BioLC columns

# Thermo Scientific DNAPac columns

## Superior oligonucleotide analysis

thermo scientific

DNAPac
RP column
2024

DNAPac
RP column
2016

DNAPac
PA200 RS column
2013

DNASwift
SAX-1S column
2009

DNAPac
PA200 column
2004

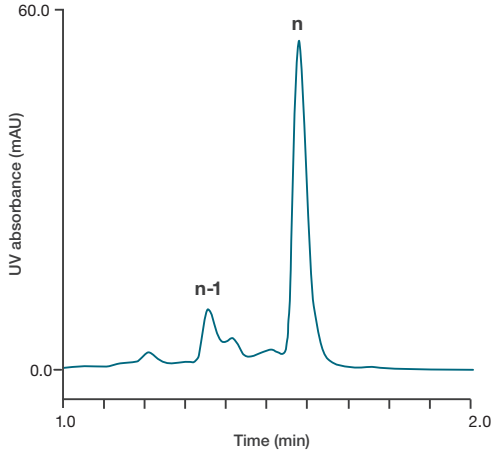
DNAPac
PA100 column
1990

For over 30 years, the Thermo Scientific™ DNAPac™ family of columns has been the go-to for high resolution 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 column series. 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™ PA200 RS Columns, scientists now experience high resolution ion exchange separations for their oligonucleotide purity analysis by liquid chromatography-ultra violet (LC-UV). DNAPac 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)



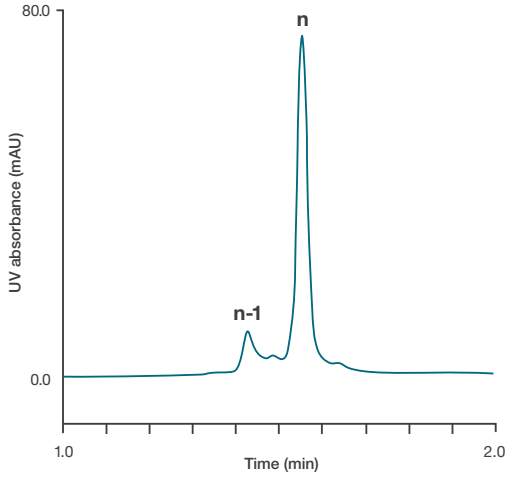

| | |
|---|---|
| **Column:** | **DNAPac PA200 RS** |
| **Format:** | 4.6 x 50 mm |
| **Mobile phase A:** | 20 mM Tris, pH 8.0 |
| **Mobile phase B:** | 20 mM Tris, 1 M NaCl, pH 8.0 |

| **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 50 mm |
| **Mobile phase A:** | 0.1 M TEAA |
| **Mobile phase B:** | 0.1 M 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) |

Learn more at **gene therapy workflow brochure**

# We invest in your chromatography

## Technology that delivers benefits

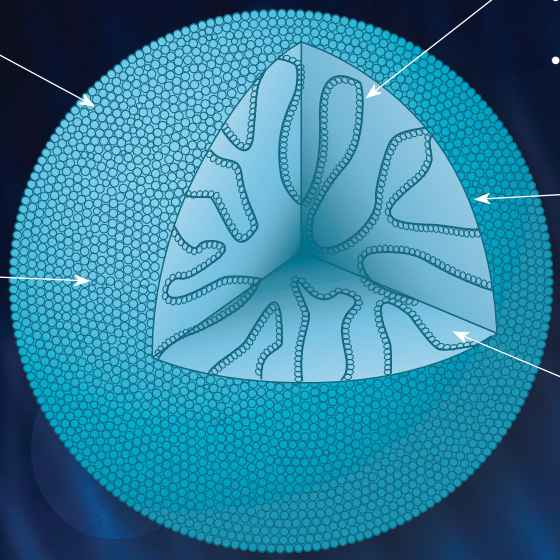
### DNAPac PA200 and PA200 RS columns

**Non-porous resin with latex nano beads on the surface**

- Minimizes diffusion
- Offers high-resolution separations

**Quaternary amine functionalized nano beads**

- Delivers single base resolution of up to 100mer oligonucleotides
- Achieves a higher loading capacity compared to conventional non-porous columns



