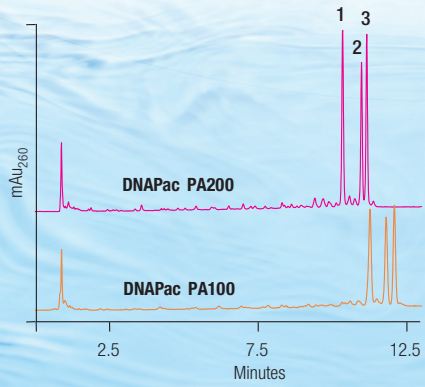
DNAPac PA200 and PA200 RS Columns

Solutions for Nucleic Acid Analysis

Thermo Scientific™ DNAPac™ PA200 HPLC and DNAPac PA200 RS (Rapid Separation) UHPLC columns for high-resolution separations of ssDNA and RNA:

- Resolution of oligonucleotide linkage and stereoisomers
- Improved resolution and throughput
- Selectivity/retention control with eluent pH, salt, and solvent
- High resolution n from n-1 separations

Separation of 20–22mers Showing Improved Column Performance



Column:	**DNAPac PA200 Guard and Analytical**
Eluent A:	20 mM Tris, pH 8.0
Eluent B:	Eluent A with 1.25 M NaCl
Flow Rate:	1.2 mL/min
Injection. Volume:	8 µL
Temperature:	25 °C
Gradient:	22.8% B to 43.7% in 12 min
Sample:	$d(AC)_{10-11}$
Peaks:	1. 20mer 2. 21mer 3. 22mer

Predictable, High-Resolution Separations of ssDNA and RNA by Anion-Exchange Chromatography

Oligonucleotides are among the most widely used reagents in modern molecular biology, where they participate in a variety of applications, from primers for DNA sequencing and PCR to therapeutic agents and tools to study gene function. Because of the nature of these applications, the quality of synthesized oligonucleotides can be of vital importance.

Demonstration of purity, especially of clinical-grade oligonucleotides is a critical step that ensures the efficacy and safety of these reagents. HPLC is an analytical tool capable of rapidly resolving an oligonucleotide from its failure sequences and other contaminants. Reversed-phase HPLC purified oligonucleotides are often contaminated with failure sequences so verification of purity should employ an orthogonal technique. Anion-exchange chromatography on the DNAPac columns is the most accepted orthogonal approach. In addition, Thermo Scientific offers methods to couple anion-exchange UHPLC with high resolution mass spectrometry for purity confirmation, identification of product and quantification of the peak component.

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In anion-exchange chromatography, separation is driven by the negative charge on the molecule, which approximates length. Each oligonucleotide residue adds one negative charge. Thus at pH 6–8, all oligonucleotides of the same length will have essentially the same net charge. At these pH values, oligonucleotides are separated in the "Trityl-off" state (see Figure 1), and unlike the reversed-phase approach, often under conditions that do not disrupt hydrogen bonds.

The DNAPac PA200 HPLC and DNAPac PA 200RS UHPLC columns are both anion exchange columns developed for controlled resolution of ssDNA and RNA molecules. Oligonucleotide selectivity is controlled using a variety of parameters, including eluent pH, solvent, and the use of different salts, enabling the researcher to tailor the separation to the requirements of the specific application.

Phase Stability Over a Broad pH Range

Strong anion-exchange chromatography, using pH-stable polymeric resins, is a simple and effective technique for separating oligonucleotides. Denaturing conditions obtained with high-pH eluent eliminate Watson-Crick hydrogen bonding and allow resolution of problem sequences such as self-complimentary sequences or poly-G stretches. As a consequence, anion-exchange chromatography at high pH has become the preferred approach for oligonucleotide analysis. High-pH methods at room temperature are highly effective at resolving problem sequences. Occasionally, methods are developed using high-pH conditions at elevated temperature. Under these conditions, ion-exchange columns may rapidly degrade, resulting in short column lifetime. These columns employ a chemistry that improves phase stability under alkaline conditions (Figure 2) and offers narrower peak widths for improved peak capacity and resolution (figure on cover).

