thermo scientific

Thermo Scientific DNAPac Family of Columns

Superior Oligonucleotide Analysis

ThermoFisher SCIENTIFIC

DNAPac RP
2016

DNAPac PA200 RS
2013

DNASwift SAX-1S
2009

DNAPac PA200
2004

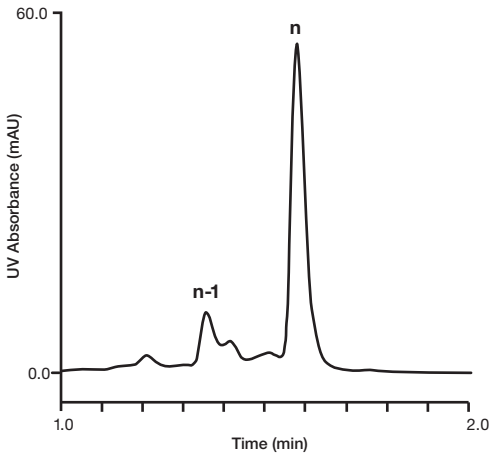
DNAPac PA100
1990

For over 30 years, the Thermo Scientific™ DNAPac™ family of columns has been the go-to for high resolution U/HPLC separation of oligonucleotides. When purity and characterization is critical to your work, you can rely on our anion-exchange and reversed-phase column chemistries. These columns have the performance and robustness to meet your oligonucleotide analysis needs so you can be confident in your data while meeting the throughput demands of your laboratory.

Our legacy began with the Thermo Scientific™ DNAPac™ PA100 series of columns. These high-resolution anion-exchange columns helped transition scientists from a laborious capillary gel electrophoresis approach to high performance liquid chromatography (HPLC). As oligonucleotide separations became more demanding, our columns evolved to meet the challenge.

With the introduction of the Thermo Scientific™ DNAPac™ PA200 and Thermo Scientific™ DNAPac™ PA200RS columns, scientists now experience high resolution ion-exchange separations for their oligonucleotide purity analysis by LC-UV. Our Thermo Scientific™ DNAPac™ reversed-phase (RP) columns allow you to couple reversed-phase oligonucleotide separations directly to a mass spectrometer, for accurate mass determinations of oligonucleotide sequences. Our innovative chemistries are widely referenced in hundreds of peer reviewed journal articles.

Ion Exchange separation of 22mer DNA (n), 21mer DNA (n-1; 3' truncated)



Column:	**DNAPac PA200 RS**
Format:	4.6 x 50mm
Mobile phase A:	20mM Tris, pH 8.0
Mobile phase B:	20mM Tris, 1 M NaCl, pH 8.0

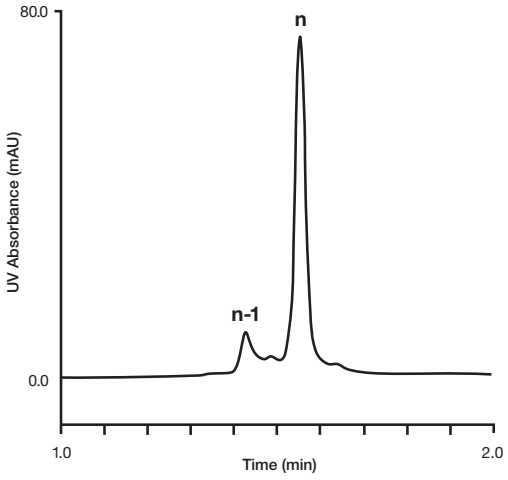
Gradient:

Time (min)	**%A**	**%B**
0.0	58	42
2.0	46	54
2.1	0	100
3.0	0	100
3.1	58	42
7.0	58	42

Flow rate:	1.3 mL/min
Inj. volume:	4 µL
Temperature:	30 °C
Detection:	UV (260 nm)
Sample:	22mer DNA (n), 21mer DNA (n-1; 3' truncated)

A = Adenine
MeC = 5-methyl-Cytosine
** = phosphothioate linkage*
All ribose are 2'-O-methylated

Reversed-Phase separation of 22mer DNA (n), 21mer DNA (n-1; 3' truncated)



Column:	**DNAPac RP**
Format:	2.1 x 50mm
Mobile phase A:	0.1M TEAA
Mobile phase B:	0.1M TEAA in Water/ Acetonitrile (75:25 v/v)

Gradient:

