DNAPac PA100 Columns

034411 Revision 10 • November 2015

Thermo SCIENTIFIC

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Product Manual

for

DNAPac PA100 Guard
2 × 50 mm (P/N 088761)

DNAPac PA100
2 × 250 mm (P/N 088760)

DNAPac PA100 Guard
4 × 50 mm (P/N 043018)

DNAPac PA100
4 × 250 mm (P/N 043010)

DNAPac PA100
9 × 250 mm (P/N 043011)

DNAPac PA100
22 × 250 mm (P/N 088759)

Revision History:

Revision 10, November, 2015, Rebranded for Thermo Scientific. Converted special part numbers to standard part numbers.

Safety and Special Notices

Make sure you follow the precautionary statements presented in this guide. The safety and other special notices appear in boxes.

Safety and special notices include the following:

 **SAFETY**	***Indicates a potentially hazardous situation which, if not avoided, could result in death or serious injury.***
 **WARNING**	***Indicates a potentially hazardous situation which, if not avoided, could result in damage to equipment.***
 **CAUTION**	***Indicates a potentially hazardous situation which, if not avoided, may result in minor or moderate injury. Also used to identify a situation or practice that may seriously damage the instrument, but will not cause injury.***
 **NOTE**	***Indicates information of general interest.***
IMPORTANT	***Highlights information necessary to prevent damage to software, loss of data, or invalid test results; or might contain information that is critical for optimal performance of the system.***
Tip	***Highlights helpful information that can make a task easier.***

Contents

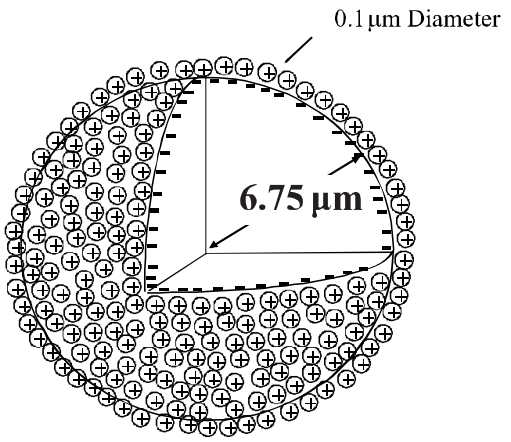
1. Introduction

The DNAPac PA100 anion exchange column is specifically designed to provide high-resolution separations of nucleic acids with scaleup capability. The DNAPac PA100 can:

- achieve N, N-1 resolution over a wide range of oligomer lengths;
- perform separations under high temperature or high pH denaturing conditions;
- be scaled from analytical to lab-scale preparative sizes without changing methods;
- utilize volatile buffers for sample recovery without desalting.

The packing material is composed of 100 nm quaternary amine functionalized MicroBeads™ bound to a 13 µm solvent-compatible, non porous substrate (Figure 1, "DNAPac PA100 Packing Material"). Benefits include rapid mass transport, higher loading capacity than conventional nonporous materials, and good durability. The result is a scaleable column with the same resolution as non-scaleable anion exchange columns that use 2 to 3 µm resins.

Figure 1 DNAPac PA100 Packing Material



Column	Particle Size µm	Substrate X-Linking %	Latex Diameter nm	Latex X-Linking %	Column Capacity µeq/column	Functional Group	Hydrophobicity
DNAPac PA100 Analytical 4x250	13.5	55	100	5	40	Alkyl quaternar	Low
DNAPac PA100 Guard 4x50	13.5	55	100	5	5	Alkyl quaternar	Low
DNAPac PA100 Semi-Prep 9x250	13.5	55	100	5	200	Alkyl quaternary amine	Low

2.Installation

2.1 Operational Parameters

pH Range:	pH 2 to 10 (unrestricted eluents)
and counterion, such as	pH 10 to 12.5 (requires equimolar concentrations of hydroxide chloride, see Section 3.2, “Denaturing Conditions”)
Temperature Limit:	90 °C
Pressure Limit:	5,000 psi (34.5 MPa) (4 x 250 mm)
Organic Solvent Limit:	100% Acetonitrile.
Detergent Compatibility:	Nonionic, cationic or zwitterionic detergents.
	Do not use anionic detergents.
Chaotrope Limit:	30% formamide, 6 M Urea
Typical Eluents:	Sodium, potassium, or lithium salts of chloride, bromide,
perchlorate or acetate.	

2.2 Eluent Limitations

The DNAPac PA100 is generally compatible with typical eluents such as sodium chloride up to their limit of solubility. Test the solubility limit of eluents in the presence of organic solvents. Some combinations of eluent salts and organic solvents are not miscible. Anionic detergents will irreversibly bind to the column.

2.3 Compatibility with Stainless Steel Systems

Use of stainless steel tubing, ferrule and bolt assemblies is not recommended because they will strip the threads of the PEEK end fittings. Use of a stainless steel pump is not recommended because the halide salt eluents typically use for the DNAPac PA100 will cause corrosion of metallic components. Such corrosion may lead to decreased column performance.

Therefore, a metal-free pump system is recommended.

2.4 System Void Volume

Minimize the number of unions and the length of all liquid lines. Tubing between the injection valve and detector should be

≤ 0.010" i.d. PEEK tubing. The use of larger diameter tubing may decrease peak resolution.

3. Example Applications

3.1 Sample Preparation

Matrix Interferant	Effect	Possible Removal
Halides	High concentrations of salts in the sample will affect the retention time of analytes.	Dialysis, dilution, ethanol precipitation
Anionic Detergents	Will bind irreversibly to column.	Dialysis, dilution, solid phase extraction using the OnGuard RP Cartridge.