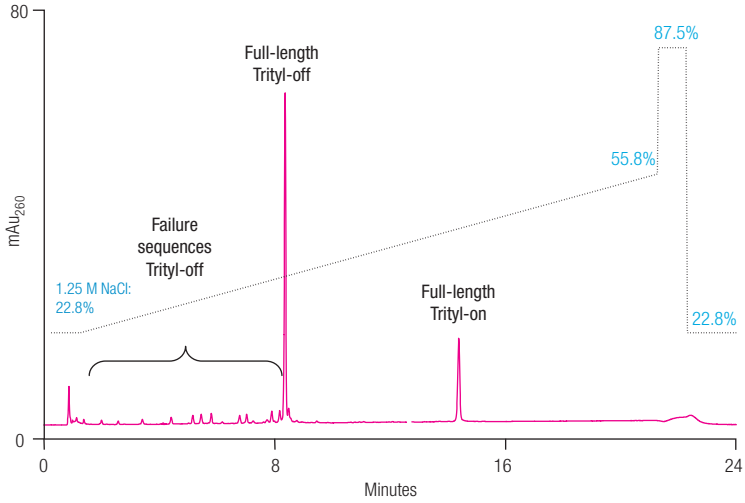


Figure 1: Resolution of target, failure, and trityl-on oligonucleotides

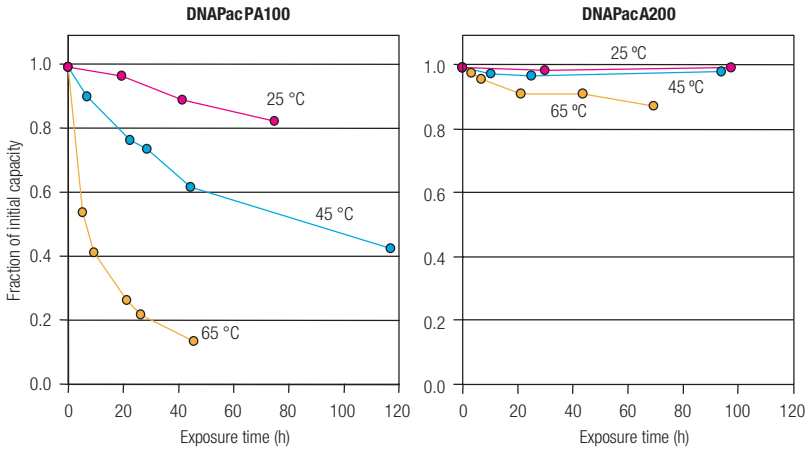


Figure 2: Improved stability of the DNAPac PA200 to alkaline conditions, using a NaCl eluent salt

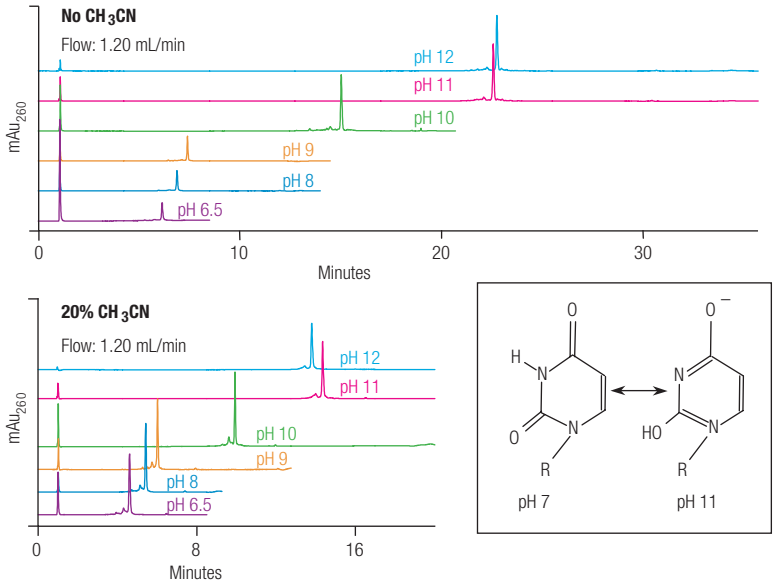


Figure 3: Influence of pH on the retention of G and T containing oligonucleotides using a NaCl eluent salt