**Polymeric base media**

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

**Polymeric base media**

- Long-lasting column lifetimes
- Optimizes pH control selectivity with column compatibility up to pH 12
- Compatible with high temperature (85 °C) up to pH 8

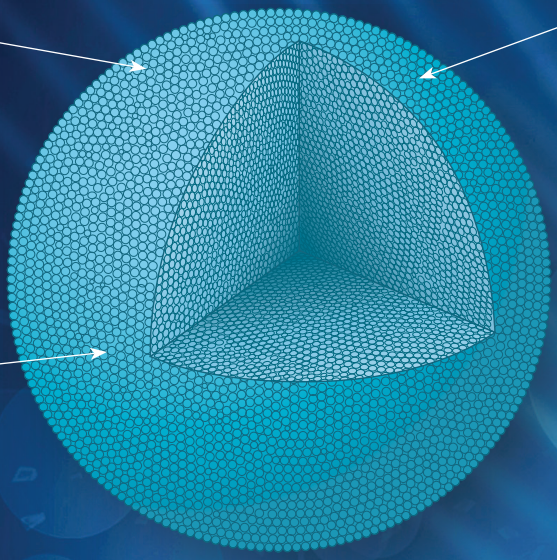
**Format options**

- High sample throughput is available from standard HPLC and rapid separation (RS) UHPLC formats

### DNAPac RP column

**Polymeric (PS-DVB) resin**

- Higher temperature stability (up to 110 °C)
- Can operate up to pH 14
- Long-lasting column lifetime compared to conventional silica column



**Optimized surface chemistry**

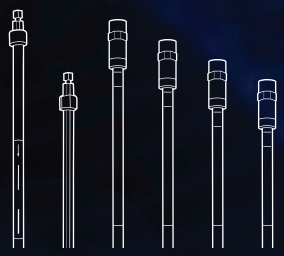
- Long-lasting column lifetime for siRNA, ASOs, and other short oligonucleotides sequences
- Compatible with liquid chromatography-mass spectrometry (LC-MS)
- Reliable data

**Wide pore size**

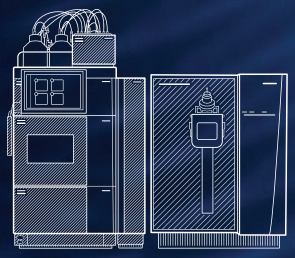
- Supermacroporous structure up to 2,000 Å for excellent separation of short [siRNA] and long [mRNA] oligonucleotide samples
- Very low carryover
- Long-lasting column lifetime

# Oligonucleotide analysis

## Solutions for oligonucleotide therapeutic analysis



HPLC Columns



LC-MS systems

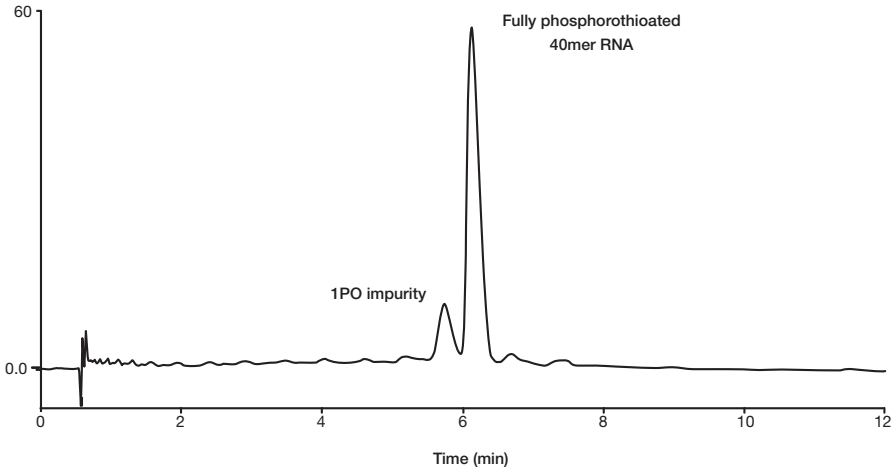

Software and data analysis

### DNAPac RP columns

The DNAPac RP 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 temperatures. The supermacroporous structure has small pores as well as 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 column. 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.

