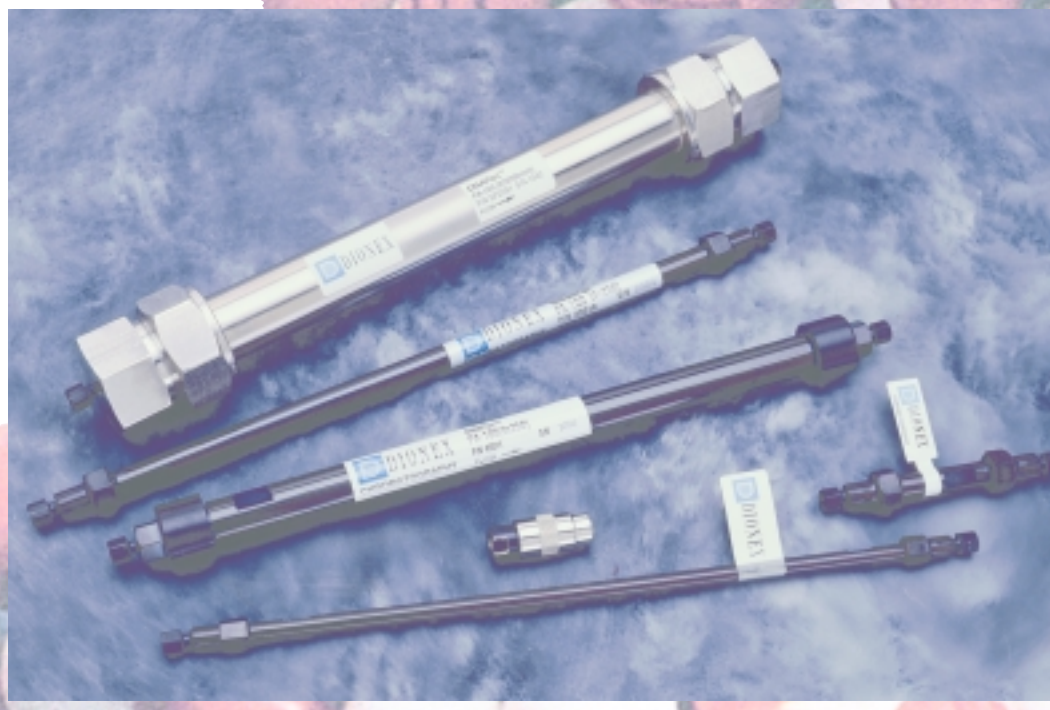
DNAPAC™ PA-100

DIONEX

Now sold under the
Thermo Scientific brand

Thermo SCIENTIFIC

The DNAPac PA-100 columns set the industry standard for oligonucleotide purity checks, fast screenings, and purification.



The Dionex DNAPac columns combine high resolution and scalability for DNA analysis.

- Achieve *N*, *N-1* resolution for oligonucleotides.
- Easy scale up.
- Assay phosphorothioate purity.
- Resolve oligonucleotides with secondary structures.
- Analyze phosphorothioate-based clinical samples.
- Compatible with solvent, high pH, and high temperatures.

N, N-1 RESOLUTION OF SYNTHETIC OLIGONUCLEOTIDES

N, N-1 resolution of single-stranded oligonucleotides up to 60 bases in length can easily be achieved.

Synthetic oligonucleotides can be screened for production yield and failure sequences on a routine basis. Figure 1 shows a chromatogram of a -20 sequencing primer. Failure sequences are separated from the main product in less than 20 minutes.

Figure 2 demonstrates the complete resolution of a synthetic pd(A)$_{12–18}$ sample. Note that the minor peaks observed in this separation are due to the resolution of the dephosphorylated and intact oligonucleotides by the DNAPac column.

Figure 3 shows a separation of a pd(A)$_{40–60}$. These oligonucleotides are well resolved using a sodium chloride gradient in 20 minutes.