Time (min)	**%A**	**%B**
0.0	90	18
2.0	64	36
2.1	0	100
3.0	0	100
3.1	90	18
7.0	90	18

Flow rate:	0.6 mL/min
Inj. volume:	2 µL
Temperature:	80 °C
Detection:	UV (260 nm)
Sample:	22mer DNA (n), 21mer DNA (n-1; 3' truncated)

We invest in your Chromatography

Technology that delivers benefit

DNAPac PA200 and PA200RS

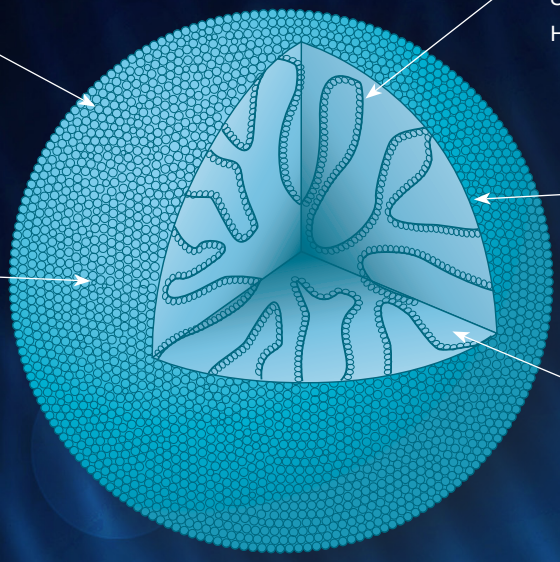
Non-porous resin with latex nano beads on the surface

Minimizes diffusion, offers high resolution separations

Quaternary amine functionalized nano beads

Single base resolution of up to 100mer oligonucleotides

Higher loading capacity vs conventional non-porous



Polymeric Base media

Compatible with high temperature (85 °C) up to pH8

Polymeric Base media

Long column lifetimes and compatible up to pH12 allowing you to utilize pH control to optimize selectivity

Available in both Standard HPLC and Rapid Separation (RS) UHPLC formats.

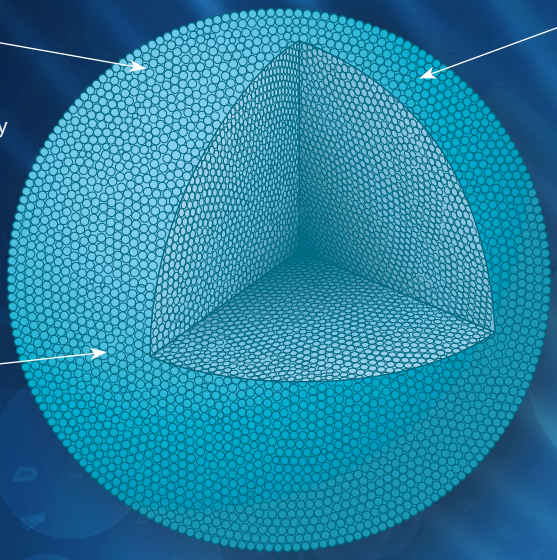
High sample throughput

DNAPac RP

Polymeric (PS-DVB) resin

Higher temperature (100 °C) tolerance, even at elevated pH (1-14) stability

Long column lifetimes due to the ability to run at higher pH and temperatures than conventional silica column



Optimized surface chemistry

Long lifetime for siRNA, ASOs, and other short oligo sequences

Compatible with LC-MS

Reliable data

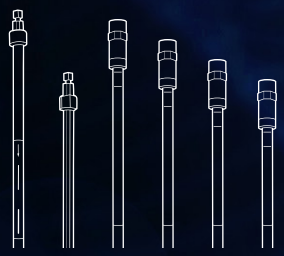
Wide pore size

Supermarcoporous structure up to 2,000 Å for excellent separation of short [siRNA] and long oligonucleotides [mRNA] samples