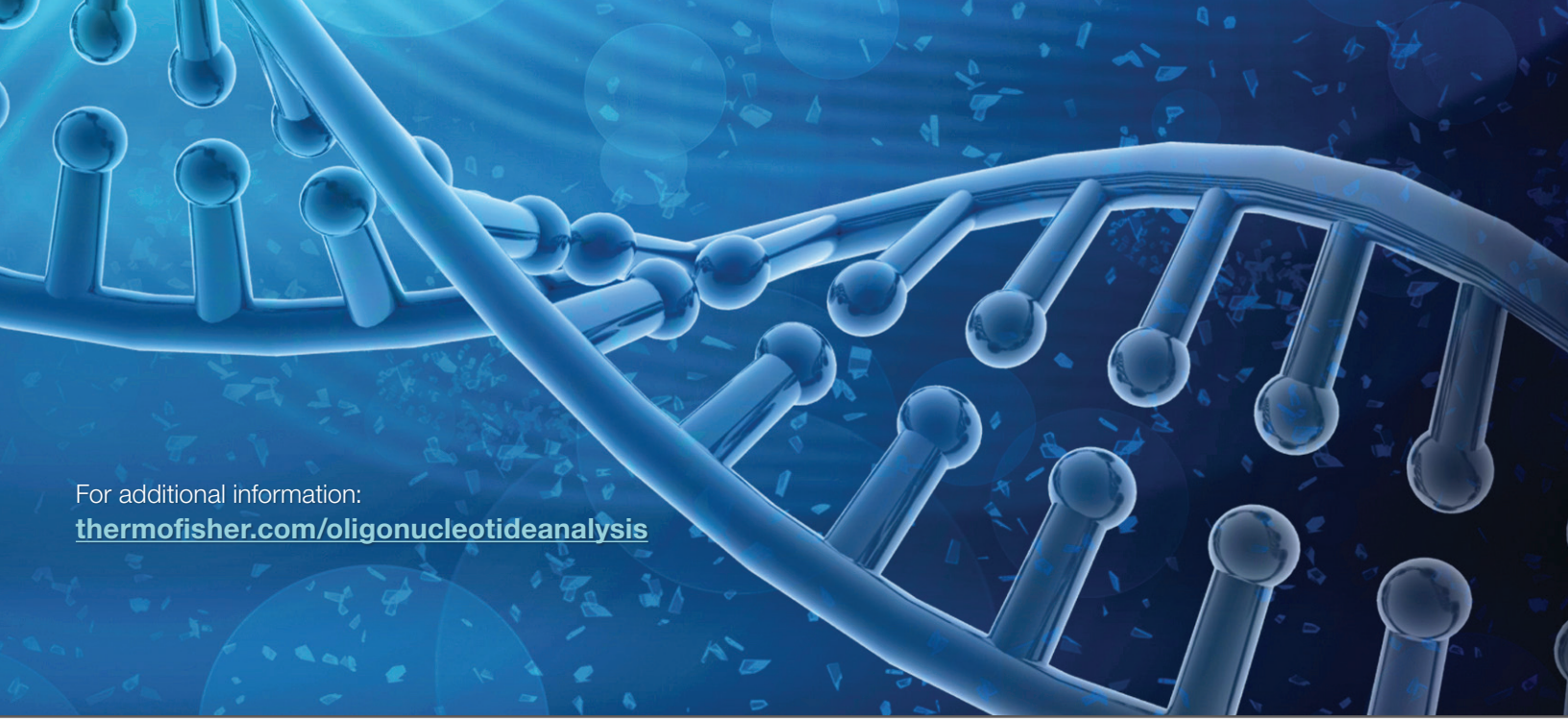
Learn more **about this analysis**




**1PO separation from fully phosphorothioated 40mer RNA**

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

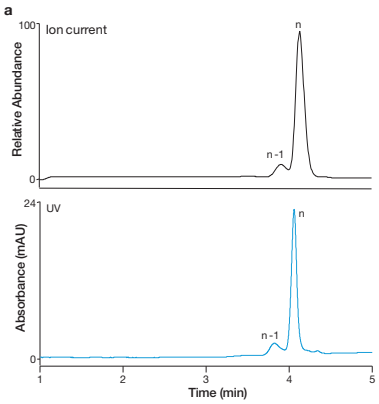



## Identification and quantification of oligonucleotides using LC-MS

The use of 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 to determine structural changes and quantitate 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.

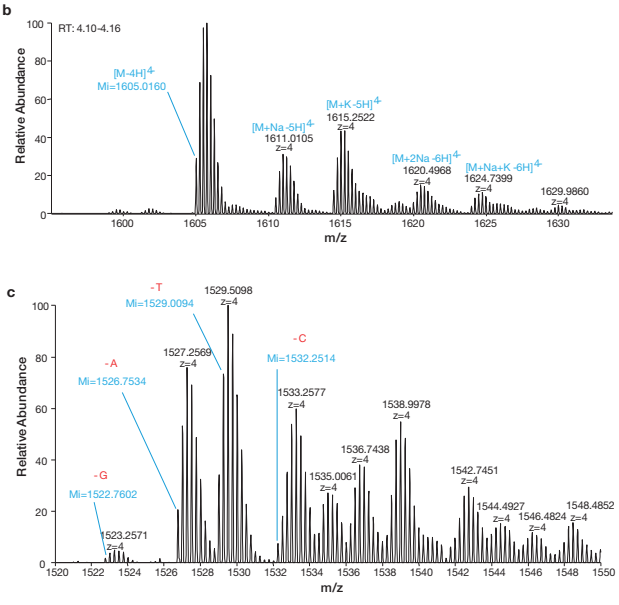