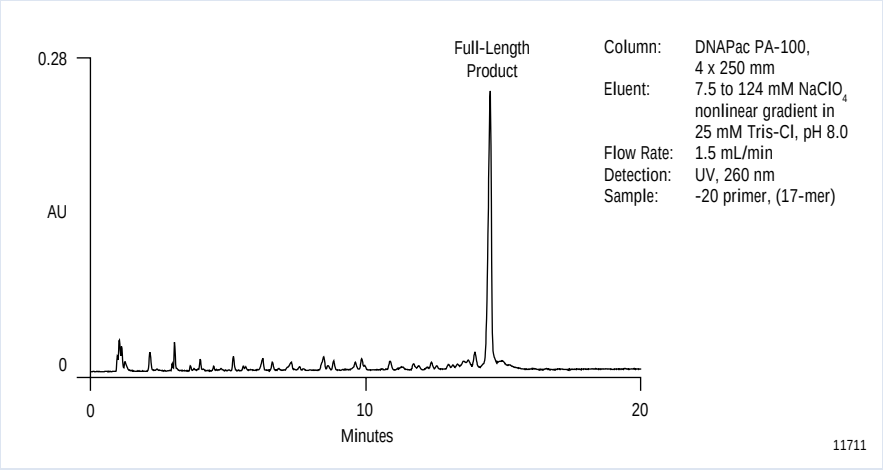


Figure 1 *DNAPac PA-100 separation of crude synthetic -20 sequencing primer (17-mer) from its failure sequences.*

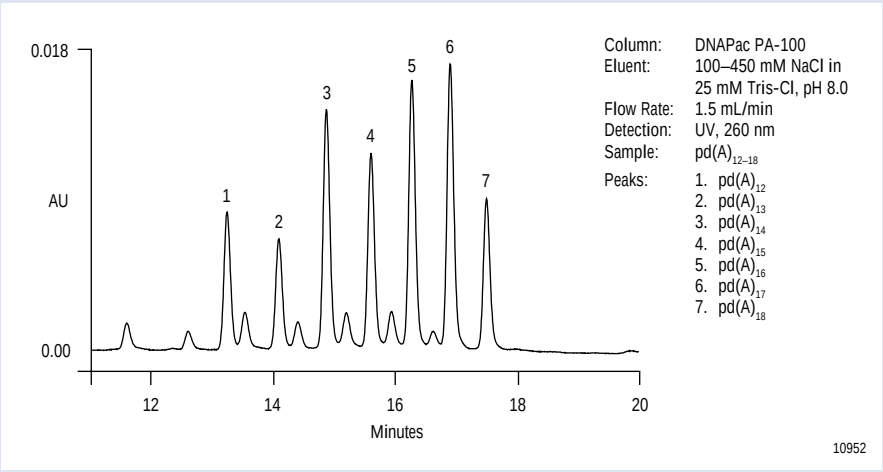


Figure 2 *Separation of 1 µg of pd(A)$_{12–18}$ at pH 8.0 with a 100 to 450 mM NaCl gradient. Small peaks represent dephosphorylated impurites.*

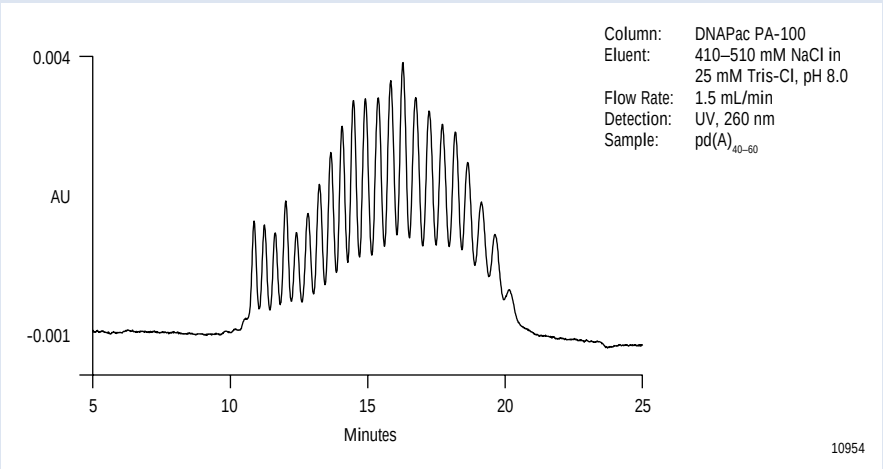


Figure 3 *Separation of 1.5 µg of pd(A)$_{40–60}$. These oligonucleotides are well-resolved in 20 minutes.*

CONVENIENT SCALE-UP

Analytical separations on the 4-mm diameter column can be scaled directly to larger diameter columns so preparative methods can be conveniently developed using small samples. The loading capacity of the DNAPac PA-100 (4 x 250 mm) column is demonstrated from 1 to 100 µg (see Figure 4A). Scaling the flow rate and sample size up for the 22 x 250 mm column yields almost identical chromatography (see Figure 4B).

FAST-CARTRIDGES—ANALYSIS OF PHOSPHOROTHIOATE-BASED CLINICAL SAMPLES

The DNAPac Fast-Cartridges are designed for quick purity check and purification. The cartridges are packed with DNAPac PA-100 resins, and are installed on-line in an HPLC system as reusable "fast columns."

