High-Resolution Separations of Oligonucleotides on a New Strong Anion-Exchange Column

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The DNAPac® PA200 is a new high-efficiency, strong anion-exchange column with enhanced stability under alkaline conditions. This new column offers outstanding selectivity, resolution, and peak capacity when compared with existing anion-exchange columns, and allows precise control of selectivity with the use of eluent modifiers, alternate salts, and changes in pH.

Strong anion-exchange chromatography, using pH-stable polymeric resins, is a simple and effective technique for separating oligonucleotides. Denaturing conditions established at high pH can be used to eliminate 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 analyses. Chromatographic oligonucleotide analyses are used to evaluate the coupling efficiency of a synthesis, assess purity and verify the nature of the components in a nucleic acid amplification mixture. This note illustrates the use of a new strong anion-exchange column, the DNAPac PA200, for oligonucleotide analysis. The DNAPac PA200 employs a new monomer that improves phase stability under alkaline conditions and offers improved peak width, peak capacity, and resolution.

Experimental

A Dionex ICS 2500 (GP50 Gradient Pump, AD20 Absorbance Detector, and AS50 Autosampler with Thermal Compartment) was used for this study. The DNAPac PA200 column (4 x 250 mm) was operated at 1.2 mL/min. Control of the chromatograph and data acquisition was achieved using Dionex Chromelion® Chromatography Management Software.

Results

Figure 1 shows the resolution of a 25-base target Trityl-off oligonucleotide from the “n – x” (x = 1 - 13) and “n + 1” failure sequences, as well as from the Trityl-on component. The peak width at half height for all 15 peaks with retention times between 1.5 and 16 min was 3.48 ±0.5 s, demonstrates outstanding peak shape and capacity.

Figure 2 is an overlay of 10 chromatograms showing separation based primarily on size between 10 oligonucleotides using a NaClO₄ eluent at pH 6.5. Each set of oligonucleotides differs in length from the next set by 1 base. Within each set of identical length oligonucleotides, the oligonucleotides differ from one another by the nature of the 5’ or 3’ terminal sequences.

Conclusions

DNAPac PA200 is packed with 8 µm substrate particles functionalized with a new monomer that improves latex stability for improved efficiency in both size-based and composition-based separations. The new phase produces improved peak width and resolution.


Figure 2. Oligonucleotide elution by length: Elution by the indicated gradient of NaClO₄ at pH 6.5 with 20% CH₃CN. The 21–25 base oligonucleotides represent the earliest and latest eluting components at each length from a set of 21 oligonucleotides.
the ability to rapidly resolve oligonucleotides from both their failure sequences and their identical length, derivatized version. The phase also produces sufficient resolution to support increased throughput applications.

References

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