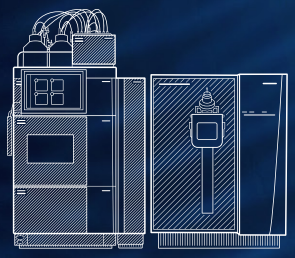
Very low carryover and long lifetime

Oligonucleotide Analysis

Solutions for oligonucleotide therapeutic analysis



HPLC Columns



LC-MS Systems

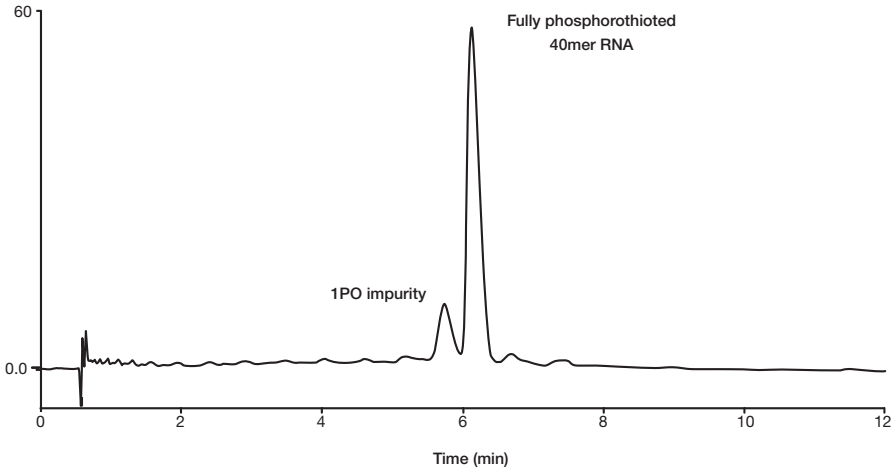
Software & Data Analysis

Thermo Scientific DNAPac RP

The DNAPac RP (reversed-phase) column is designed for analysis of oligonucleotides by LC-UV or LC-MS. The polymeric backbone provides excellent lifetime under a wide range of pH and temperature. The supermacroporous structure has small pores in addition to very large pores to allow high resolution separations of short and large oligonucleotides on the same column.

Even the most challenging samples can be separated by the DNAPac RP. The data below shows antiviral nucleic acid polymer (NAP) targeting Hepatitis B (REP 2139) separated from its phosphodiester (PO) and truncation impurities. The antiviral NAP is a fully phosphothioated and 2'- O-methylated 40mer RNA comprised of alternating adenosine and 5-methylcytidine nucelotides.

To learn more about this analysis please see poster PO21721-EN 0517S.



1PO Separation from fully phosphorothioated 40mer RNA

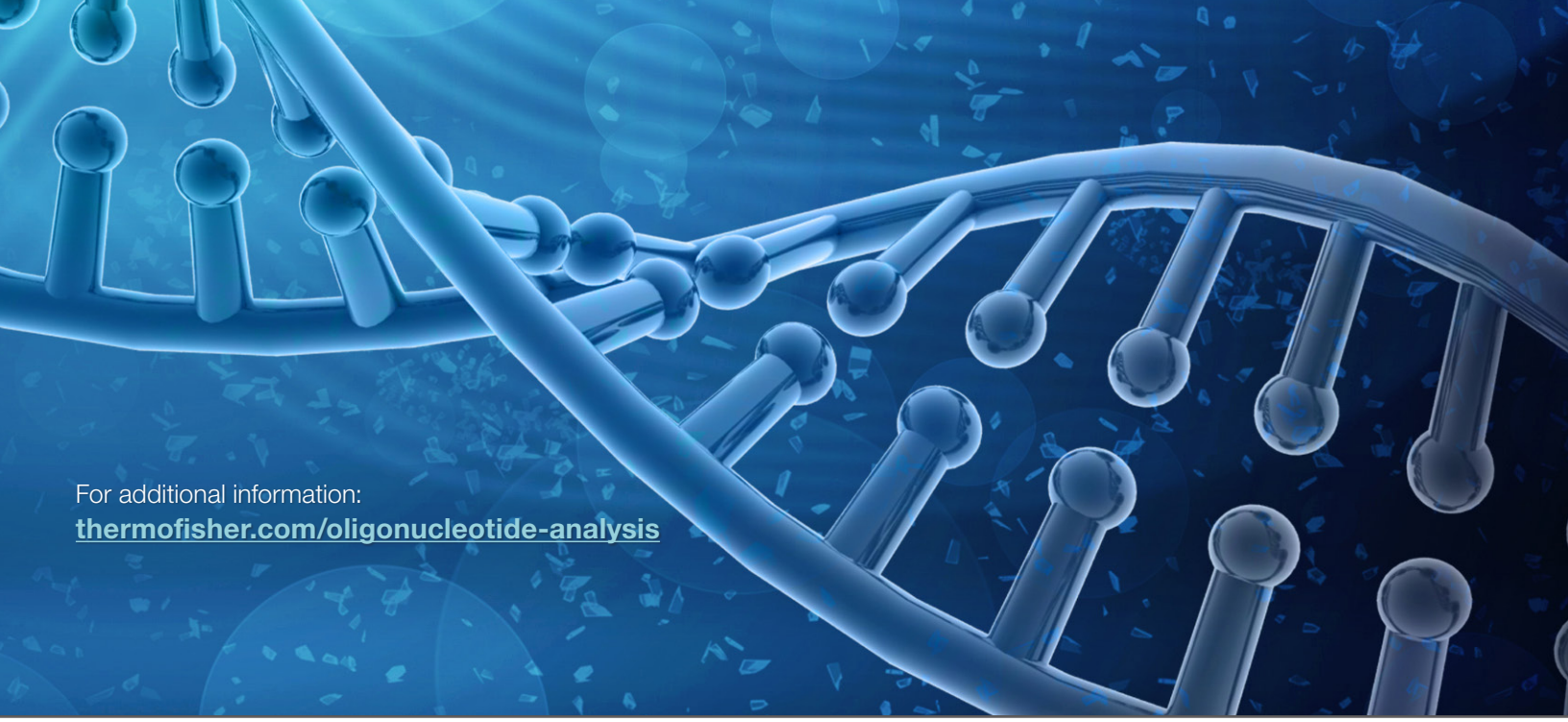
Column:	**DNAPac RP**, 4 µm
Format:	3.0 x 100mm
Mobile phase A:	Water
Mobile phase B:	Acetonitrile
Mobile phase C:	0.2 M TEAA, pH 7.0
Mobile phase D:	50mM EDTA