The Fast-Cartridges are used for the analysis of phophorothioate-based clinical samples. Phosphorothioates in plasma samples are selectively retained on-cartridge, and subsequently characterized by analytical DNAPac columns.

The DNAPac Fast-Cartridge.

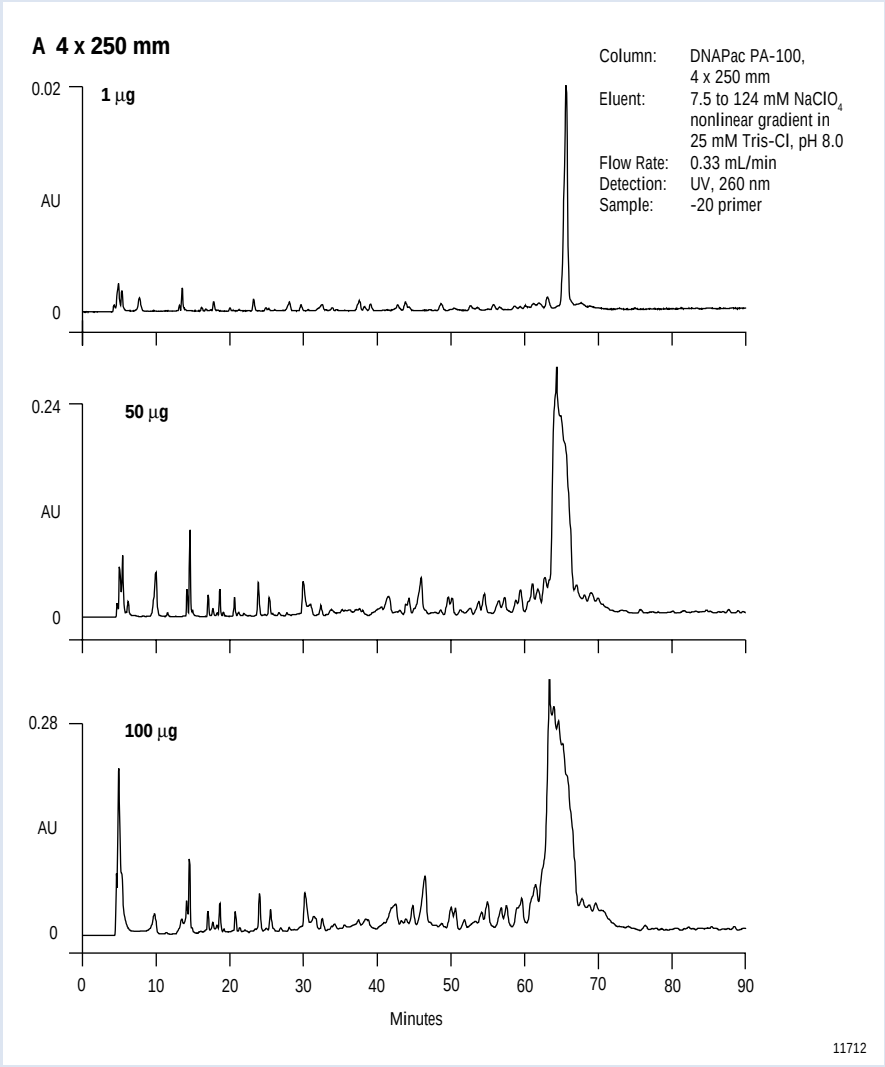


Figure 4A *The DNAPac PA-100 (4 x 250 mm) column can be scaled 100-fold from 1 to 100 µg with minimal loss in resolution.*

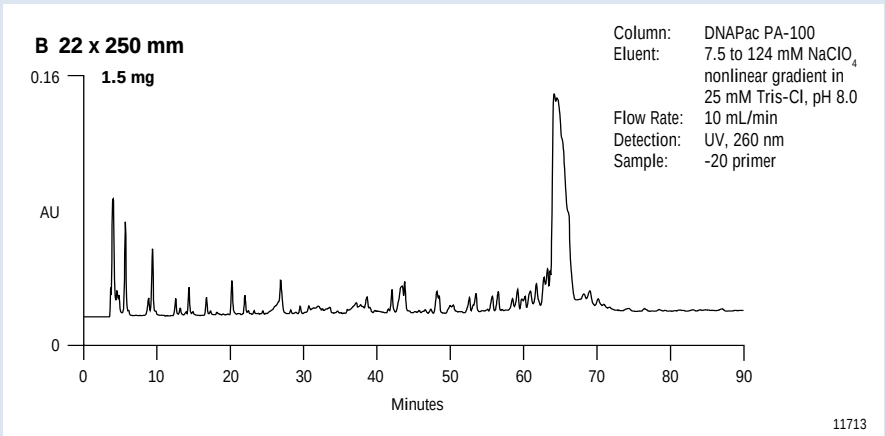


Figure 4B *Transfer to preparative scale. The 1.5-mg injection on the 22 x 250 mm column is equivalent to a 50-µg injection on the 4 x 250 mm column. Note that the analytical separations in Figure 4A are run at a reduced flow rate (0.33 mL/min) so that for comparative purposes, the preparative separations in Figure 4B can be run on the same analytical pump at 10 mL/min.*

