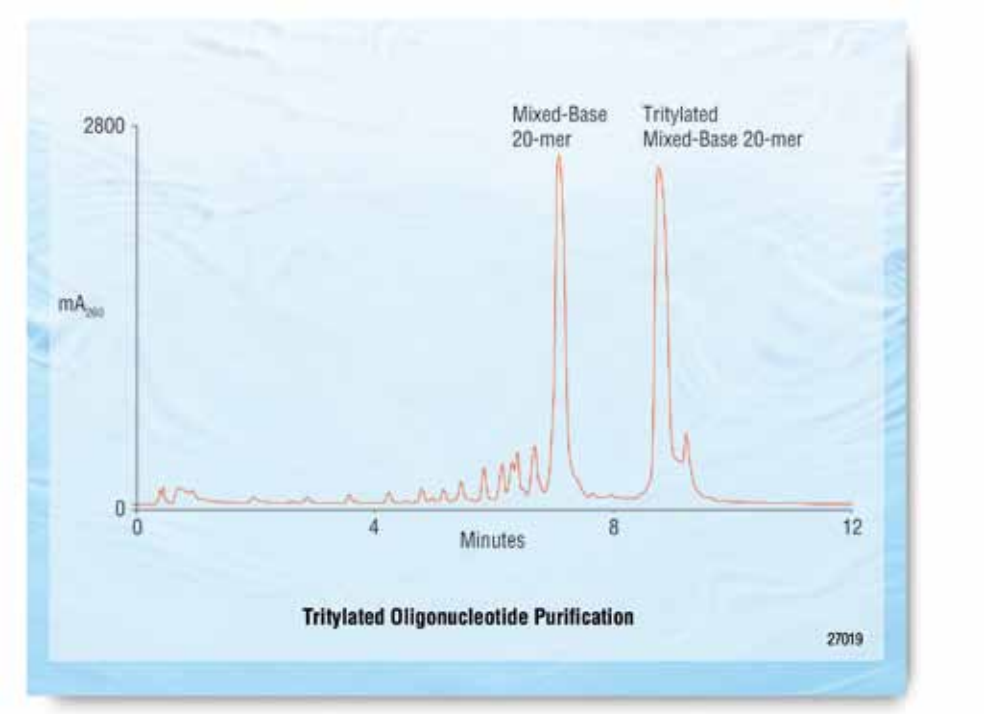
columns

DNASwift Monolith Column for DNA and RNA Oligonucleotide Purification



The DNASwift™ monolith column is specifically designed to provide exceptionally high-resolution, laboratory-scale purification of DNA and RNA oligonucleotides by anion-exchange chromatography. The use of oligonucleotides in therapeutic and diagnostic research is rapidly expanding. DNA and RNA oligonucleotides for these areas of research must be of the highest purity. DNASwift monolith columns provide high resolution and capacity, typically resulting in superior purity and yield compared to other ion-exchange columns.

Now sold under the Thermo Scientific brand

Thermo SCIENTIFIC

Major Column Features and Benefits

The DNASwift monolith is a high performance column for DNA and RNA oligonucleotide purification. The unique monolith design provides:

- High resolution
- High capacity
- High purity and yields
- Refined selectivity control
- Fast flow rates
- Excellent pH stability
- Outstanding reproducibility
- Long column lifetime

DIONEX

Passion. Power. Productivity.

Column Chemistry

The DNASwift column is a unique porous polymer monolith specifically designed for superior oligonucleotide purification. The column is a pressure and chemically stable monolith support coated with functionalized latex nanobeads. These nanobeads with strong anion-exchange functional groups, optimized for oligonucleotide separations, are similar to those of the industry leading DNAPac® columns. These nanobeads contribute to the DNASwift column's exceptionally high resolution and selectivity control.

The monolith, a polymer cylinder with interconnected flow-through channels, contributes to the high capacity and the exceptionally high resolution of the DNASwift column, maintaining high resolution even at elevated flow rates. The combination of functionalized latex nanobeads and monolith technology results in the DNASwift column having very high capacity, exceptionally high resolution, and refined selectivity control, which results in superior purity and yield of full-length oligonucleotide products purified from crude oligonucleotide samples. This makes the DNASwift column ideal for DNA and RNA oligonucleotide therapeutic and diagnostic research. Additionally, it provides DNASwift columns with the capability to successfully purify difficult DNA and RNA oligonucleotide products.