Gradient:

Time (min)	%A	%B	%C	%D
-8.0	27	13	50	10
0.0	27	13	50	10
12.0	24	16	50	10
12.1	15	25	50	10
14.0	15	25	50	10

Flow rate:	0.80 mL/min
Inj. volume:	2 µL
Temperature:	100 °C
Detection:	UV (260 nm)
Sample:	5'-[A*MeC*]$_{20}$-3' (0.4 mg/mL)

A = Adenine
MeC = 5-methyl-Cytosine
** = phosphothioate linkage*
All ribose are 2'-O-methylated

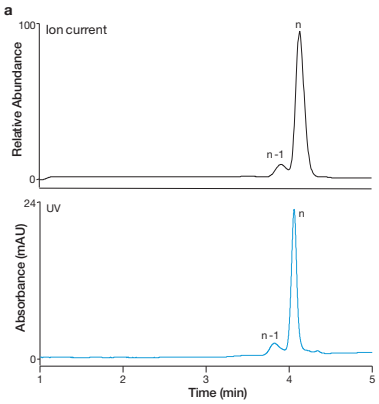


Identification and Quantification of Oligonucleotides using LC/MS

The use of liquid chromatography mass spectrometry (LC-MS) for oligonucleotide analysis is a growing tool for those in research and quality control environments. Within a single analysis users can confidently identify and measure oligonucleotides, determining structural changes and quantitating minor components in their oligonucleotide sample.

The data below, demonstrates increased productivity and confidence levels in the analysis of synthetic oligonucleotides by high resolution mass spectrometry and separated using the DNAPac RP column.

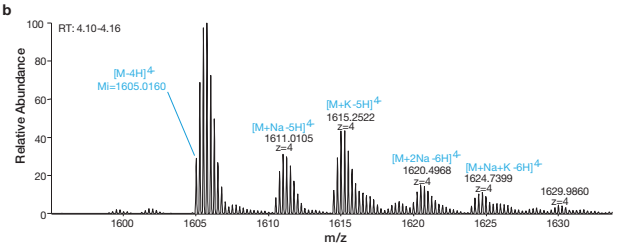
More application references by high resolution accurate mass or single quadrupole mass spectrometry can be found at www.thermofisher.com/oligonucleotides

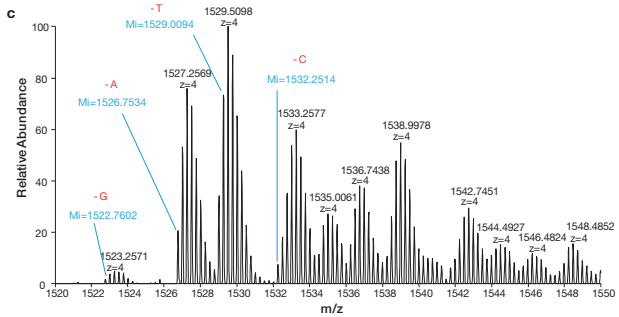


Column:	**DNAPac RP**, 4 µm
Format:	2.1 x 50 mm
Mobile phase A:	15 mM TEA 400 mM HFIP, pH 7.9
Mobile phase B:	15 mM TEA 400 mM HFIP in Water / Methanol (50:50 v/v)