OLIGONUCLEOTIDE PURIFICATION

The DNAPac PA-100 column exhibits substantially greater loading capacity than other pellicular columns. Typically, pellicular columns deliver sharp peaks below their loading capacity (usually between 0.5 and 5 µg), and increasingly broad peaks as that limit is exceeded. Figure 5A shows that a standard DNAPac PA-100 (4 x 250 mm) column delivers good analytical chromatography even when 89 µg is loaded.

A further capability offered by the DNAPac MicroBead™ technology is the ability to severely overload the analytical capacity of the column, and still collect fractions of high purity. An example of this process is shown in Figure 5B.

The same gradient used in Figure 5A is used to elute a 1000-µg sample of the same oligonucleotide on the same 4 x 250 mm column. Under these conditions, the full-length oligonucleotide can act as an eluent, forcing the smaller failure sequences to elute ahead of the full-length 25-mer.

Fractions collected across the main peak in Figure 5B are rechromatographed in a different eluent system (0.35–0.55 M LiCl, 2% ACN) in Figure 5C. The purity of each fraction, from the top of the main peak (fraction 1) to the beginning of the following shoulder (fraction 7), exceeded 93%. This approach resulted in an 83% yield of the target oligonucleotide at a purity >95%, from a sample containing only 39% of the target sequence.

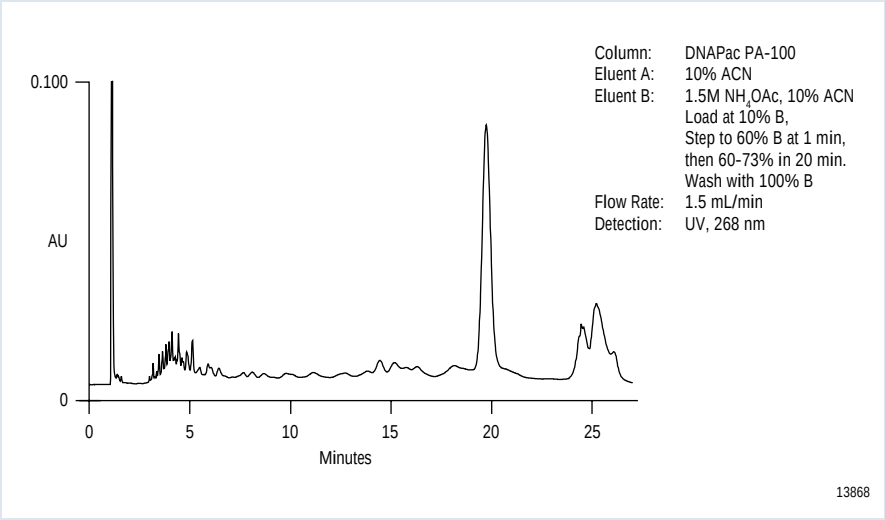


Figure 5A *Analytical chromatography of Cetus PCR-01 $A_5C_5G_8T_7$, (3.55 O.D.; 89 µg). The crude sample contains ~39% full-length primer (by area %).*