3.2 Denaturing Conditions

Self-complementary sequences or poly-G stretches can result in intra- or intermolecular associations of oligonucleotides. These associations can prevent efficient separations under non-denaturing conditions. For example, the attempted separation of pd(G)12-18 at ambient temperature with a 100–900 mM NaCl gradient at pH 8.0 results in a single broad peak on the DNAPac PA100. The DNAPac PA100 can be run under denaturing conditions to analyze samples containing such sequences.

Denaturing conditions can be achieved by running at high pH (up to pH 12.5), or high temperature (up to 90 °C), or with chaotropic agents (such as 6 M urea). Operating at pH 12.4 eliminates hydrogen bonding, dramatically improving the chromatography of samples such as G-rich oligonucleotides (see Section 3.7, “High G-Content Oligonucleotide Analysis ”). High pH eluents may also improve the separation of other analytes. The DNAPac PA100 is sensitive to high pH eluents, but is compatible with hydroxide when used with at least equimolar concentrations of selective salt (example: 20 mM NaOH, with at least 20 mM NaCl).

Guanosine (G) and thymidine (T) both begin to lose protons at pH values >= 9.2. This confers a more negative charge at higher pH values, resulting in stronger binding to the DNAPac resin. This property permits selectivity changes by changing the eluent pH (Thayer et. al., 1996, 2005).

High temperature and high pH conditions in the same method will cause premature column degradation. Use only high pH or high temperature or chaotropic agents to denature the analyte.

3.3 Modified DNA

Peptide-DNA complexes have been successfully purified away from non-cross-linked peptides (Allen et. al., 1991). Same length oligomers with 6-substituted guanines and 4-substituted thymines have been separated (Xu and Swann, 1992). DNA labeled with biotin and horseradish peroxidase are easily separated from unlabeled DNA.

3.4 Production Test Chromatograms

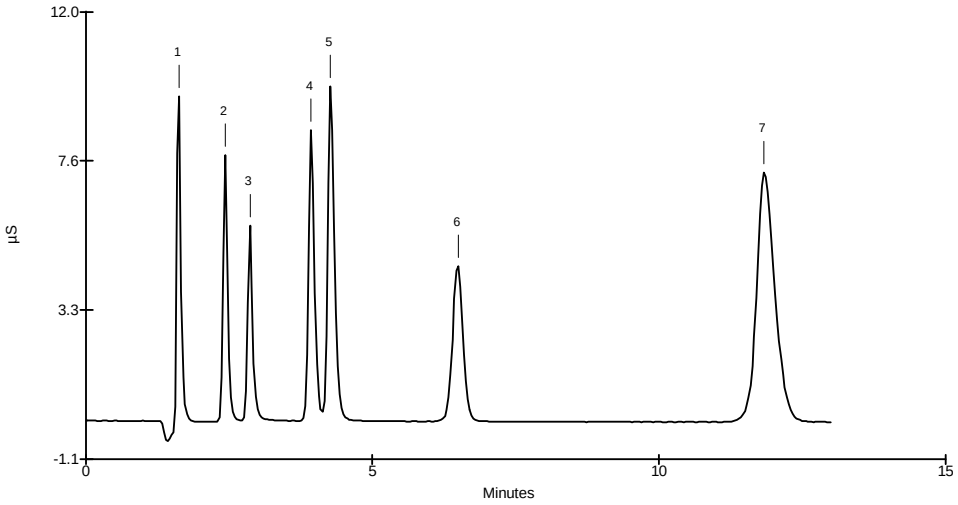
To guarantee that all DNAPac PA100 Analytical Columns meet high quality and reproducible performance specification standards, all columns undergo the following production control test. Because gradient separation is not an accurate test for determining column capacity and packing quality, an isocratic separation of seven inorganic anions is designed to measure individual column performance utilizing a sodium carbonate eluent.

Production and Performance Parameters Measured by the Seven Inorganic Anions

Retention time of sulfate is used to measure the capacity of the column with an acceptable range of 10–13 minutes. Peak efficiency and peak symmetry of sulfate are used to measure the packing quality of the column. Retention times and resolution of fluoride, chloride, nitrite, bromide, nitrate and phosphate are used to measure the overall selectivity of the column.

Sample Volume:	25 µL
Column:	DP PA100
Eluent:	1.8 mM Na_2CO_3/1.7 mM $NaHCO_3$
Eluent Flow Rate:	1.0 mL/min
SRS Suppressor:	Anion Self-Regenerating Suppressor ULTRA, ASRS ULTRA II AutoSuppression® Recycle Mode
or MMS Suppressor:	Anion MicroMembrane Suppressor, AMMS III
MMS Regenerant:	50 mN H_2SO_4
Regenerant Flow Rate:	10–15 mL/min
Expected Background Conductivity:	15–20 µS
Storage Solution:	800 mM NH_4Cl/25 mM Tris / 10% Acetonitrile

Figure 2 DNAPac PA100 Production Test Chromatogram



	Analyte	mg/L
1.	Fluoride	2.0
2.	Chloride	3.0
3.	Nitrite	5.0
4.	Bromide	10.0
5.	Nitrate	10.0
6.	Phosphate	15.0
7.	Sulfate	15.0

(where 1 mg/L = 1 ppm)