Visit **thermofisher.com/oligonucleotides** to view more high-resolution accurate mass or single quadrupole mass spectrometry applications.




| | |
|---|---|
| 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 |

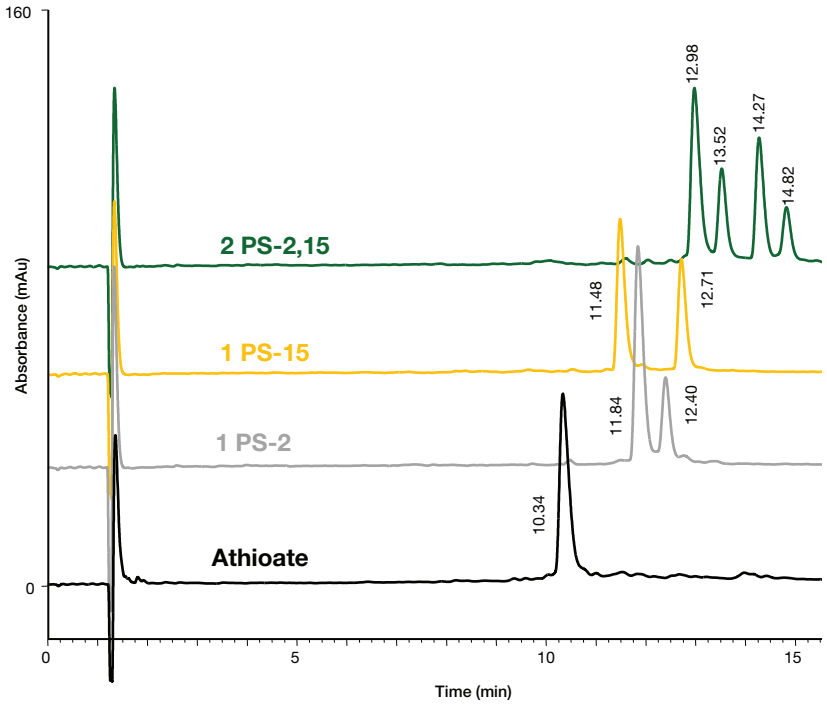
| | |
|---|---|
| Temperature: | 60 °C |
| Flow rate: | 0.25 mL/min |
| Inj. volume: | 4 µL |
| Detection: | UV (260 nm) MS (Negative-ion mode) |
| Mass Spec: | Q Exactive Plus |
| Sample: | 21mer DNA GATTGTAGGTTCTCTAACGCT |