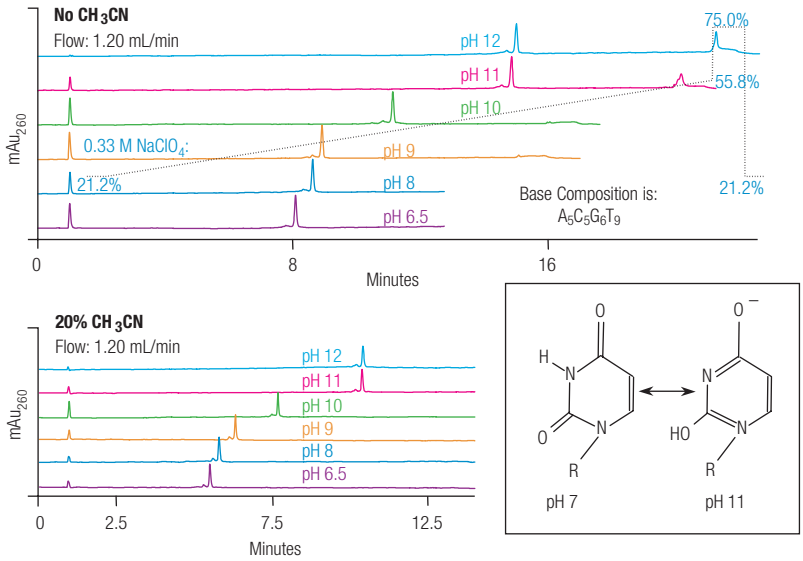


Figure 4: Influence of pH on the retention of G and T containing oligonucleotides using a $NaClO_4$ eluent salt

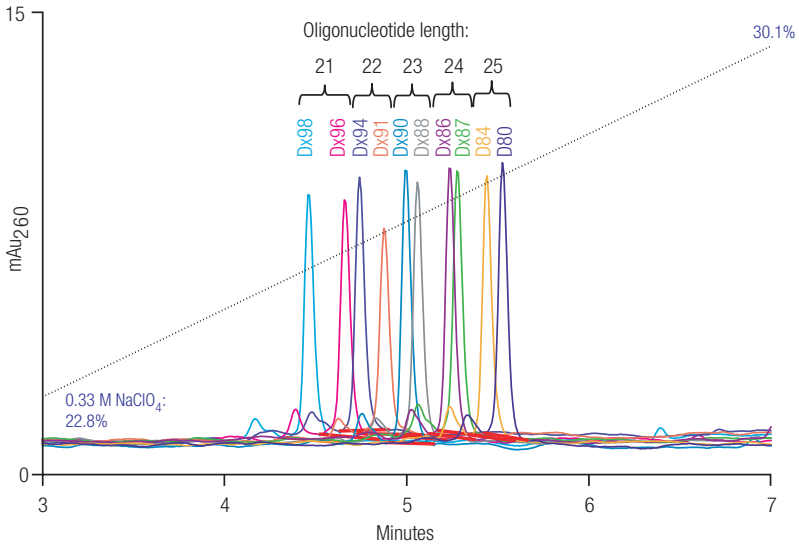
Retention Control by Eluent pH

Increasing eluent pH results in a pronounced increase in retention of mixed-base oligonucleotides (Figures 3 and 4). This effect occurs because between pH 9 and 11 ionization of the tautomeric oxygen on G and T bases confers a formal increase in negative charge for each G and T base unit.

This effect is not observed when the oligonucleotide contains only C and A bases. Because different oligonucleotides often contain different proportions of G and T, the impact of the pH on formal charge allows resolution of same-length oligonucleotides due to differences in base content. Figures 3 and 4 also demonstrate that the addition of solvent dramatically reduces retention and can alter the pH-based selectivity as well.