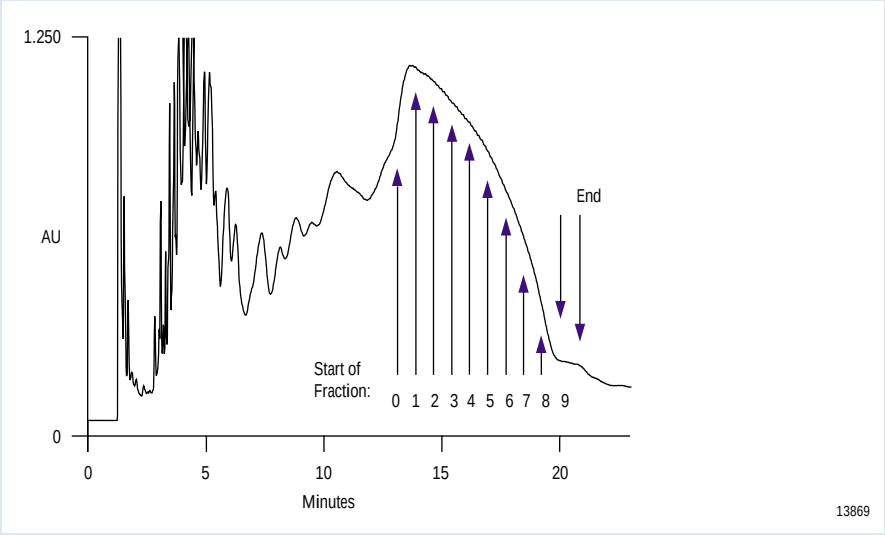


Figure 5B *Quasi-displacement chromatography of Cetus PCR-01 (1.0 mg 40 O.D. in 75 µL).*

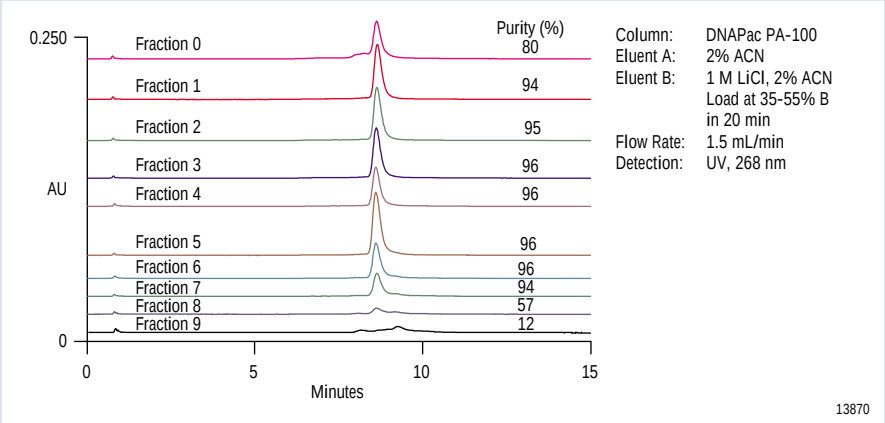


Figure 5C *Purity of 1.0 mg (40 A260) Cetus PCR-01 fractions, assayed with LiCl eluent.*

RESOLVES PROBLEM SEQUENCES

Self-complementary sequences or poly-G stretches can result in intra- and intermolecular associations of oligonucleotides. These associations can prevent efficient oligonucleotide separations under nondenaturing conditions. The DNAPac PA-100 can be operated under denaturing conditions. Either high temperature (up to 90 °C) or high-pH eluents (up to pH 12.5) can be used to eliminate hydrogen bonding, allowing resolution of these problem sequences (see Figure 6).

ASSAY PHOSPHOROTHIOATE PURITY

Phosphorothioate analog syntheses can fail in two ways. The first kind of failure creates sequences with deletions. The second type of failure creates sequences with a mixed phosphodiester/phosphorothioate backbone. The unique DNAPac PA-100 column effectively separates most of these species, even when they vary only by their degree of thioation (see Figure 7).