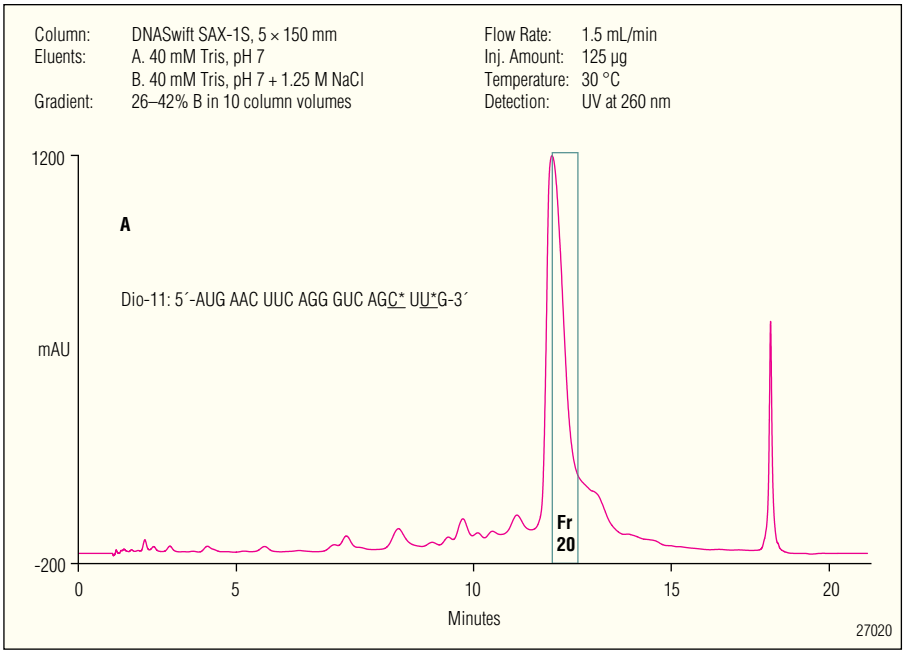


Figure 1A. Purification of a 21-base RNA sample with aberrant 2'-5' linkages at the 1 and 3 positions from the 3' end.

High Resolution

The DNASwift column combines functionalized nanobeads and the monolith stationary phase to deliver exceptionally high resolution and the highest efficiency for the purification of DNA and RNA oligonucleotides. Figure 1A shows the purification of a 21-base RNA oligonucleotide from a crude synthesis. It clearly demonstrates the high resolution capability of the DNASwift column required for high purity and yield. Chromatograms B and C in Figure 1B show analysis of the crude sample and a purified fraction of the 21-base RNA oligonucleotide sample using the DNAPac PA200 column.

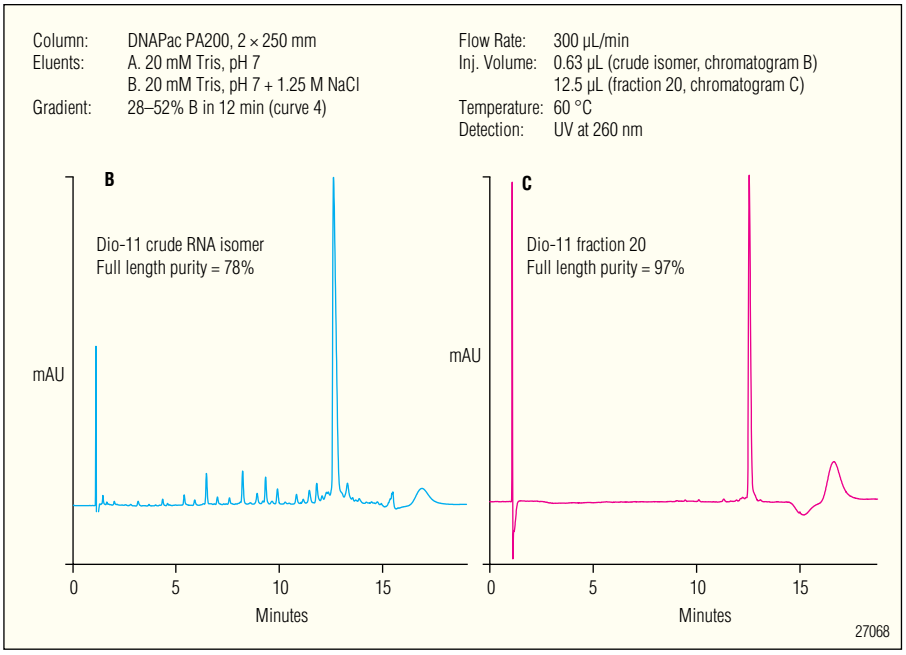


Figure 1B. Analysis of a crude sample and a DNASwift-purified fraction of the 21-base RNA sample shown in Figure 1A.

High Yields and High Purity

The DNASwift column's superior capacity and resolution provides exceptionally high yields and high purity of full-length oligonucleotide products purified from crude oligonucleotide samples even under highly overloaded conditions. Figure 2 compares the purification of an 8.25 mg oligonucleotide sample on a DNASwift column and a leading competitor's anion-exchange column. The DNASwift column produced higher yields with higher purity. Fractions were collected from each full-length oligonucleotide purified product and analyzed on a DNAPac PA200 column to determine purity and yield. Figure 2C shows the analysis of one fraction from the DNASwift column compared to an equivalent fraction obtained from the competitor's column. The yield-purity plot of arithmetically combined analyzed fractions (Figure 2D), clearly shows the DNASwift can provide significantly higher yield at any given purity as well as significantly higher purity at any given yield.