3.4.1 Certificate of Performance - Resin Batch Testing

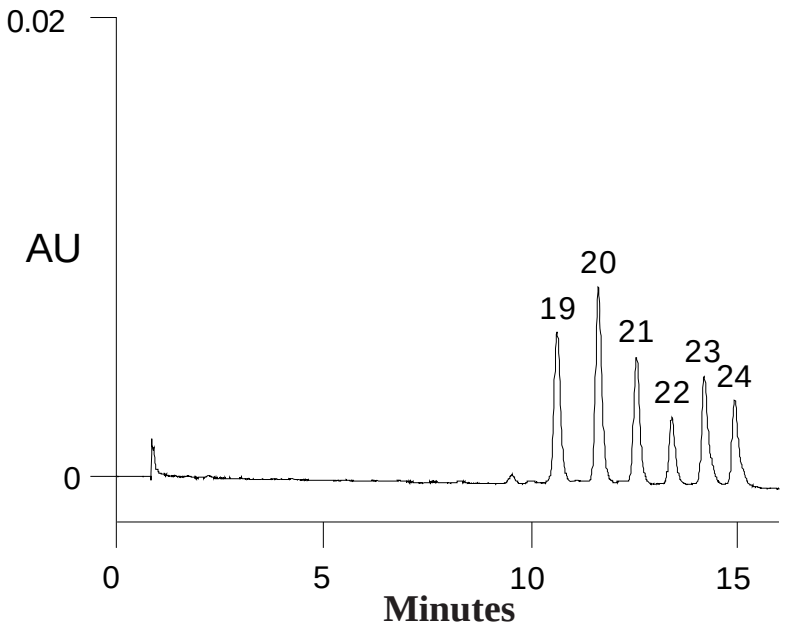
Each resin batch used for packing the DNAPac PA100 columns is tested to ensure reliable performance. Each batch is tested by separating pd(T)$_{19-24}$ with a DNAPac column packed with the production resin. This procedure ensures that resins with the highest quality are used for column production.

Sample Volume:	25 µL
Column:	DNAPac PA100
Eluent 1:	25 mM Tris/0.5% Acetonitrile, final pH adjusted to 8.0
Eluent 2:	25 mM Tris/800 mM NH_4Cl/0.5% Acetonitrile
Eluent Flow Rate:	1.5 mL/min
Storage Solution:	90% Eluent 2/10% Acetonitrile
Detection:	Absorbance at 254 nm, 0.1 AUFS
Standard:	pd(T)$_{19-24}$, 10 mL Loop containing 2.5 mg of sample

Gradient:

Time	%1	%2	Comments
0.0	55	45	Equilibration of Column
8.0	55	45	Sample Injection
9.0	55	45	1 min isocratic
25.0	35	65	16 min Gradient Ramp
26.0	0	100	0.8 M salt wash
29.0	0	100	Hold 3 min
30.0	55	45	reset

Figure 3 Certificate of Performance DNAPac PA100



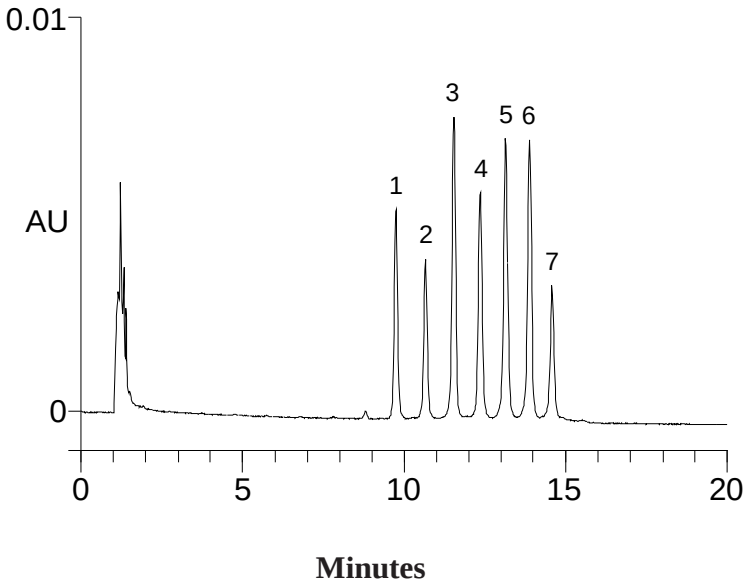
3.5 Single-stranded Phosphodiester Analysis

High resolution (typically single-base) separations of phosphodiester oligonucleotides can be accomplished using a NaCl gradient with a moderate pH buffer. A typical method for oligomers less than 30 bases long is a 100 to 450 mM NaCl gradient in the presence of 25 mM Tris-Cl, pH 8. Injection at 100 mM NaCl ensures that the sample binds efficiently to the resin. Longer oligomers can be analyzed by loading at 100 mM NaCl and eluting with NaCl gradients up to 2 M. The stronger eluent, $NaClO_4$, will elute a given oligomer at a lower eluent concentration than NaCl. A ten minute equilibration after the gradient prepares the column for the next injection.

Sample: 1 mg pd(A)12-18 in 200 mL TE
Column: DNAPac PA100, 4 x 250 mm
Eluent: 100 to 450 mM NaCl convex gradient in 25 mM Tris-Cl, pH 8.0
Flow Rate: 1.5 mL/min.
Detection: UV, 260 nm

	Time (min.)	%A	%B	%C	Curve *		
Eluent A: Deionized H_2O	0.00	80	10	10	5		
Eluent B: 0.25 M Tris-Cl, pH 8.0	20.00	45	10	45	4		
Eluent C: 1.0 M NaCl	20.50	0	10	90	5		
	24.50	0	10	90	5		
	25.00	80	10	10	5		
	35.00	80	10	10	5		

Figure 4 Single-stranded Phosphodiester Analysis



Component Name
1. pd(A) 12
2. pd(A) 13
3. pd(A) 14
4. pd(A) 15
5. pd(A) 16
6. pd(A) 17
7. pd(A) 18

* Refers to the DIONEX GP40 pump gradient shape. Gradient 5 is linear. Gradient 4 is convex.