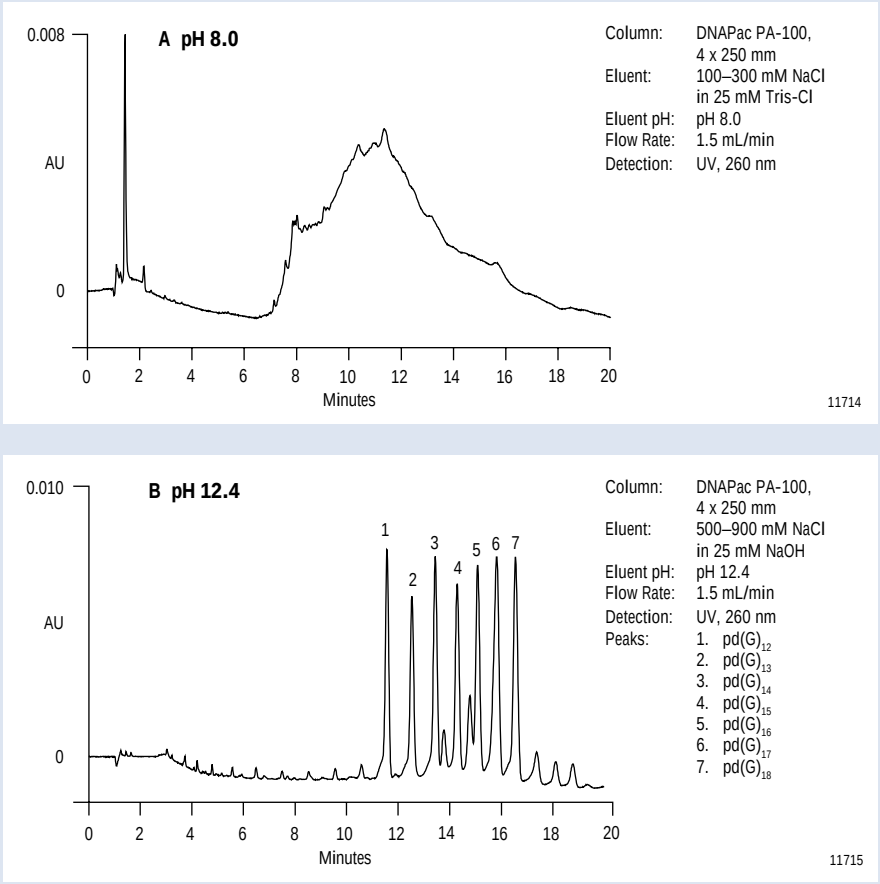


Figure 6 *(A) Attempted separation of 3 µg of pd(G)$_{12–18}$ with a NaCl gradient of pH 8.0. Poly-G regions in DNA can prevent oligonucleotide separation under nondenaturing conditions. (B) Resolution of pd(G)$_{12–18}$ homopolymers at pH 12.4 with a 500 to 900 mM NaCl gradient. At high pH, hydrogen bonding between poly-dG sequences is eliminated.*

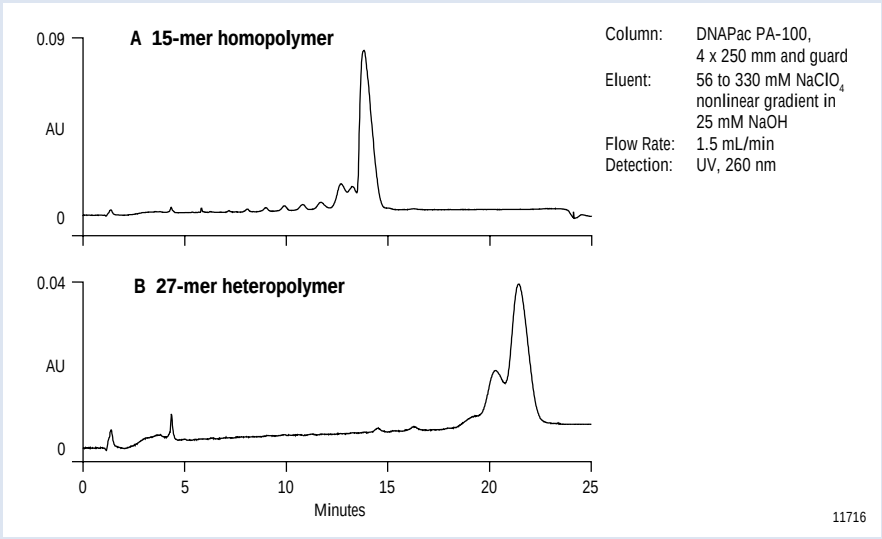


Figure 7 *Analysis of phosphodiester content in phosphorothioates. (A) 15-mer homopolymer. (B) 27-mer heteropolymer. Both samples have been previously purified to remove deletion failure sequences, leaving only full-length oligomers. In sample (B), P31-NMR verified that the peaks eluting before the main product peak contain progressively larger numbers of bonds in the phosphodiester form, and fewer in the phosphorothioate form.*

SEPARATES RNA AND DOUBLE-STRANDED DNA

The DNAPac PA-100 is ideally suited for the purification and analysis of synthetic RNA. Figure 8 shows an example of the analysis of a synthetic RNA 12-mer. Failure sequences are easily separated from the full-length product. Longer RNA can also be purified with intact biological activity using methods developed by Sproat et. al.*

Double-stranded DNA, such as PCR products and restriction fragments, have also been successfully separated on the DNAPac PA-100. Figure 9 shows a separation of *ΦX174/Hae III* restriction fragments with excellent resolution from 72 to 1353 bp.

THE DIONEX DIFFERENCE: HIGH-EFFICIENCY MICROBEAD RESINS

The DNAPac PA-100 packing material is composed of 100-nm, quaternary-ammonium functionalized MicroBeads bound to a 13-µm diameter, solvent-compatible, nonporous substrate (see Figure 10). Benefits include rapid mass transport, higher loading capacity than conventional nonporous materials, and remarkable durability. The result is a scalable column with single-base resolution.