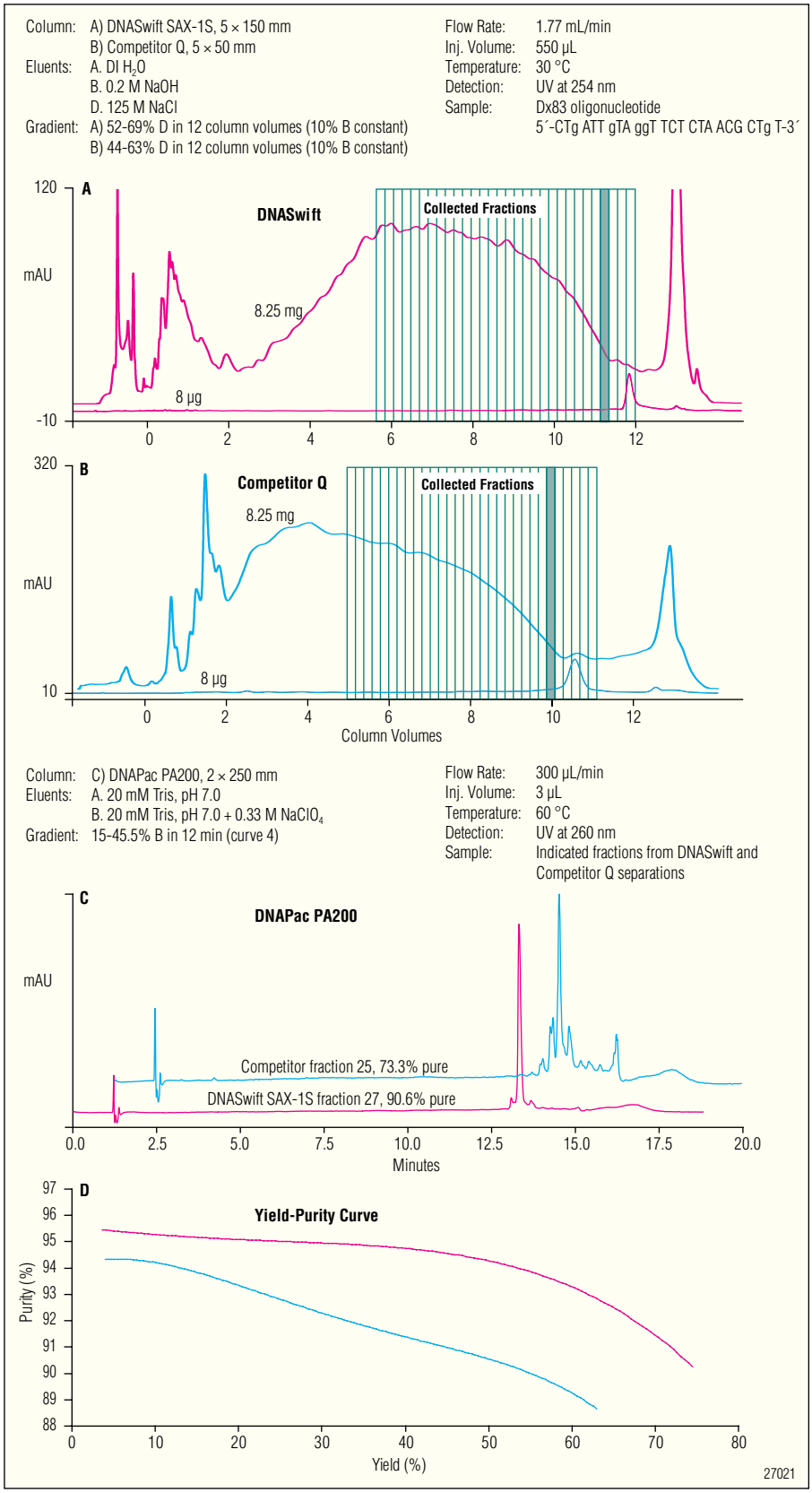


Figure 2. Purification of a 25-base oligonucleotide using A) the DNASwift SAX-1S, and B) a benchmark, monodisperse bead-based competitor. Analysis of equivalent fractions C) carried out on the DNAPac PA200, then the arithmetically combined fractions of the target oligonucleotide D) are plotted.