Gradient:	Time (min)	%A	%B
	0.0	70	30
	3.0	48	53
	3.1	10	90
	5.0	10	90
	5.1	70	30
	11.0	70	30

Temperature:	60 °C
Flow rate:	0.25 mL/min
Inj. volume:	4 µL
Detection:	UV (260 nm) MS (Negative-ion mode)
Mass Spec:	Q Exactive Plus
Sample:	21mer DNA GATTGTAGGTTCTCTAACGCT

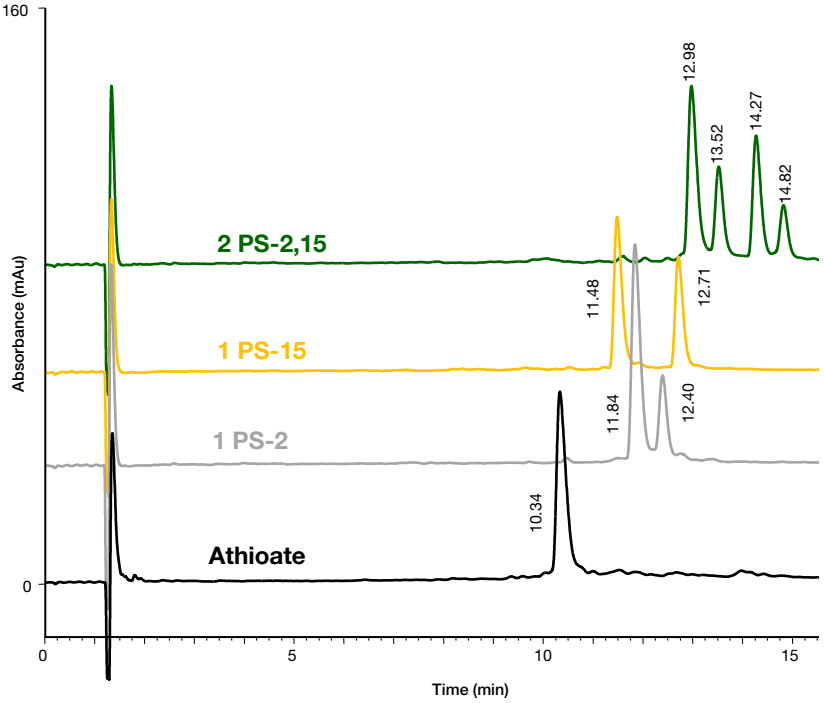




LC/MS analysis of failure sequences. a) UV and ion current traces. b) Mass spectrum of 21mer at -4 charge state. c) Mass spectrum of n-1 failure sequence at -4 charge state.

DNAPac PA200/ DNAPac PA200 RS Columns

The Thermo Scientific DNAPac PA200 and DNAPac PA200RS are ideal for oligonucleotide separations by HPLC, offering unsurpassed resolution for full-length oligonucleotides and n-1, n+1 separations based on the size and charge of the sample. Applications that may pose a challenge by reversed-phase chromatography, such as diastereoisomers of phosphorothioated RNA, resolve with ease on these strong anion exchange columns. Our proprietary non-porous particles deliver high-resolution separations with minimal sample diffusion. Users can optimize their selectivity through the control of salt concentration and mobile phase pH. The polymeric media is compatible with mobile phases up to pH 12, providing maximum flexibility in method design.