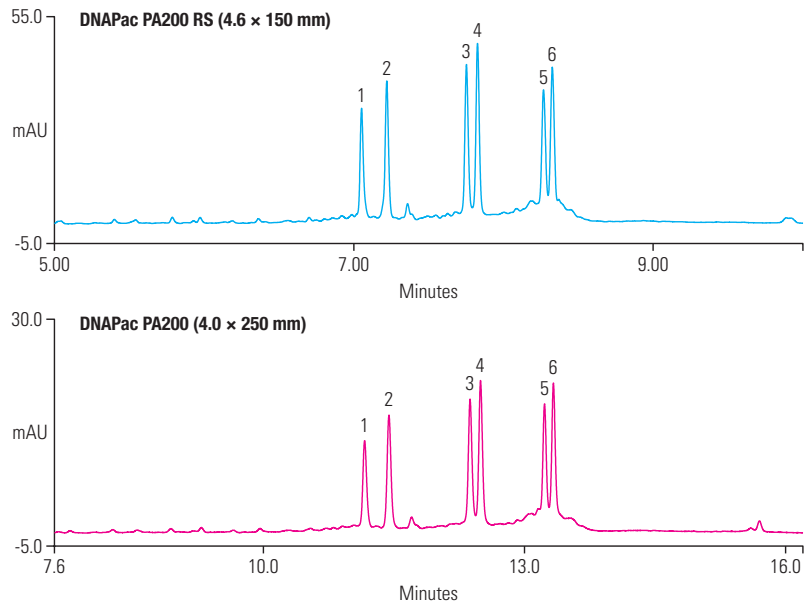
Size Based Separations

Twenty-one oligonucleotides ranging in length from 21–25 bases, and differing only in their 3' and 5' terminal base sequence, were separated under identical conditions. Figure 5 shows only the first and last eluting oligonucleotides in each length set, demonstrating n from n-1 and n+1 resolution. As can be seen, each length set is resolved from all other length sets. In addition, resolution increases with increasing length. This is also shown in Figure 5 and 6, where under the conditions shown, the resolution is based primarily on size. In Figure 6, resolution comparable to a 250 mm long PA200 is achieved in 25% less time on a 4.6 × 150 mm DNAPac PA200 RS UHPLC column. In addition, this column provides approximately twice the detection sensitivity of the DNAPac PA200 HPLC column.



Column: **DNAPac PA200**
Eluent: $NaClO_4$, pH 6.5 with 20% CH_3CN
Flow Rate: 1.2 mL/min
Injection Volume: 8 µL
Temperature: 25 °C
Detection: UV, 260 nm

Figure 5: Separation of oligomers by length



Column: **DNAPac PA200 RS (4.6 × 150 mm)**
Eluent A: 20 mM Tris pH 8
Eluent B: A + 1.25M NaCl
Flow Rate: 1.2 mL/min
Injection Volume: 7.5 µL
Temperature: 30 °C
Gradient: 28-54% B in 4 CV*
Sample: Oligonucleotide mix
* CV = column volumes

Peaks:
1: ON 34
2: ON 35
3: ON44
4: ON45
5: ON54
6: ON55

Column: **DNAPac PA200 (4.0 × 250 mm)**
Eluent A: 20 mM Tris pH 8
Eluent B: A + 1.25M NaCl
Injection Volume: 7.5 µL
Temperature: 30 °C
Flow Rate: 1.2 mL/min
Gradient: 28-54% B in 4 CV*
Sample: Oligonucleotide mix
* CV = column volumes

Figure 6: Oligonucleotides differing by one nucleic acid are separated. The DNAPac PA200 RS in 4.6 × 150 mm format exhibits improved oligonucleotide sensitivity compared to the standard DNAPac PA200 4.0 x 250 mm format

Separation of Identical Length Oligonucleotides

Figure 7 shows the effect of pH on the retention time and selectivity of two 23-base oligonucleotides differing only at their 5' and 3' terminal bases, as indicated. At pH 9 and 9.5, the peaks are unresolved. As the pH is increased, not only are the peaks resolved, but their retention order reverses (compare traces at pH 10 and pH 10.5).