Selectivity

The ability to control selectivity, by changing chromatographic parameters such as pH or temperature, represents a significant advantage of the DNASwift anion-exchange column for DNA and RNA oligonucleotide purification. This is the same refined capability as the industry leading DNAPac column to control selectivity of oligonucleotide analysis. This selectivity control can be used to directly affect the resolution and the ability to obtain high purity and yields of purified oligonucleotides. A DNASwift column's selectivity can be controlled or adjusted through the change of pH value, solvent concentration, salt form or temperature.

Adjusting Selectivity Using pH

The pH value of the mobile phase of the DNASwift column can be adjusted to optimize selectivity for oligonucleotides. On increasing the mobile phase pH between 8 and 11, an increase of net oligonucleotide charge is generated due to the ionization of the tautomeric oxygen on the Thymine (T) (or U for RNA) and Guanine (G) bases. An increase of net negative charge increases retention time in proportion to the number of T and G bases on an oligonucleotide. Figure 3 shows the elution pattern of two 23-base oligonucleotides differing only in their 3´and 5´ terminal bases when compared between pH 8 and 10. In this example, the elution order is shown to reverse as the eluent pH rises. Therefore, changing the eluent pH value between pH 8 and pH 11 can change the selectivity between two different oligonucleotides on a DNASwift column. This clearly demonstrates that refined and significant changes in oligonucleotide selectivity can be achieved by simple eluent pH modifications on a DNASwift column.

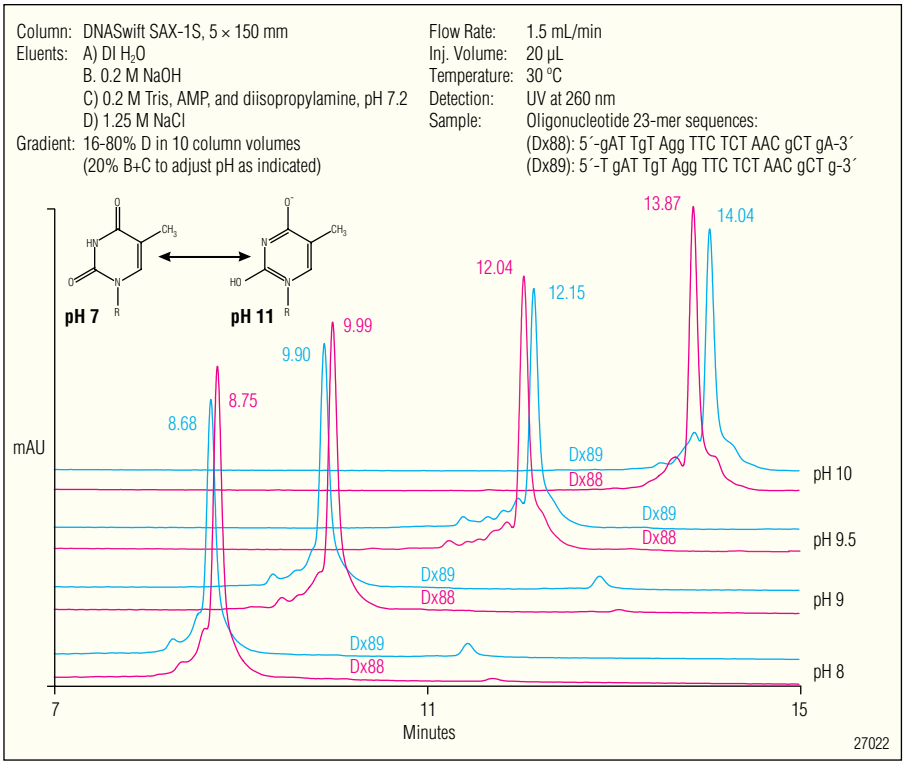


Figure 3. Effect of pH on oligonucleotide retention and selectivity.

Adjusting Selectivity Using Temperature

Temperature can also be used to control selectivity effectively on a DNASwift column. (Note: take care to limit the eluent pH at temperatures above 45 °C to pH 6–8.5.) Figure 4 shows the same oligonucleotide sample analyzed several times using different temperatures under the same gradient conditions. In this example, overall selectivity and resolution of the major component from minor failure sequences improves with increasing temperature on the DNASwift column.

Reproducibility and Durability

The DNASwift column exhibits excellent longevity under normal operating conditions. Figure 5 compares the elution profile for a 20-base oligonucleotide for over 300 runs at pH 12.6 and 3 mL/min. The chromatograms clearly show that retention time, peak width, and asymmetry all remain stable for over 300 runs. This demonstrates that DNASwift users can expect a long, useful operating life-span.