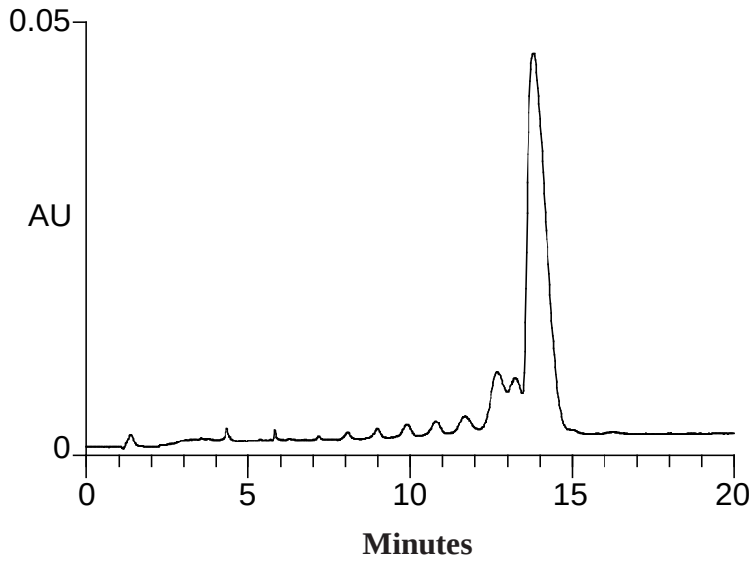
3.6 Phosphorothioate Oligonucleotide Analysis

Substitution and deletion failure sequences from synthetic phosphorothioate oligonucleotides can analyzed by using a $NaClO_4$ gradient with a moderate pH buffer. Since phosphorothioates are retained much more strongly on the DNAPac PA100 than phosphodiester oligonucleotides, the conventional NaCl gradient is replaced in this example with a $NaClO_4$ gradient. The 15-mer has been previously purified to remove the deletion failure sequences, so the separation is due primarily to the degree of thioation.

Sample: 15-mer (T15) phosphorothioate, previously purified to remove deletion failures
Column: DNAPac PA100, 4 x 250 mm
Eluent: 56 to 323 mM $NaClO_4$ convex gradient
Flow Rate: 1.5 mL/min.
Detection: UV, 260 nm

	Time (min.)	%A	%B	%C	Curve *		
Eluent A: Deionized H_2O	0.00	88	10	2	5		
Eluent B: 0.25 M Tris-Cl, pH	8.00	0.10	75	10	15	5	
Eluent C: 0.375 M $NaClO_4$	20.10	4	10	86	4		
	21.50	0	0	100	5		
	29.50	0	0	100	5		
	30.00	88	10	2	5		
	45.00	88	10	2	5		

Figure 5 Phosphorothioate Oligonucleotide Analysis



* Refers to the DIONEX GP40 pump gradient shape. Gradient 5 is linear. Gradient 4 is convex.

3.7 High G-content Oligonucleotide Analysis

Hydrogen bonding in samples with self-complementary sequences or poly-G stretches promote intra and intermolecular associations that restrict access of these sequences to the stationary phase. Hydrogen bonds can be broken by operating at pH 12.4, resulting in greatly improved chromatography compared to operating at moderate pH. The analysis of pd(G)12-18 can be accomplished with a NaCl gradient in the presence of 25 mM NaOH, pH 12.4.

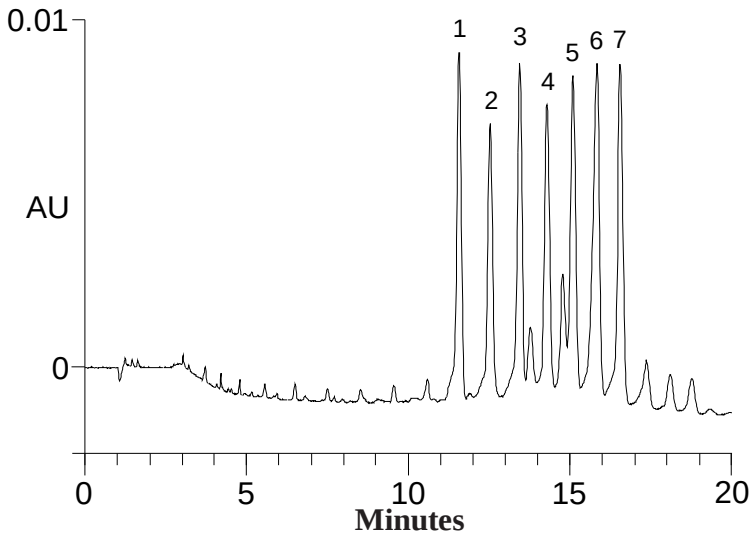
Sample: pd(G)12-18 1 mg in 200 mL
Column: DNAPac PA100, 4 x 250 mm
Eluent: 500 to 900 mM NaCl convex gradient, pH 12.4
Flow Rate: 1.5 mL/minutes
Detection: UV, 260 nm

	Time (min.)	%A	%B	Curve *		
Eluent A: 25 mM NaOH		0.00	90.0	10.0	5	
Eluent B: 1 M NaCl in 25 mM NaOH		2.00	50.0	50.0	5	
	20.00	10.0	90.0	4		
	20.50	0	100.0	5		
	24.50	0	100.0	5		
	25.00	90.0	10.0	5		
	40.00	90.0	10.0	5		

Component Name

1. pd (G) 12
2. pd (G) 13
3. pd (G) 14
4. pd (G) 15
5. pd (G) 16
6. pd (G) 17
7. pd (G) 18

Figure 6 High G-content Oligonucleotide Analysis



* Refers to the DIONEX GP40 pump gradient shape. Gradient 5 is linear. Gradient 4 is convex.