The exceptional benefits provided by MicroBead resins include:

- Higher flow rate for faster separations
- Broad pH and temperature compatibility for separations of problem sequences and easy column clean-up
- Noncompressible resins that simplify scale-up
- Higher loading capacity than conventional nonporous materials
- Higher mechanical and chemical stability for exceptionally long column life

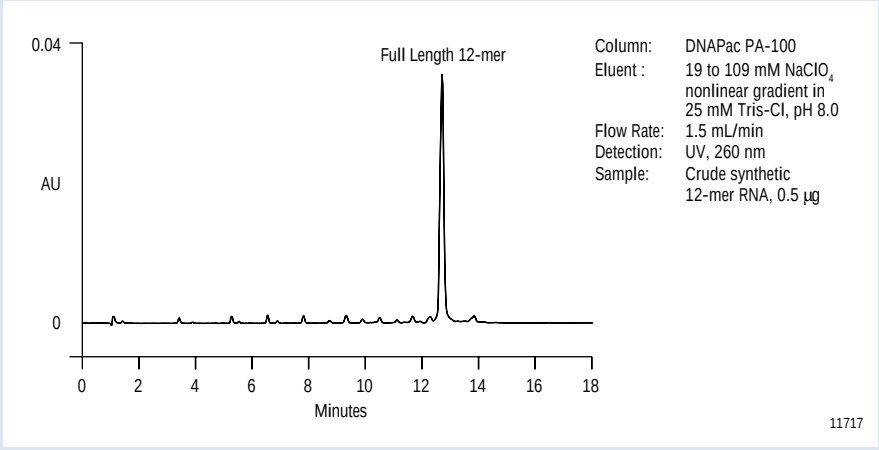


Figure 8 *Analysis of a crude synthetic RNA 12-mer.*

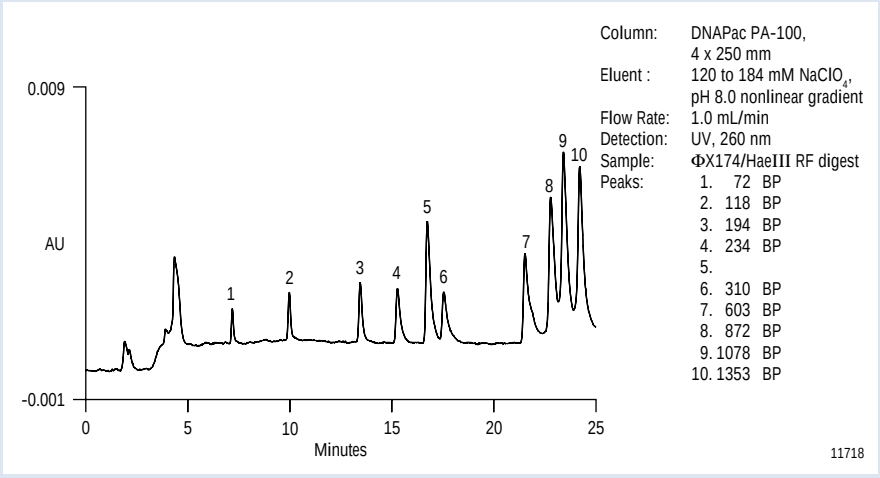


Figure 9 *Separation of ΦX174/Hae III restriction fragments on the DNAPac PA-100. Note that the gradient can be modified to improve the separation of fragments within a specific size range.*

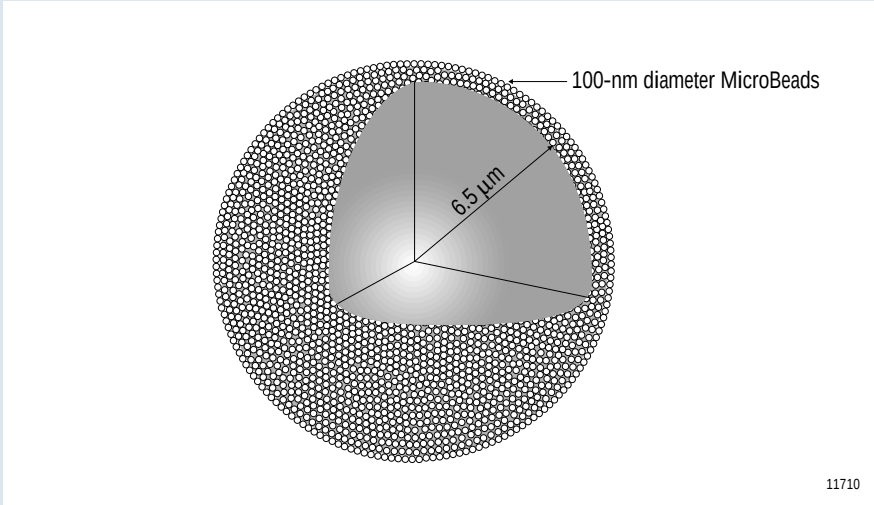


Figure 10 *Schematic of a DNAPac PA-100 pellicular anion-exchange resin. The 13-µm diameter, nonporous substrate is covered with 100-nm diameter, quaternary ammonium-functionalized MicroBeads, creating a thin surface rich in anion-exchange sites. The large resin scales up easily, and eliminates the high backpressure associated with smaller diameter substrates.*