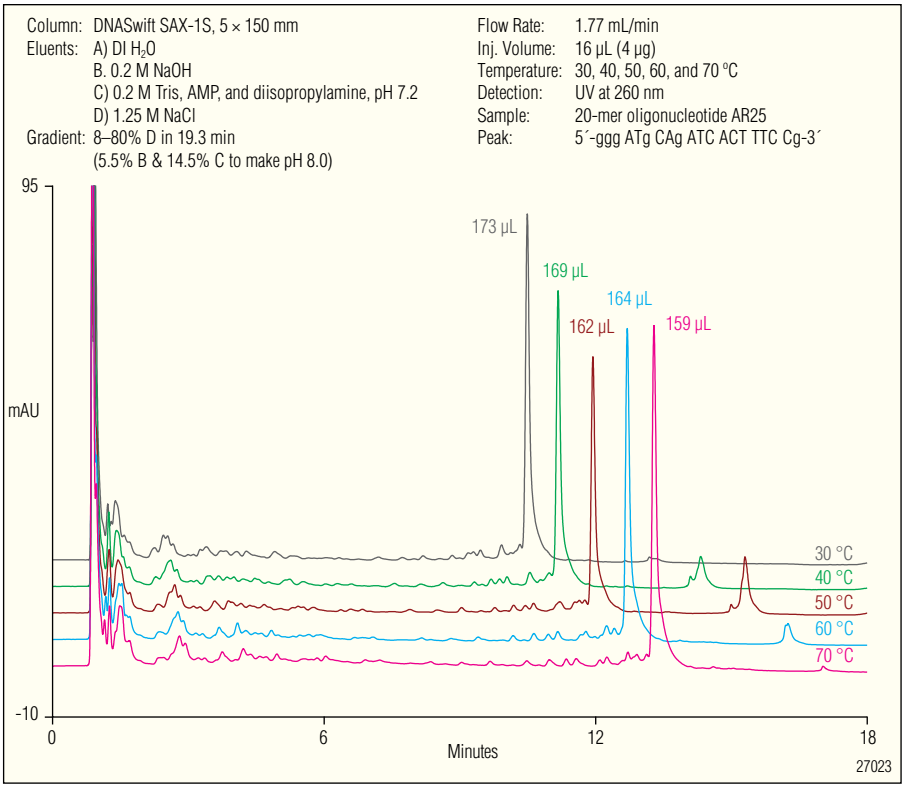


Figure 4. Effect of temperature on oligonucleotide resolution.

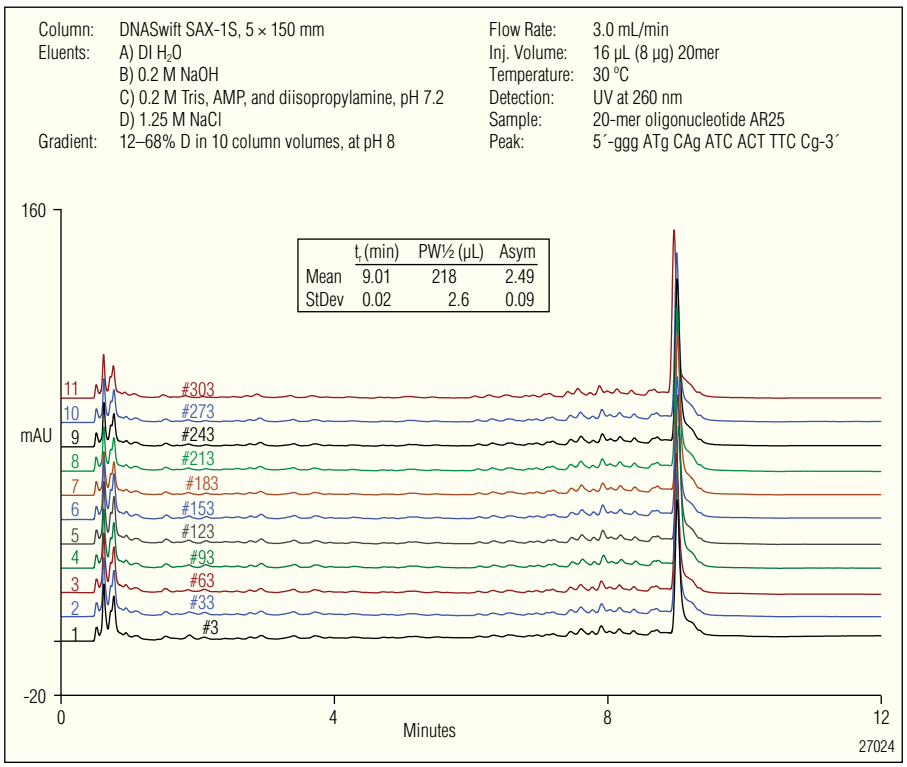


Figure 5. Run-to-run reproducibility of the DNASwift column at pH 12.6, 3 mL/min.

Applications

The DNASwift column has the capability to purify a broad range and various types of oligonucleotide targets from crude oligonucleotide samples. The column is capable of purifying single- and double-stranded DNA and RNA, and has the versatility and capability to purify modified forms of DNA and RNA.