High-Resolution Separations

The improved resolution of the DNAPac PA200, permits separation of normal-length oligonucleotides (13–25 nucleotides) under high-throughput conditions where it resolves even diastereoisomers arising from phosphorothioate linkages. Figure 8 shows results from the DNAPac PA200 RS, UHPLC compatible column, packed with smaller 4 µm resin particles. Columns packed with smaller particles improve resolution and offer better performance than standard DNAPac PA200 columns with significantly higher throughput. The DNAPac PA200 RS columns are packed in bioinert PEEK-lined Stainless steel (SST) bodies. The inner sleeve of PEEK eliminates unwanted oligonucleotide interactions with SST, while the SST shell provides the necessary support for higher pressures generated by the smaller, higher-efficiency resin beads. The high throughput separation of phosphorothioate diastereoisomers using three DNAPac PA200 RS column formats are shown in Figure 8. In this example, a resolution of 4 is achieved in approximately 2.5 minutes on the 4.6 × 50 mm column, demonstrating a substantial throughput improvement on this UHPLC column compared with the longer formats columns.

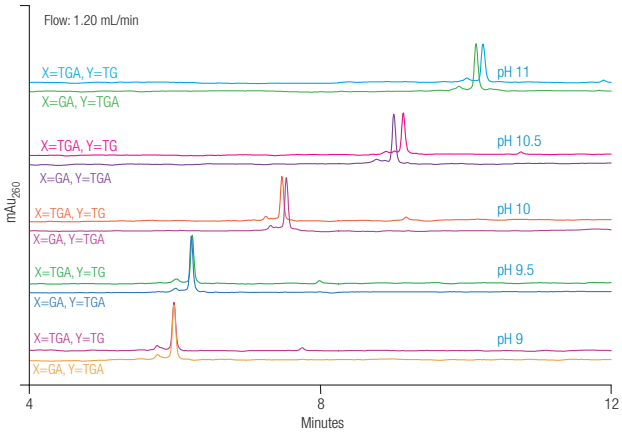
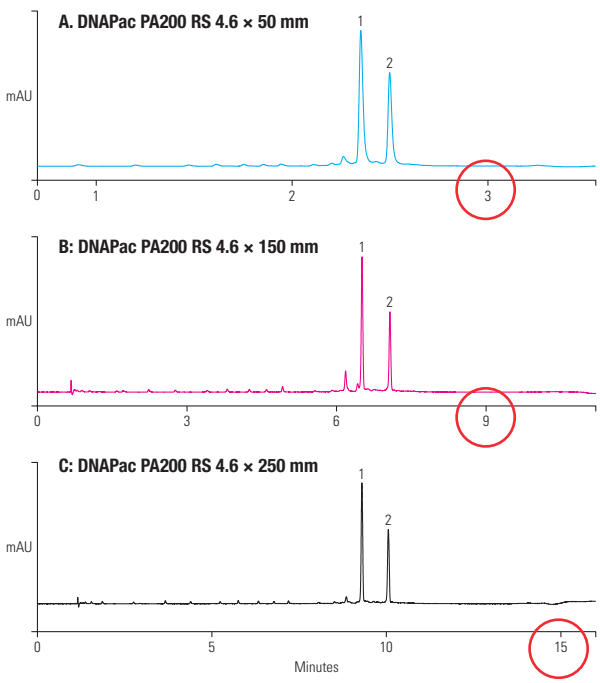


Figure 7: Control of 23mer oligonucleotide selectivity (5'X-$G_4C_4A_3T_7$-Y3') using pH with a NaCl eluent