**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 and DNAPac PA200 RS columns

The DNAPac PA200 and DNAPac PA200 RS 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.



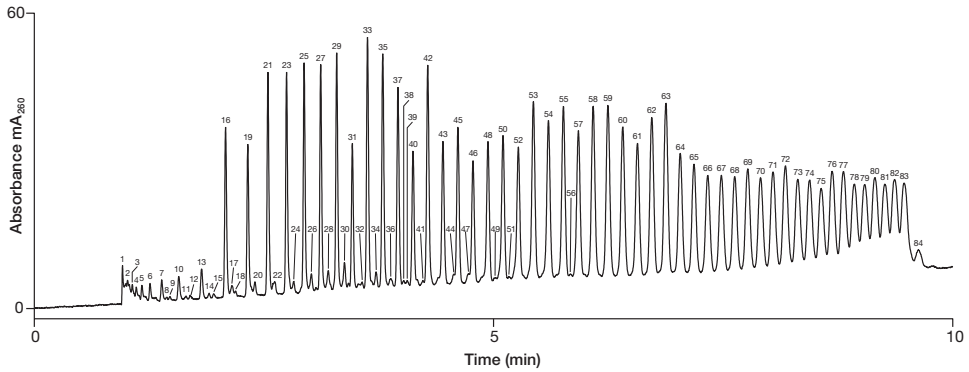

**Separation of diastereoisomers of phosphorothioated RNA**

| | |
|---|---|
| Column: | **DNAPac PA200**, 8 µm |
| Format: | 4.6 x 250 mm |
| 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 PA200 RS anion exchange columns offer you the resolving power for your most challenging sample. The 8 µm DNAPac PA200 column is optimal for conventional HPLC users. Those looking for UHPLC separations, or running more complicated samples will prefer the 4 µm, PEEK-lined SST, DNAPac PA200 RS column for the highest resolution separation.



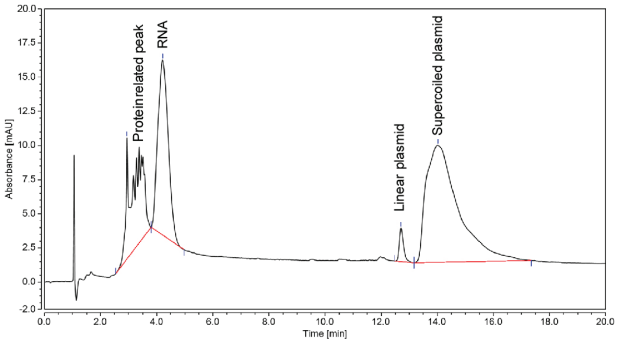

| | |
|---|---|
| Column: | **DNAPac PA200 RS**, 4 µm |
| Format: | 4.6 x 150 mm |
| Mobile phase A: | 40 mM Tris, pH 8.0 |
| Mobile phase B: | 40 mM Tris, pH 8.0, 1.0 M NaCl |