RNA Isomer Purification

An example of the versatility of the DNASwift column is its ability to separate and purify RNA isomers. Size-based separations are often desired for oligonucleotides separations and purifications but due to synthetic failures that are not fully resolved, many deleterious impurities may be under-represented or missed entirely by this approach. One example of such impurities are RNA 2′-5′ linkage isomers. These can be generated during synthesis and release from oligonucleotide synthesizers and have identical length, charge and mass as their normally linked counterparts. Since these aberrant linkages are known to influence biochemical activities, determination of their presence in therapeutic and diagnostic RNA preparations is necessary. Figure 6 shows resolution of several 21-base RNAs having identical sequence (length and mass) but which harbor aberrant 2′-5′ linkages at different positions within the sequence. Purification on the DNASwift, followed by MS and other analyses can identify the position of the linkages in each variant.

Resolution of Phosphorothioate Diastereoisomers

Another RNA isomer example is resolution of phosphorothioate (PS) linkage isomers. Use of 1-2 PS linkages in RNA is common and results in two diastereoisomers at each linkage. These isomers co-elute in size-based separations. Figure 7 shows the elution of a 21-base RNA without PS linkages (Trace A) as a single peak. However, addition of two PS linkages results in four RNA isomers that are resolved on the DNASwift column(Trace B).

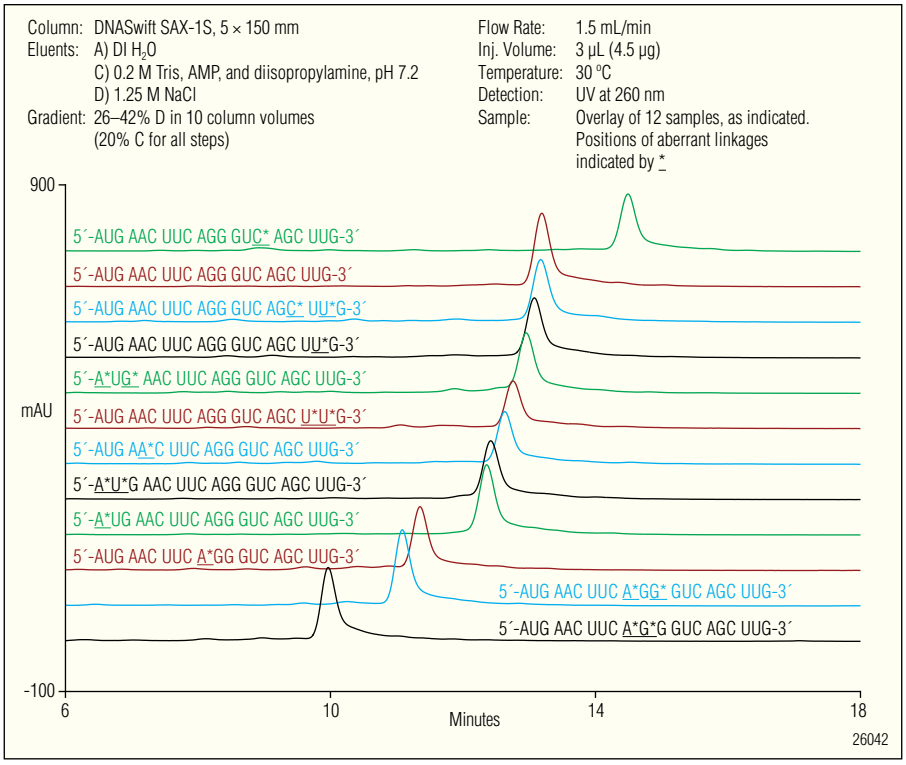


Figure 6. Differential retention of 21-base RNAs with identical sequences but with aberrant linkages at different positions.