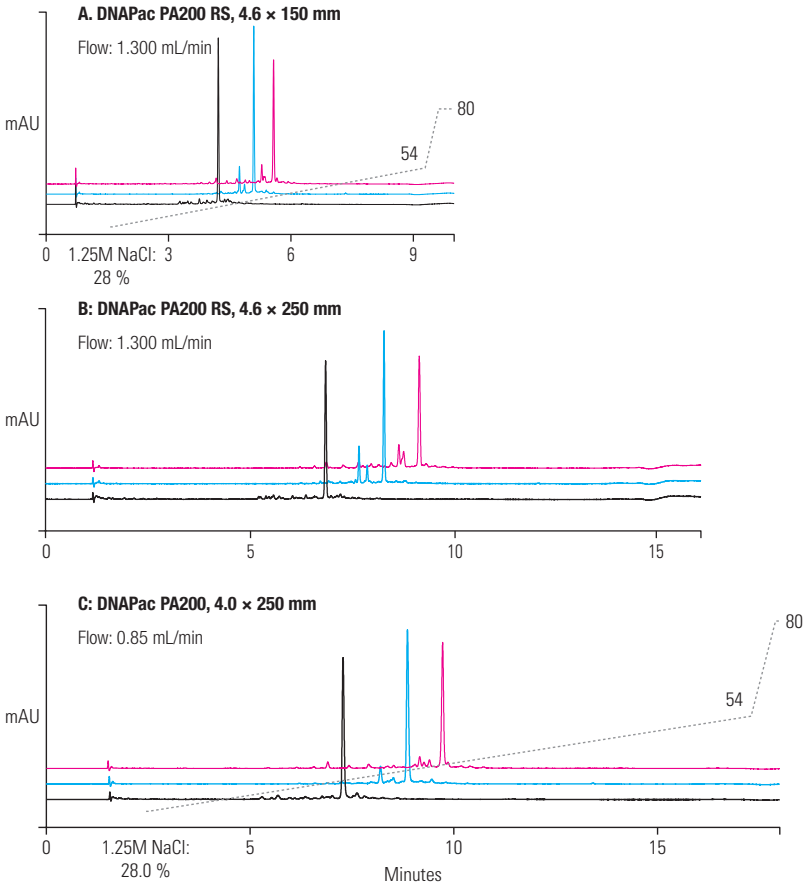


Columns:	**A. DNAPac PA200 RS 4.6 × 50 mm** **B: DNAPac PA200 RS 4.6 × 150 mm** **C: DNAPac PA200 RS 4.6 × 250 mm**
Eluent A:	20 mM Tris pH 8
Eluent B:	A + 1.25 M NaCl
Flow Rate:	A: 1.30 mL/min B: 1.30 mL/min C: 1.30 mL/min
Injection Volume:	10 µL
Temperature:	30 °C
Gradient:	28-54% B in 4 CV*
Sample:	2′,5′ linkage isomers

* CV = column volumes

Peaks:
1: Phosphorothioate isomer "A"
2: Phosphorothioate isomer "B"

Figure 8: Phosphorothioate diastereoisomer separations comparison using different column lengths for faster, higher throughput separations



Columns: **A. DNAPac PA200 RS, 4.6 × 150 mm**
B: DNAPac PA200 RS, 4.0 × 250 mm
C: DNAPac PA200, 4.0 × 250 mm
Eluent A: 20 mM Tris pH 8
Eluent B: A + 1.25M NaCl
Flow Rate: A: 1.30 mL/min
B: 1.12 mL/min
Injection Volume: 6 µL
Temperature: 30 °C
Gradient: 28-54% B in 4 CV*
Sample: 2′,5′ linkage isomers

* CV = column volumes

Peaks:
1: Dio-6 (two 2′,5′-linkage isomers)
2: Dio-1 (no 2′,5′-linkage isomers)
3: Dio-9 (one 2′,5′-linkage isomers

Figure 9: RNA Oligonucleotide linkage isomer separations

Resolution of Linkage Isomers

Linkage isomers can also be formed in RNA oligonucleotides by exposure to elevated temperatures, for example during annealing of double stranded RNA from single stranded forms. Figure 9 shows the resolution of three identical sequence oligonucleotides harboring zero, one, or two 2′,5′-linkages. Dio-1 harbors no such linkages, Dio-9 harbors one and Dio-6 harbors two such linkages. Both the DNAPac PA200 and the PA200 RS, in 250 mm long formats, resolve these identical sequence oligonucleotides in less than 10 minutes. Figure 9 also shows that improved throughput is available using 4.6 × 150 mm DNAPac PA200 RS column format.

