTH	TA30S05-L546PTH	TA30S05-1046PTH	TA30S05-1546PTH	TA30S05-2546PTH	TA30S05-0104GC
YMC-Triart C8	2.1	TO12S05-H3Q1PTH	TO12S05-05Q1PTH	TO12S05-L5Q1PTH	TO12S05-10Q1PTH	TO12S05-15Q1PTH	–	TO12S05-01Q1GC
	3.0	–	TO12S05-0503PTH	TO12S05-L503PTH	TO12S05-1003PTH	TO12S05-1503PTH	–	TO12S05-0103GC
	4.6	TO12S05-H346PTH	TO12S05-0546PTH	TO12S05-L546PTH	TO12S05-1046PTH	TO12S05-1546PTH	TO12S05-2546PTH	TO12S05-0104GC
YMC-Triart Bio C4	2.1	TB30S05-H3Q1PTH	TB30S05-05Q1PTH	TB30S05-L5Q1PTH	TB30S05-10Q1PTH	TB30S05-15Q1PTH	–	TB30S05-01Q1GC
	3.0	–	TB30S05-0503PTH	TB30S05-L503PTH	TB30S05-1003PTH	TB30S05-1503PTH	–	TB30S05-0103GC
	4.6	TB30S05-H346PTH	TB30S05-0546PTH	TB30S05-L546PTH	TB30S05-1046PTH	TB30S05-1546PTH	TB30S05-2546PTH	TB30S05-0104GC
Hydrosphere C18	2.1	HS12S05-03Q1WT	HS12S05-05Q1WT	HS12S05-L5Q1WT	HS12S05-10Q1WT	HS12S05-15Q1WT	HS12S05-25Q1WT	HS12S05-01Q1GC
	3.0	HS12S05-0303WT	HS12S05-0503WT	HS12S05-L503WT	HS12S05-1003WT	HS12S05-1503WT	HS12S05-2503WT	HS12S05-0103GC
	4.6	HS12S05-0346WT	HS12S05-0546WT	HS12S05-L546WT	HS12S05-1046WT	HS12S05-1546WT	HS12S05-2546WT	HS12S05-0104GC

*Guard cartridge holder required, part no. XPGCH-Q1

5 µm metal-free HPLC columns

Phase	Column ID [mm]	Column length [mm]		
		50	100	150
YMC-Triart C18	2.1	TA12S05-05Q1PTP	TA12S05-10Q1PTP	TA12S05-15Q1PTP
	4.6	TA12S05-0546PTP	TA12S05-1046PTP	TA12S05-1546PTP
YMC-Triart Bio C18	2.1	TA30S05-05Q1PTP	TA30S05-10Q1PTP	TA30S05-15Q1PTP
	4.6	TA30S05-0546PTP	TA30S05-1046PTP	TA30S05-1546PTP
YMC-Triart C8	2.1	TO12S05-05Q1PTP	TO12S05-10Q1PTP	TO12S05-15Q1PTP
	4.6	TO12S05-0546PTP	TO12S05-1046PTP	TO12S05-1546PTP
YMC-Triart Bio C4	2.1	TB30S05-05Q1PTP	TB30S05-10Q1PTP	TB30S05-15Q1PTP
	4.6	TB30S05-0546PTP	TB30S05-1046PTP	TB30S05-1546PTP

Special column connectors required.