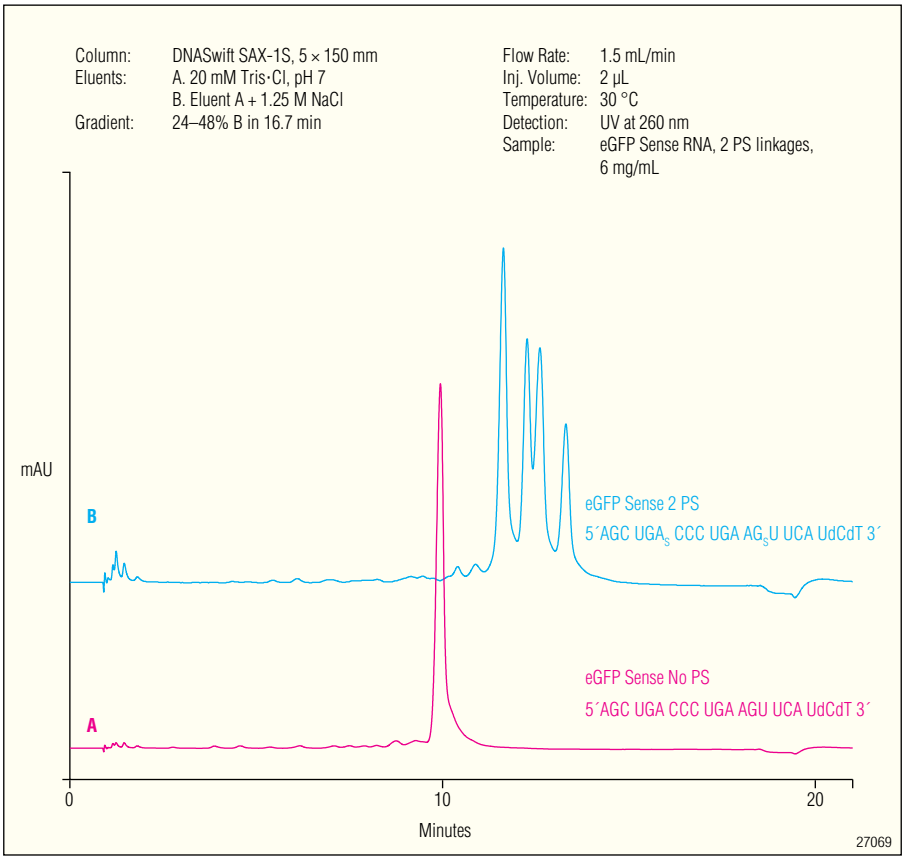


Figure 7. Trace B shows the resolution of four RNA isomers with two PS linkages. Trace A shows the elution of a 21-base RNA without PS inkages as a single peak.

Desalting and Fraction Collection with Dionex Systems

The DNASwift column can be used with Dionex UltiMate® 3000 Titanium systems for fraction collection and automated desalting. For example, a method using the DNASwift column for high resolution anion-exchange purification couples oligonucleotide separation with automated desalting to prepare oligonucleotides for direct ESI-MS analysis (Figure 8). Using two eluents for each step, a single Dionex UltiMate 3000 Titanium system with quaternary pump, thermal compartment, UV detector, and fraction-collecting autosampler first resolves on a DNASwift column and then collects the target oligonucleotide away from its failure sequences, re-injects the collected fractions through a desalting column to isolate the oligonucleotide from the contaminating salt, and directs the desalted sample to an ESI-MS, or to a vial for subsequent use. Figure 9 shows the results of automated desalting of a 21-mer RNA fraction, and the resulting mass spectra. The spectrum reveals the full length target mass, and the minor presence of sodium adducts. This demonstrates that both the DNASwift purification and the automated desalting were effective.

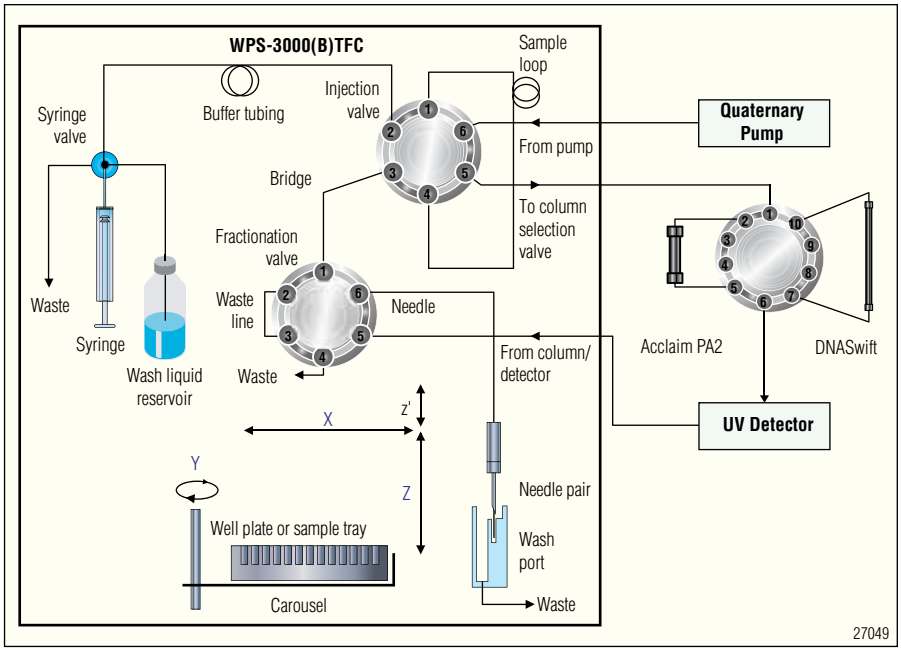


Figure 8. Schematic of setup for purification and desalting of an RNA linkage isomer using a Dionex UltiMate 3000 Titanium system.