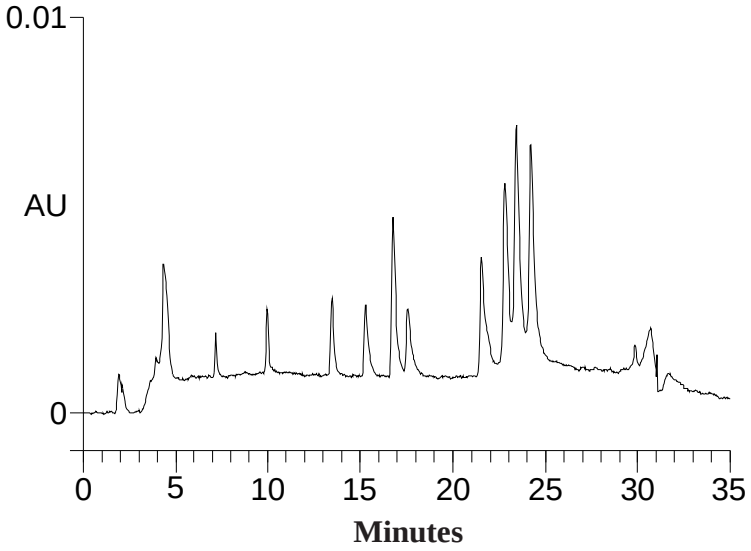
3.8 Double-Stranded DNA Analysis

Double stranded DNA such as plasmid restriction fragments and PCR products can be analyzed on the DNAPac PA100 using a $NaClO_4$ gradient in the presence of Tris-Cl, pH 8 (adapted from Thayer, et. al., 1996).

Sample: ØX174 DNA/Hae III digest
Column: DNAPac PA100, 4 x 250 mm
Eluent: 120 to 184 mM $NaClO_4$ convex gradient
Flow Rate: 1.0 mL/min.

	Time (min.)	%A	%B	%C	Curve *		
Eluent A: Deionized H_2O	0.00	88	10	2	5		
Eluent B: 0.25 M Tris-Cl, pH	8.00	0.10	58	10	32	5	
Eluent C: 0.375 M $NaClO_4$	26.10	32	10	58	3		
	26.50	0	0	100	5		
	34.50	0	0	100	5		
	35.00	88	10	2	5		
	50.00	88	10	2	5		

Figure 7 Double-stranded DNA Analysis



* Refers to the DIONEX GP40 pump gradient shape. Gradient 5 is linear. Gradient 3 is convex.

3.9 Preparative Applications

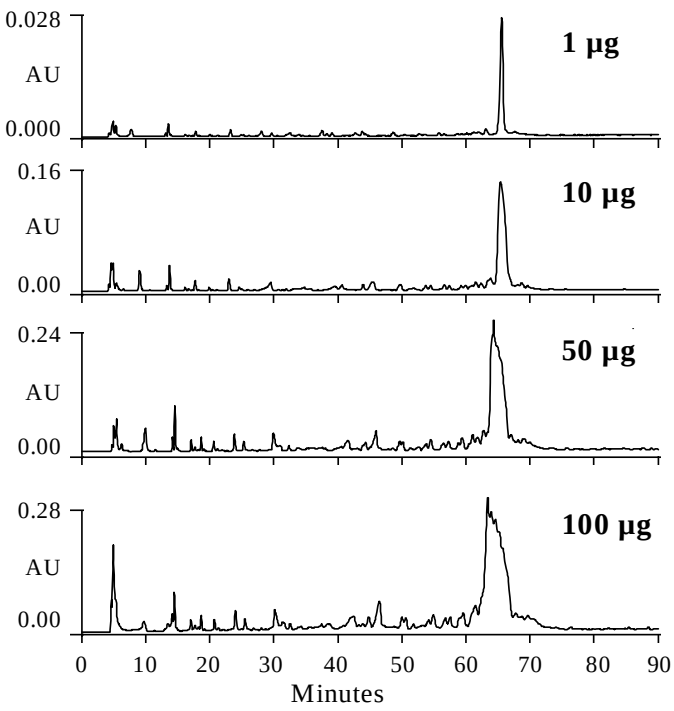
Analytical separations on the 4-mm diameter column can be scaled directly to larger columns, so that preparative methods can be conveniently developed with small samples.

First, inject a range of sample sizes to determine the optimal load. To scale up the method, calculate the new flow rate and sample load by multiplying the current flow rate and sample load by the ratio of the cross sectional areas of the preparative column and the analytical column.

3.9.1 17-mer Preparative Example

A crude synthetic 17-mer (the -20 sequencing primer) was analyzed on the 4-mm diameter column with a 1 µg load. The flow rate was set to 0.33 mL/min. to allow scale up to a 22-mm diameter column on the same analytical HPLC system (see Application Note 100, "High-Resolution Analysis and Purification of Oligonucleotides with the DNAPac PA100 Column," for complete details). The load was increased in later runs to 10, 50 and 100 µgs.