5 µm YMC-Actus high-throughput semipreparative columns

Phase	Column ID [mm]	Column length [mm]					Guard cartridges* with 10 mm length
		50	75	100	150	250	(pack of 5)
YMC-Triart C18	20	TA12S05-0520WX	TA12S05-L520WX	TA12S05-1020WX	TA12S05-1520WX	TA12S05-2520WX	TA12S05-0120CCN
	30	TA12S05-0530WX	TA12S05-L530WX	TA12S05-1030WX	TA12S05-1530WX	TA12S05-2530WX	TA12S05-0130CCN
	50	TA12S05-0553DX	–	TA12S05-1053DX	TA12S05-1553DX	TA12S05-2553DX	TA12S05-0553DXG**
YMC-Triart Bio C18	20	TA30S05-0520WX	TA30S05-L520WX	TA30S05-1020WX	TA30S05-1520WX	TA30S05-2520WX	TA30S05-0120CCN
	30	TA30S05-0530WX	TA30S05-L530WX	TA30S05-1030WX	TA30S05-1530WX	TA30S05-2530WX	TA30S05-0130CCN
	50	TA30S05-0553DX	–	TA30S05-1053DX	TA30S05-1553DX	TA30S05-2553DX	TA30S05-0553DXG**
YMC-Triart C8	20	TO12S05-0520WX	TO12S05-L520WX	TO12S05-1020WX	TO12S05-1520WX	TO12S05-2520WX	TO12S05-0120CCN
	30	TO12S05-0530WX	TO12S05-L530WX	TO12S05-1030WX	TO12S05-1530WX	TO12S05-2530WX	TO12S05-0130CCN
	50	TO12S05-0553DX	–	TO12S05-1053DX	TO12S05-1553DX	TO12S05-2553DX	TO12S05-0553DXG**
YMC-Triart Bio C4	20	TB30S05-0520WX	TB30S05-L520WX	TB30S05-1020WX	TB30S05-1520WX	TB30S05-2520WX	TB30S05-0120CCN
	30	TB30S05-0530WX	TB30S05-L530WX	TB30S05-1030WX	TB30S05-1530WX	TB30S05-2530WX	TB30S05-0130CCN
	50	TB30S05-0553DX	–	TB30S05-1053DX	TB30S05-1553DX	TB30S05-2553DX	TB30S05-0553DXG**
Hydrosphere C18	20	HS12S05-0520WX	HS12S05-L520WX	HS12S05-1020WX	HS12S05-1520WX	HS12S05-2520WX	HS12S05-0120CCN
	30	HS12S05-0530WX	HS12S05-L530WX	HS12S05-1030WX	HS12S05-1530WX	HS12S05-2530WX	HS12S05-0130CCN
	50	HS12S05-0553DX	–	HS12S05-1053DX	HS12S05-1553DX	HS12S05-2553DX	–

*Guard cartridge holder required, part no. XPGHF2P20ID (20 mm ID)
XPGHF2P30ID (30 mm ID)
no holder required for 50 mm

AEX – Ordering information

3 µm non-porous analytical columns, PEEK hardware (max. pressure 250 bar)

Phase	Column ID [mm]	Column length [mm]				Precolumn filter 2 µm*
		30 (250 bar)	50 (250 bar)	100 (250 bar)	150 (250 bar)	(pack of 5)
BioPro IEX QF	4.6	QF00S03-0346WP	QF00S03-0546WP	QF00S03-1046WP	QF00S03-1546WP	XRPRCP25

5 µm non-porous analytical columns, PEEK hardware (max. pressure 60–120 bar)

Phase	Column ID [mm]	Column length [mm]				Precolumn filter 2 µm*
		30 (60 bar)	50 (100 bar)	100 (120 bar)	150 (120 bar)	(pack of 5)
BioPro IEX QF	4.6	QF00S05-0346WP	QF00S05-0546WP	QF00S05-1046WP	QF00S05-1546WP	XRPRCP25

5 µm porous analytical columns, PEEK hardware (max. pressure 25–35 bar)

Phase	Column ID [mm]	Column length [mm]			Precolumn filter 2 µm*
		30 (25 bar)	50 (30 bar)	100 (35 bar)	(pack of 5)
BioPro IEX QA	4.6	QAA0S05-0346WP	QAA0S05-0546WP	QAA0S05-1046WP	XRPRCP25

* Holder required, part no. XRPRCP02

6 µm non-porous semiprep. columns, stainless steel hardware (max. pressure 30–90 bar)

Phase	Column ID [mm]	Column length [mm]
		100
BioPro IEX QF	10 20 30	QF00S06-1010WT QF00S06-1020WT QF00S06-1030WT

6 µm porous semiprep. columns, stainless steel hardware (max. pressure 40 bar)

Phase	Column ID [mm]	Column length [mm]
		100
BioPro IEX QA	10 20	QAA0S06-1010WT QAA0S06-1020WT

Other dimensions on demand

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