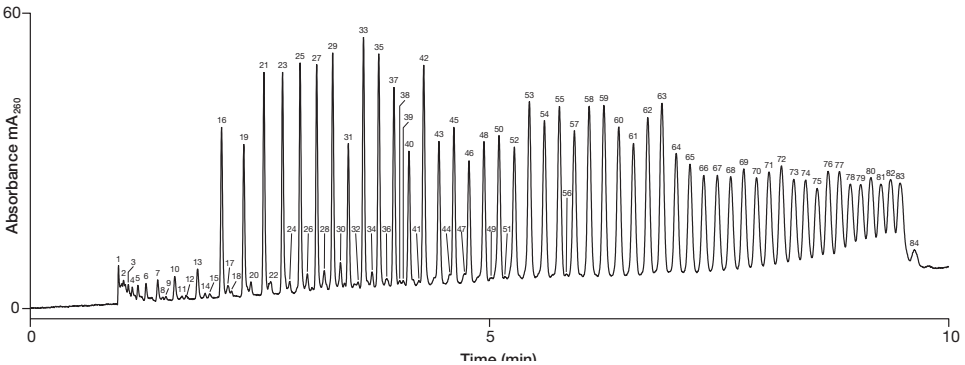
Separation of Diastereoisomers of Phosphorothioated RNA

Column:	**DNAPac PA200**, 8 µm
Format:	4.6 x 250mm
Mobile phase A:	40 mM Tris, pH 7.0
Mobile phase B:	40 mM Tris, 1.0 M NaCl

Gradient:	Time (min)	%A	%B
	-10.0	60.0	40.0
	0.0	60.0	40.0
	14.6	48.7	51.3
	22.0	20.0	80.0

Flow rate:	1.0 mL/min
Temperature:	41 °C
Detection:	UV (260 nm)
Sample:	37mer RNA (phosphorothioate at 2, 15)
Peak label:	RT

Whether your main application is the routine screening of synthetic oligonucleotides for production yield and failure sequences, or resolving a high molecular weight mRNA sample, our DNAPac PA200 and DNAPac PA200RS anion-exchange columns offer you the resolving power for your most challenging sample. The 8µm DNAPac PA200 is optimal for conventional HPLC users. Those looking for UHPLC separations, or running more complicated samples will prefer the 4µm, PEEK-lined SST, DNAPac PA200RS for the highest resolution separation.



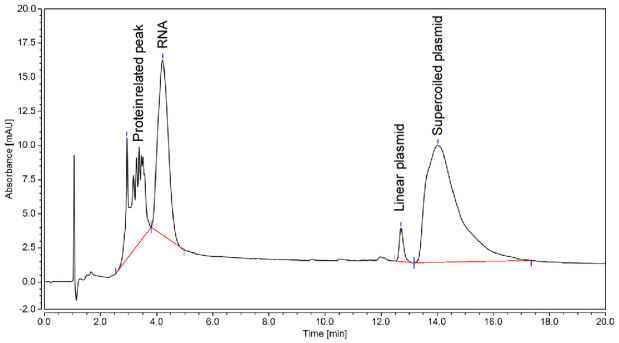
Column:	**DNAPac PA200 RS**, 4 µm
Format:	4.6 x 150mm
Mobile phase A:	40 mM Tris, pH 8.0
Mobile phase B:	40 mM Tris, pH 8.0, 1.0 M NaCl

Gradient:	Time (min)	%A	%B
	-10.0	59	41
	0.0	59	41
	8.4	35	65
	8.5	20	80

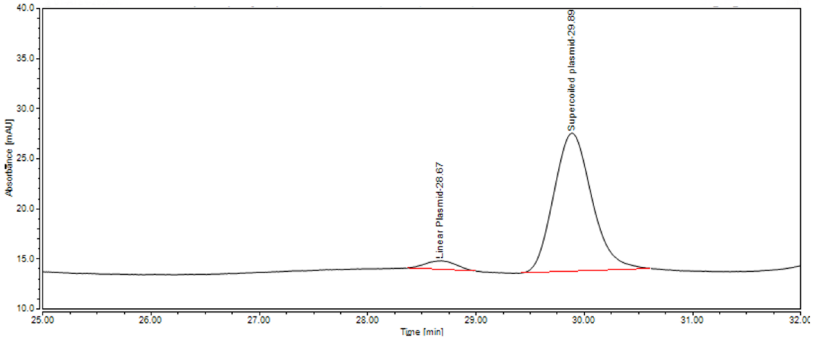
Gradient Curve:	3
Flow rate:	1.0 mL/min
Inj. volume:	15 µL
Temperature:	30 °C
Detection:	UV (260 nm)
Sample:	poly dT12-60 (0.4 A/mL)

Plasmid Separations

Biopharmaceutical advances in gene therapy and RNA/DNA vaccinations has driven the need to analyse and purify larger biomolecules, such as plasmid DNA (pDNA). As sample complexity increases so does the need for analytical flexibility, as demonstrated below, our anion-exchange and ion-pair reversed-phase chromatography columns each deliver high resolution and sample throughput for these samples. Whether your lab preference is anion exchange or ion-pair reversed phase chromatography, you will find the separation of linear and supercoiled plasmids from proteins, RNA, and other sample components.



