Oligonucleotide Purification and Analysis by Anion-Exchange and Reversed-Phase Chromatography

Phosphodiesters

Syntheses of phosphodiester oligonucleotides often result in high yields of the target oligonucleotide and lesser amounts of n-1, n-2, etc. High performance anion-exchange chromatography on a Vydac 301VHP column will separate oligonucleotides differing in length by one nucleotide residue, as shown by the oligonucleotide ladder chromatogram of Figure 1.

The separation of impurities from a synthetic 10-mer is shown in Figures 2-4. Typical of scale-up separations, NaCl gradient slope was reduced by a factor of five in Figure 3 to improve resolution with the larger sample. Further reduction in gradient slope permits even larger sample loads, up to 5 mg or more, with the 7.5 x 50 mm column.

Phosphorothioates

Phosphorothioate oligonucleotides have previously been separated using high-pH mobile phases to avoid aggregation. We found that incorporating 50% isopropanol (IPA) in the eluting buffer allows both trityl-on and trityl-off phosphorothioates to be separated by anion exchange at near neutral pH on a Vydac 301VHP column (Figure 5, below). The trityl-off phosphorothioate elutes slightly earlier than the trityl-on S-oligonucleotide. The trityl group elutes near the void volume.
Synthetic Oligonucleotides by C4 Reversed-Phase

Joe Kosmoski of Washington State University has successfully purified oligos ranging from 20 to 135 bases in length using reversed-phase chromatography on a Vydac C4 column (Cat# 214TP1010). Figures 6 and 7 show chromatograms of the crude 135-base oligo with the 5'-dimethoxytrityl, and subsequent purification of the deprotected oligo. By first running the 5'-DMTr oligonucleotide, which is more strongly retained, and then the detritylated oligo with a shallower gradient, this two-step procedure favors a high degree of purification from extraneous compounds.

Figure 6 shows data from polyacrylamide gel electrophoresis used as a quality control.

**ORDERING INFORMATION:**

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Description</th>
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<tbody>
<tr>
<td>301VHP575</td>
<td>Column, Anion-Exchange, Tertiary Amine, 'DEAE' type, 5µm, 900Å, 7.5mm ID x 50mm L</td>
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<td>214TP1010</td>
<td>Column, C4, 10µm, 300Å, 10mm ID x 250mm L</td>
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<td>214TP54</td>
<td>Column, C4, 5µm, 300Å, 4.6mm ID x 250mm L</td>
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<tr>
<td>214TP5415</td>
<td>Column, C4, 5µm, 300Å, 4.6mm ID x 150mm L</td>
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Other analytical and preparative column dimensions available upon request.

To place an order, call 800-247-0924 or contact your local Vydac distributor.

Vydac’s appreciation to

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