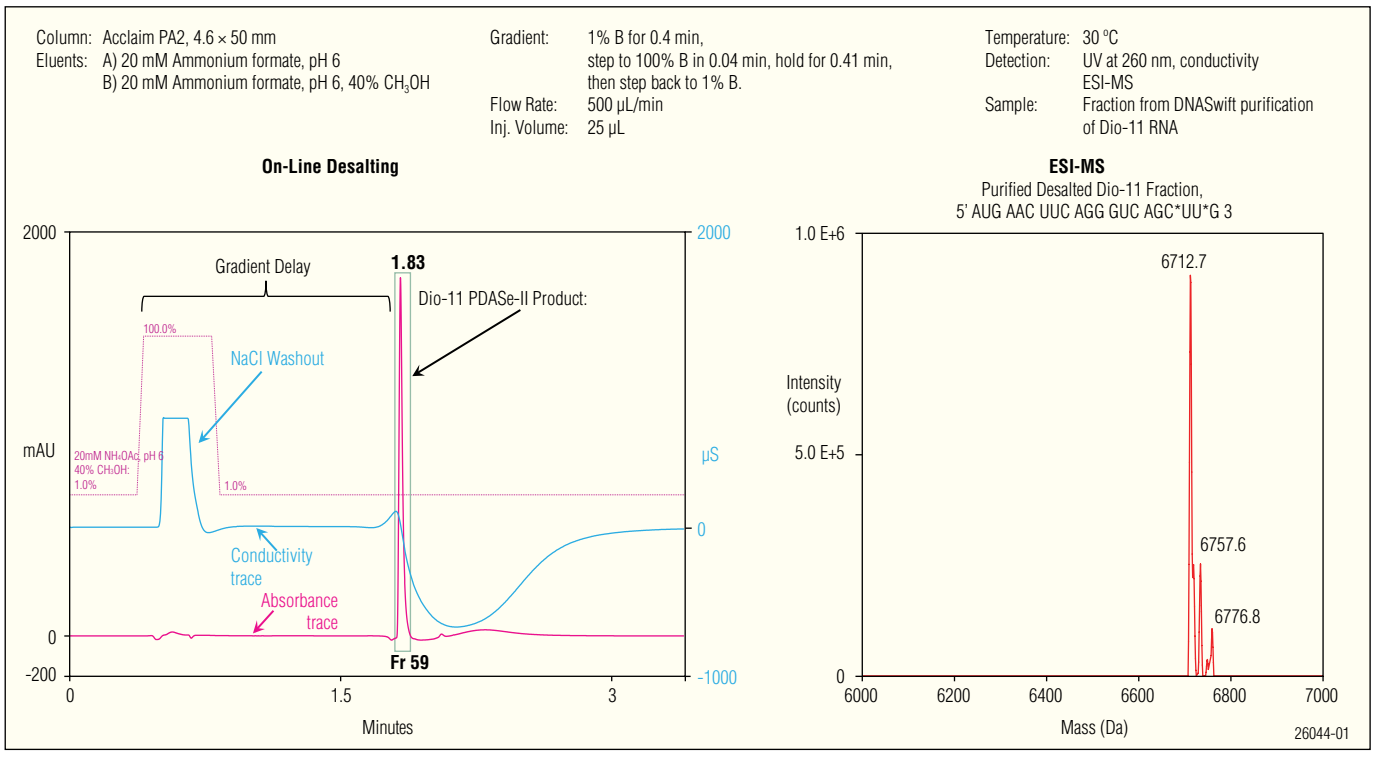


Figure 9. Desalting of a purified RNA fraction and the resulting mass spectrum.

SPECIFICATIONS

Parameter	DNASwift
Column dimension	5 × 150 mm
Base matrix material	Poly(meth)acrylate
Surface chemistry	Quaternary amine
Oligonucleotide binding capacity per mL of polymer	20 mg/mL, 20-mer
Total binding capacity per column	50 mg, 20-mer
Bed height	130 mm
Bed volume	2.5 mL
pH range	6–12.4 for operation 2–14 for cleaning Note: [Salt] ≥ Equimolar to [NaOH] required.
Recommended flow rate	0.5–2.5 mL/min
Maximum flow rate	3 mL/min
Operating column pressure	< 1500 psi 10.3 MPa
Maximum operating temperature	85 °C
Solvent compatibility	Most common organic solvents (e.g., CH_3CN, CH_3OH)

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 following part numbers.

DNASwift Monolith Columns	Part Number
DNASwift Monolith SAX-1S, 5 × 150 mm	066766

Dionex Corporation
1228 Titan Way
P.O. Box 3603
Sunnyvale, CA
94088-3603
(408) 737-0700

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