Separation on a DNAPac PA200 anion exchange column showing the resolution of linear from supercoiled plasmid, in a sample rich with RNA and protein related material.

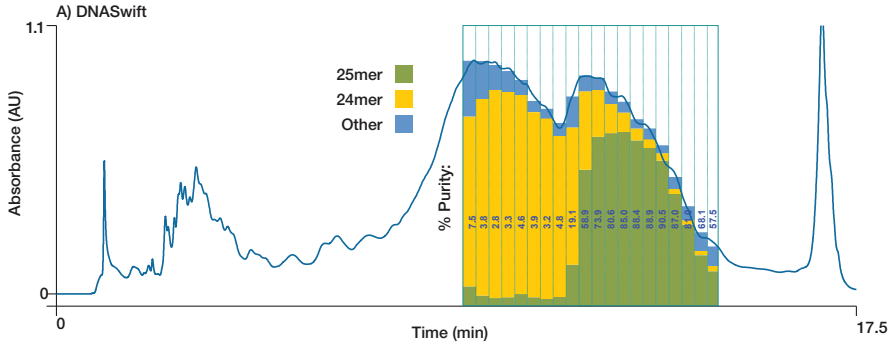


The supermacroporous DNAPac RP (2.1x100mm) supermacroporous column offers high resolution separation of linear and supercoiled plasmid forms.

Thermo Scientific DNASwift SAX-1

Thermo Scientific™ DNASwift™ SAX-1 columns deliver high yield analytical scale purifications. The high surface area, monolithic support of this column provides a robust option for scientists looking for anion-exchange purifications with a high load capacity. The DNASwift is recommended for mg-scale separations of crude mixtures when purification is of greater priority than resolution.

The figure below shows the chromatography of a mixed DNA sample. Fractions were collected during the gradient, highlighted by the shaded areas, and evaluated using a DNAPac PA200 for purity and yield. The purity of each fraction is plotted onto the chromatogram in blue, yellow and green.



Separation of Diastereoisomers of Phosphorothioated RNA

Column:	**DNASwift**
Format:	5 × 150 mm
Mobile phase A:	Water
Mobile phase B:	0.2 M NaOH
Mobile phase C:	0.2 M Tris, 0.2 M AMP, 0.2 M Diisopropylamine, pH 7.2
Mobile phase D:	1.25 M NaCl

Gradient:

Time (min)	%A	%B	%C	%D
0.00	73.6	12.1	7.9	6.4
0.01	73.6	12.1	7.9	6.4
1.00	32.0	12.1	7.9	48.0
15.01	16.0	12.1	7.9	64.0
15.51	0.0	12.1	7.9	80.0
16.50	0.0	12.1	7.9	80.0
17.00	73.6	12.1	7.9	6.4

Flow rate:	1.77 mL/min
Inj. volume:	1 mL
Temperature:	30 °C
Detection:	UV (295 nm)
Sample:	Mixture of full length DNA (45%) and n-1 DNA (55%) Full length: CTGATTGTAGGTTCTCTAACGCTGG n-1: CTGATTGTAGGTTCTCTAACGCTG

‡ *AMP: 2-amino-2-methyl-1-propanol*

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Ordering Information

DNAPac PA200 column

Particle Size (µm)	Format	Length (mm)	2.0 mm ID	4.0 mm ID	9.0 mm ID	22.0 mm ID
8	Standard Analytical Column	250	063425	063000	063421	088781
5	Guard Column	50	063423	062998	063419	088780

DNAPac PA200 RS, high resolution analytical column

Particle Size (µm)	Format	Length (mm)	4.6 mm ID
4	UHPLC	50	082508
		150	082509
		250	082510

DNAPac RP column

Particle Size (µm)	Format	Length (mm)	2.1 mm ID	3.0 mm ID
4	Analytical Column	100	088923	088919
		50	088924	088920
	Guard Cartidges (2/pk)	10	088925	088921
Guard Cartridge Holder			069580	069580

DNASwift SAX-1S column

Particle Size (µm)	Format	Length (mm)	5.0 mm ID
Monolith	HPLC Column	150	066766

Find out more at **thermofisher.com/BioLC**

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