* Sproat, B.; Colonna, F.; Mullah, B.; Tsou, D.; Andrus, A.; Hampel, A.; and Vinayak, R., "An efficient method for the isolation and purification of oligoribonucleotides, " *Nucleosides & Nucleotides*, **1995**, *14*, 255-273.

RELATIVE LOADING CAPACITY

A variety of DNAPac PA-100 column sizes are available for analytical and preparative separations of DNA samples. The relative loading capacities for the DNAPac PA-100 column family are listed in Table 1.

TABLE 1 RELATIVE LOADING CAPACITIES OF THE DNAPAC COLUMNS

	Analytical Scale/ Overloading Purification
2 x 10 mm Fast-Cartridge	1
2 x 50 mm Microbore Guard	5X
2 x 250 mm Microbore Analytical	25X
4 x 50 mm Guard	20X
4 x 250 mm Analytical	100X
9 x 250 mm Semipreparative	500X
22 x 250 mm Preparative	3000X

UNIQUE DIONEX METHODS

Specially designed protocols using the Dionex DX-500 BioLC® system are developed for the maximum resolution of a wide range of DNA samples. These methods, using curved gradients, are designed to resolve:

- Normal-length oligonucleotides (8- to 30-mer) at pH 8 and 12.4
- Extended-length oligonucleotides (30- to 70-mer) at pH 8 and 12.4
- Phosphorothioates (antisense oligonucleotides) at pH 8 and 12.4
- Linear double-stranded DNA at pH 8
- Supercoiled versus nicked/linear DNA at pH 8

DIONEX DX-500 BIOLC SYSTEM

The Dionex DX-500 BioLC (see Figure 11) combines precision flow (artificial intelligence control), curved gradient capabilities, solvent-compatibility and a high-pH-compatible PEEK flow path to deliver the best separations of your DNA samples using Dionex protocols.

Figure 11 *The Dionex PEEK-based DX-500 BioLC is designed to analyze DNA samples using Dionex protocols.*

CUSTOM PRODUCTION SERVICE

For customers who intend to purchase DNAPac columns in quantity and require a high degree of reproducibility on column performance, Custom Lot Select service is available. Upon entering a Custom Lot Select agreement with Dionex, specific resin production batches are set aside for a particular customer. DNAPac columns can be manufactured using these specific resin batches and shipped to the customer following a pre-arranged schedule. Please contact your local Dionex representative for details of this service.

SPECIFICATIONS

pH Range:
2–10, no eluent limitations
pH Range:
10–12.5, requires an equimolar ratio of hydroxide to another anion, such as chloride
Temperature Range:
4–90 °C
Pressure Limit:
4000 psi (27.6 MPa) for the analytical column
Column Construction:
PEEK column body with 10–32 ferrule-style fittings and polymeric frits

Chemical Compatibility
Organic Limit:
100% Acetonitrile or methanol
Detergents:
Cationic or zwitterionic
Chaotropic Agent Limit:
40% Formamide or 6 M urea
Typical Eluents:
Chloride, acetate, bromide, perchlorate: in lithium, sodium, or ammonium forms. Tris-Cl.

ORDERING INFORMATION

In the U.S., call 1-800-346-6390 or contact the Dionex Regional Office nearest you. Outside the U.S., order through your local Dionex Office or distributor. Refer to the part numbers listed below.

DNAPac PA-100 Guard
(4 x 50-mm) ..... P/N 43018

DNAPac PA-100 Analytical
(4 x 250-mm)..... P/N 43010

DNAPac PA-100 Semipreparative
(9 x 250-mm)..... P/N 43011

DNAPac PA-100 Preparative
(22 x 250-mm)..... SP2091

DNAPac PA-100 Fast-Cartridges
(2 x 10-mm)
For pkgs. of 10 cartridges..... SP4757

DNAPac PA-100 Fast-Cartridges
(2 x 10-mm)
In pkgs. of 150 cartridges
For 1-4 pkgs. SP4008
For 4 or more pkgs. SP3229

DNAPac PA-100 Microbore, Analytical
(2 x 250-mm)..... SP3686

DNAPac PA-100 Microbore, Guard
(2 x 50-mm) SP4016

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