Figure 8 DNAPac Loading Study



Column:	DNAPac PA100, 4 x 250 mm
Eluent:	7.5 to 124 mM $NaClO_4$ Nonlinear gradient in 25 mM Tris-Cl, pH 8.0
Flow Rate:	0.33 mL/min
Detector:	UV, 260 nm
Sample:	-20 primer

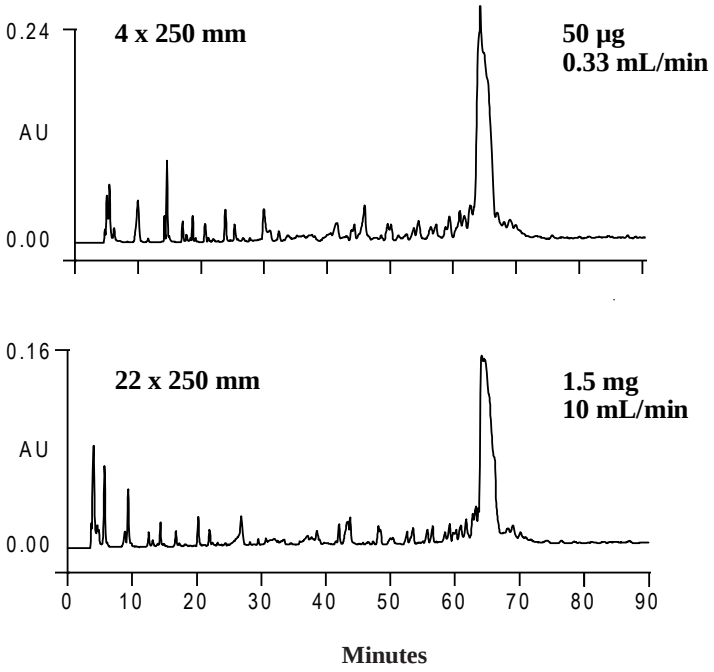
To scale up to a 22 mm diameter column, the flow rate was increased to 10 mL/min. and the load increased to 1.5 mg.

(0.33 mL/min.)(π(11.0 mm)^2 / π(2.0 mm)^2) = 10.0 mL/min.

(50 µg) (π(11.0 mm)^2 / π(2.0 mm)^2) = 1.5 mg

Figure 9 Transfer to Preparative Scale

The 50 µg load was selected to be scaled to a 22 mm diameter column.

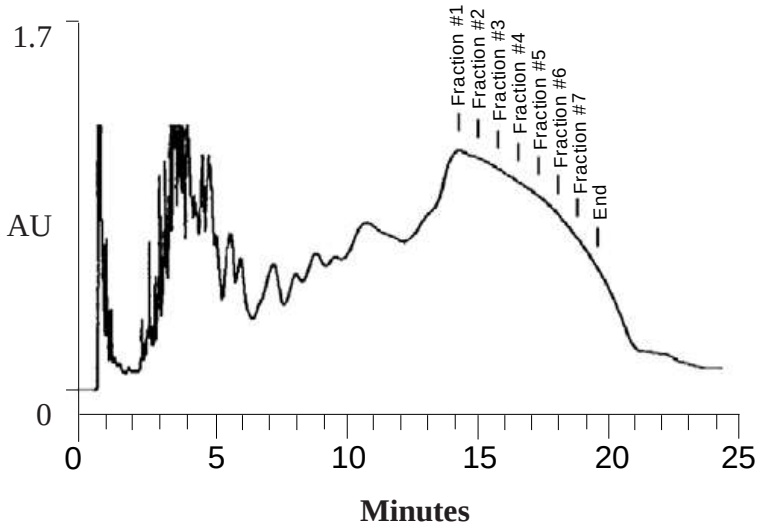


Column:	DNAPac PA100, 4 x 250 mm
Eluent:	7.5 to 124 mM $NaClO_4$ Nonlinear gradient in 25 mM Tris-Cl, pH 8.0
Flow Rate:	0.33 mL/min
Detector:	UV, 260 nm
Sample:	-20 primer

3.9.2 25-mer Preparative Example

The DNAPac PA100 can be loaded further with an apparent loss in resolution. The following example of a crude 25-mer purification demonstrates the loss of resolution with a 1.0 mg load on a 4 x 250 mm column. However, reinjecting a sample of the pooled fraction reveals a relatively pure product. This is possibly due to sample self-displacement chromatography