High-Efficiency Thermo Scientific™ MicroBead™ Resins

The DNAPac PA200 8 µm diameter packing material and the DNAPac PA 200 RS 4 µm packing material are composed of 130-nm quaternary ammonium-functionalized MicroBeads bound to a solvent-compatible, nonporous substrate (see Figure 10). Benefits include rapid mass transport, higher loading capacity than traditional nonporous materials, and remarkable durability.

Guaranteed Performance

The unique pellicular resin in the DNAPac PA200 and PA200 RS columns offers exceptional selectivity and stability over the entire pH range. Its highly cross-linked structure ensures long column life and easy cleanup. The entire manufacturing process (resin synthesis, amination, and packing and testing of the chromatographic columns) is carefully controlled to ensure that every DNAPac PA200 and PA200 RS delivers reproducible performance. DNAPac columns are tested with a $dT_{19–24}$ standard mix to ensure lot-to-lot reproducibility.

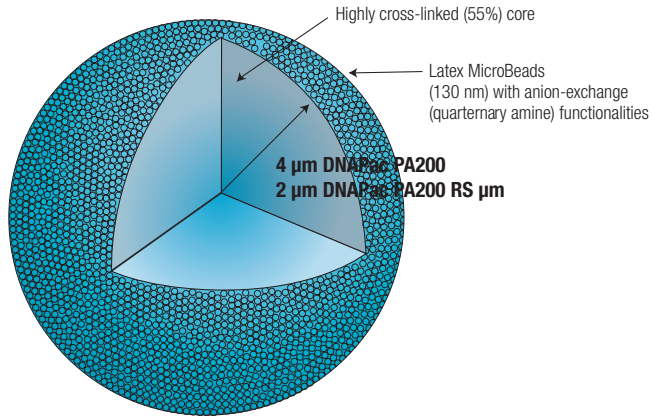


Figure 10: Schematic of a DNAPac PA200 and PA200 RS pellicular anion-exchange resin

Specifications and Operational Parameters

	DNAPac PA200	DNAPac PA200 RS
Column chemistry	55% crosslinked nonporous polymer with with quaternary amine fuchtionalized latex MicroBeads	55% crosslinked nonporous polymer with with quaternary amine fuchtionalized latex MicroBeads
Particle size	8 µm	4 µm
Ion exchange capacity	~ 14 µeq/mL	~ 20 µeq/mL
pH range	4 to 10 (12.5 with salt, see manual)	
Pressure maximum	4,000 psi	10,000 psi
Temperature	≤ 85°C	≤ 85°C
Flow rates	Recommend 0.1 to 1.5 mL/min	
Organic solvent limit	100% acetonitrile or methanol for cleaning	
Typical eluents	High purity water (18 megohm-cm), sodium chloride, sodium perchlorate, buffers, sodium acetate and sodium hydroxide.	
Detergent compatibility	Compatible with nonionic, cationic or zwitterionic detergents.	

Ordering Information

Analytical, 4 µm, UHPLC Columns	Part Number
DNAPac PA200 RS, 4 µm High Resolution Analytical Column (4.6 × 250 mm)	082510
DNAPac PA200 RS, 4 µm Analytical (4.6 × 150 mm)	082509
DNAPac PA200 RS, 4 µm, Fast Analytical Column (4.6 × 50 mm)	082508

Analytical, 8 µm, HPLC Columns and Guards	Part Number
DNAPac PA200 Analytical, 8 µm (2 × 250 mm)	063425
DNAPac PA200 Guard, 8 µm (2 × 50 mm)	063423
DNAPac PA200 Analytical, 8 µm (4 × 250 mm)	063000
DNAPac PA200 Guard, 8 µm (4 × 50 mm)	062998
DNAPac PA200 Analytical, 8 µm (9 × 250 mm)	063421
DNAPac PA200 Guard, 8 µm (9 × 50 mm)	063419
DNAPac PA200 Semi-Preparative, 8 µm (22 × 250 mm)	088781
DNAPac PA200 Guard, 8 µm (22 × 50 mm)	088780

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