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

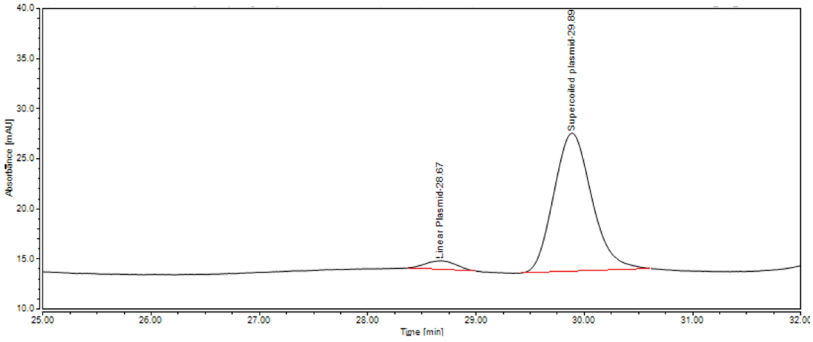
| | |
|---|---|
| Gradient Curve: | 3 |
| Flow rate: | 1.0 mL/min |
| Inj. volume: | 15 µL |
| Temperature: | 30 °C |
| Detection: | UV (260 nm) |
| Sample: | poly dT12-60 (0.4 A/mL) |

## 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**

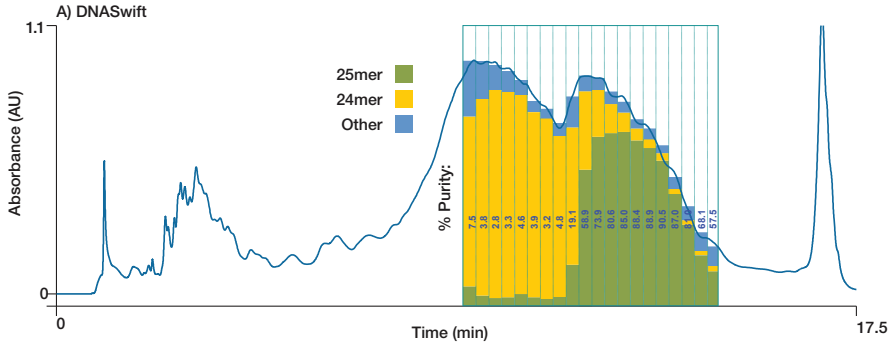



**The supermacroporous DNAPac RP (2.1 x 100 mm) column offers high resolution separation of linear and supercoiled plasmid forms**

## DNASwift SAX-1S column

Thermo Scientific™ DNASwift™ SAX-1S column delivers 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 SAX-1S column 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 column 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 SAX-1S** |
| **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%)<br>Full length:<br>CTGATTGTAGGTTCTCTAACGCTGG<br>n-1: CTGATTGTAGGTTCTCTAACGCTG |

[‡] *AMP: 2-amino-2-methyl-1-propanol*

# Ordering information

| Description | | | | |
|---|---|---|---|---|
| **DNAPac PA200 columns** | 2.0 mm ID | 4.0 mm ID | 9.0 mm ID | 22.0 mm ID |
| Analytical column, 8 µm, 250 mm | 063425 | 063000 | 063421 | 088781 |
| Guard column, 8 µm, 50 mm | 063423 | 062998 | 063419 | 088780 |
| **DNAPac PA200 RS columns** | | 4.6 mm ID | | |
| HPLC or analytical column, 4 µm, 250 mm | – | 082510 | – | – |
| HPLC or analytical column, 4 µm, 150 mm | – | 082509 | – | – |
| HPLC or analytical column, 4 µm, 50 mm | – | 082508 | – | – |
| **DNAPac RP columns** | 2.1 mm ID | 3.0 mm ID | 10.0 mm ID | 21.2 mm ID |
| Analytical column, 4 µm, 250 mm | 303324 | – | – | – |
| Analytical column, 4 µm, 100 mm | 088923 | 088919 | – | – |
| Analytical column, 4 µm, 50 mm | 088924 | 088920 | – | – |
| Semi-preparative column, 4 µm, 150 mm | – | – | SP6998 | 080922-1521232 |
| Guard cartridges (2/pack) | 088925 | 088921 | – | 080922-0121232 |
| Guard cartridge holder | 069580 | 069580 | – | 950-00 |
| **DNASwift SAX-1S column** | | 5.0 mm ID | | |
| HPLC column, monolith, 150 mm | – | 066766 | – | – |

Learn more at **thermofisher.com/biolc**