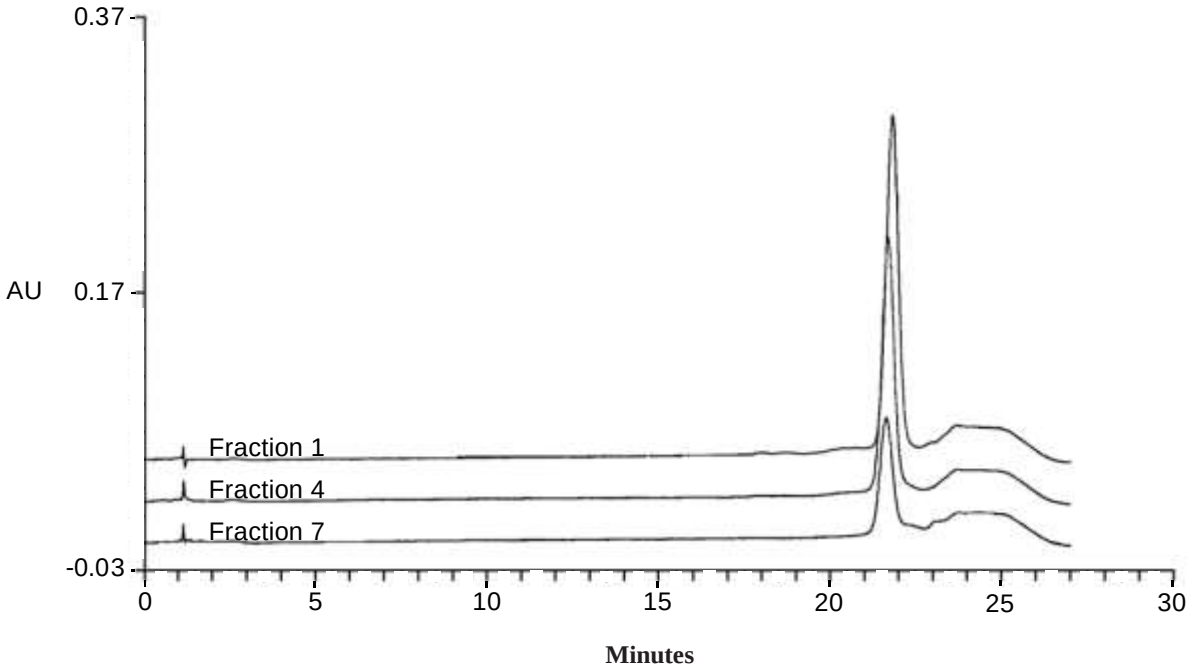
Figure 10



Three of the resulting fractions (1, 4, and 7) were re-chromatographed as shown in Figure 9. Fractions 1 through 7 comprise more than 60% of the applied crude oligonucleotide.

Re-chromatography of fractions from the 25-mer of composition G8, A5, C5, T7 on a 4 x 250 mm DNAPac PA100. The synthetic oligomer was applied to the column in 870 mM ammonium acetate and eluted with a gradient of 0.87 to 1.1 M ammonium acetate pH 6.0 at 1.5 mL/min.

Figure 11



4. Troubleshooting Guide

4.1 Finding the Source of High System Back Pressure

A. A significant increase in system back pressure may be caused by a plugged inlet frit.
B. Before replacing the inlet bed support assembly of the column, make sure that the column is the cause of the excessive back pressure.
C. Check for pinched tubing or obstructed fittings from the pump outlet, throughout the eluent flow path to the detector cell outlet.

To do this, disconnect the eluent line at the pump outlet and observe the back pressure at the usual flow rate. It should not exceed 50 psi (0.3 MPa). Continue adding components (injection valve, column, detector) one by one while monitoring the system back pressure. The 4 x 250 mm DNAPac PA100 should add no more than 1,000 psi (6.9 MPa) back pressure at 1 mL/min. The 4 x 50 mm DNAPac PA100 should add no more than 400 psi (2.8 MPa) back pressure at 1 mL/min. No other component should add more than 100 psi (0.7 MPa) to the system back pressure.

4.2 Back Pressure on Column Increasing

The DNAPac PA100 (4 x 250 mm) operates at less than 1,000 psi (6.9 MPa) at 1.5 mL/min. High system backpressure maybe due to the system (such as pinched tubing or an obstructed fitting) or the column (such as a fouled bed support or compressed resin bed).

If the high back pressure is due to the column, first try cleaning the column. If the high back pressure persists, replace the column bed support.

4.3 Decreasing Peak Retention Times

Eluent contaminants may be decreasing column capacity. Use deionized water with a specific resistance of 18.2 megohm-cm. Sterile filter through a 0.45 mm filter. Also check for changes in eluent concentration and pH. Use of this column at high pH and elevated temperature will also result in decreasing retention times due to loss of phase. Avoid the combination of high pH and elevated temperature.

4.4 Decreasing Peak Efficiency and Resolution

A. If changes to the system plumbing have been made, check for excess lengths of tubing, larger than normal tubing diameter and leaks.
B. The column may be fouled. Try cleaning the column.
C. The column may have been subjected to pressures above 5,000 psi (34.5 MPa), causing irreversible bed compression.

4.5 Poor Peak Efficiency and Resolution

Try alternate eluents such as NaClO4, KBr or LiBr. Try operating under denaturing conditions, which will abolish hydrogen bonding, improving peak shape and efficiency.

4.6 Unidentified Peaks Appear

A. Intra- or intermolecular oligonucleotide associations may cause unidentified peaks. Operate under denaturing conditions.
B. The sample may be degrading. When appropriate, check for nuclease degradation and dephosphorylation.
C. The eluents may be contaminated. Use deionized, filtered water.

Appendix A – Column Care

A.1 New Column Equilibration

At a minimum, new DNAPac PA100 columns should be washed with the starting eluent for 20 minutes at 1 mL/min. (for the 4 x 250 mm column). Due to residual material from the packing process eluting from new columns, some high sensitivity applications may require an additional cleanup procedure.

The following cleanup is recommended for all new columns:

Eluent A: Deionized H2O
Eluent B: 0.8 M NaCl
Eluent C: 60% Acetonitrile
Monitor baseline at UV, 260 nm.

1. Equilibrate the column with eluent A.
2. With the column equilibrated with eluent A, use your pump to mix 50% B + 50% C for 1 hour at 1 mL/minute.
3. After 1 hour, step to 25% B + 25% C + 50% A for 30 minutes at 1 mL/minute.
4. After 30 minutes, step to 50% B + 50% C at 1 mL/minute. If the baseline rises, continue wash for 30 minutes and return to Step 3. Otherwise, continue to Step 5.
5. Equilibrate to initial conditions.

A.2 Column Cleanup

High pressure bands can be created by pumping successive eluents that are not miscible through the column. Examples of immiscible eluents are those containing eluent components in one eluent that will precipitate out when in contact with the second eluent, or use of an acidic eluent followed by a basic eluent which may create a neutralization pressure band. Salt precipitation in solvents during column washes can result in very high pressure bands.

WARNING

Do not switch between eluents quickly. This will create high pressure eluent interface bands in the column. The column bed packing can be disrupted by high pressure bands, damaging the column performance.

WARNING

Removing ionic contaminants from the column requires a small amount of electrolyte to remain in the eluent. Therefore, washes with 100% organic solvent are not recommended.

WARNING

The DNAPac PA100 resin is sensitive to eluent pH greater than 12.5, therefore cycling between high pH eluents and low pH eluents is not recommended.

A.2.1 Fouled Columns

The following cleanup is recommended for fouled columns:

Eluent A: Deionized H_2O
Eluent B: 1 M NaClO4
Eluent C: 100% Acetonitrile
Monitor baseline at UV, 260 nm.

1. Equilibrate the column with eluent A.
2. With the column equilibrated with eluent A, step to 20% A + 40% B + 40% C for 1 hour at 1 mL/minute.
3. After 1 hour, step to 50% A + 25% B + 25% C for 30 minutes at 1 mL/minute.
4. After 30 minutes, step to 20% A + 40% B + 40% C at 1 mL/minute. If the baseline rises, continue wash for 30 minutes and return to Step 3. Otherwise, continue to Step 5.
5. Equilibrate to initial conditions.

A.2.2 Metal Contamination

The following cleanup is recommended for columns fouled with acid-soluble contaminants:

Eluent A: 100 mM oxalic acid/50 mM NaOH (pH 2.5 to 3.0)

Dissolve 6.2 g of oxalic acid dihydrate with 4.2 g (2.6 m) of 50% w/w sodium hydroxide concentrate solution in 400ml of deionized water having a specific resistance of 18.2 megohm-cm. When dissolved, dilute to a final volume of 500 mL with deionized water.

1. Pump 100% Eluent A through the column for 1 hour at 1 mL/minute.
2. Equilibrate to initial conditions.

CAUTION

When cleaning an analytical column and guard column in series, move the guard column after the analytical column in the eluent flow path. Otherwise contaminants that have accumulated on the guard column can be eluted onto the analytical column causing irreversible damage.

A.3 Replacing Column Bed Support Assemblies

1. Carefully unscrew the inlet (top) column fitting. Use two open end wrenches.
2. Remove the bed support. Tap the end fitting against a hard, flat surface to remove the bed support and seal assembly. Do not scratch the wall or threads of the end fitting. Discard the old bed support assembly
3. Insert a new bed support assembly into the end fitting. Make sure the end of the column is clean and free of any particulate matter. Any resin on the end of the column tube will prevent a proper seal. Use the end of the column to carefully start the bed support assembly into the end fitting.
4. Screw the end fitting back onto the column. Tighten it finger-tight, then an additional 1/4 turn (25 in x lb). Tighten further only if leaks are observed.

CAUTION

If the column tube is not clean when inserted into the end fitting, particulate matter may prevent a proper seal between the end of the column tube and the bed support assembly. Further tightening will damage the column. Replace the outlet bed support only if high pressure persists after replacement of the inlet fitting.

A.4 Column Storage

Flush the column with five column volumes of 0.8 M NH_4Cl/ 25mM Tris Base/ 10% acetonitrile and plug both ends.

Appendix B – Literature and Bibliography

The following Application Note is on the Reference Library CD-ROM:

Application Note 100 "High Resolution Analysis and Purification of Oligonucleotides with the DNAPac PA100 Column."

Selected Bibliography

Allen, T. D., Wick, K .L. and K. S. Mathews. "Identification of Amino Acids in lac Repressor Protein Cross-linked to Operator DNA Specifically Substituted with Bromodeoxyuridine" Journal of Biological Chemistry, Vol. 266, No 10, pp. 6113-6119 (1991).

Xu, Y., and F. Swann. "Chromatographic Separation of Oligodeoxynucleotides with Identical Length: Application to Purification of Oligomers Containing a Modified Base" Analytical Biochemistry 204, pp. 185-189 (1992).

Rohrer, J. S. "Improved fractionation of sialylated glycopeptides by pellicular anion-exchange chromatography" Journal of Chromatography A, 667, pp. 75-83 (1994).

Bourque, A. J. and A. S. Cohen. "Quantitative analysis of phosphorothioate oligonucleotides in biological fluids using direct injection fast anion exchange chromatography and capillary gel electrophoresis" Journal of Chromatography B, 662, pp. 343-349 (1994).

Ausserer, W. A., and M. L. Biros, M. "High Resolution Analysis and Purification of Synthetic Oligonucleotides with Strong Anion Exchange HPLC" BioTechniques, 19(1), pp. 136-139 (1995).

Thayer, J., McCormack, R. M. and N. Avdalovic. "High Resolution Liquid Chromatographic Nucleic Acid Separations" Methods in Enzymology, volume 271, pp. 147-174 (1996).

Thayer, J. R., Barreto, V., Rao, S., and C. Pohl. Control of oligonucleotide retention on a pH-stabilized strong anion exchange column. Anal. Biochemistry 338: pp39-47 (2005)