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Essentials in biore

Large Scale Purification of Synthetic Oligonucleotides by HPLC

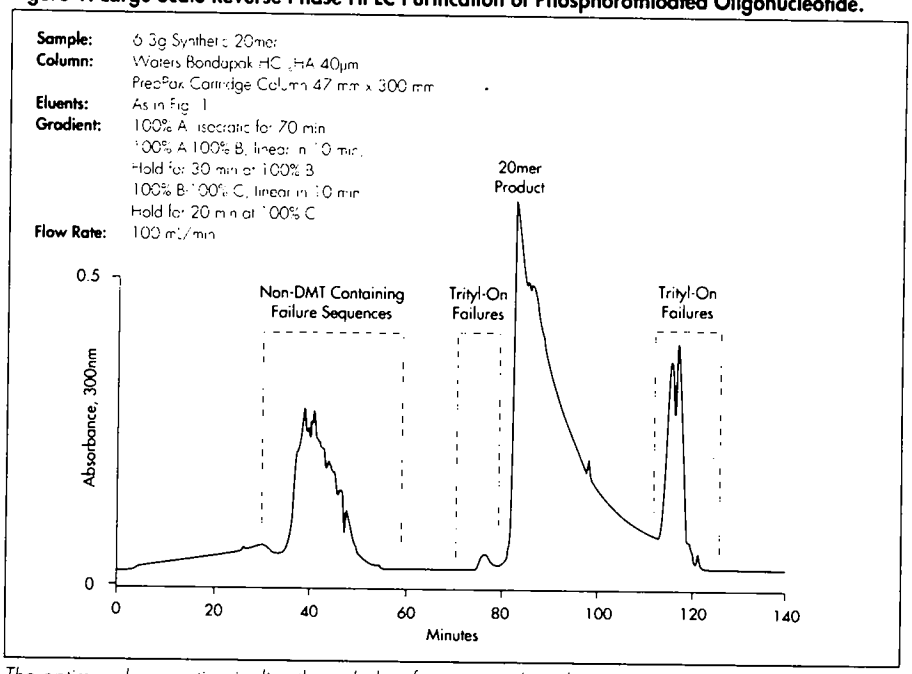
Increased demand for synthetic oligonucleotides.

The *in-vivo* use of synthetic oligonucleotides to inhibit the translation of mRNA to protein (i.e. antisense oligonucleotide intervention) or as inhibitors of transcription of genomic DNA to RNA (i.e. third strand complex inhibition) offers exciting potential for use of nucleic acids as therapeutic agents. Research and development activities in this field require the synthesis of milligram and greater quantities of synthetic oligonucleotides. Although automated instruments are capable of large scale syntheses, analysis and large scale purification strategies must also be addressed.

Why oligonucleotide purification is necessary.

Synthetic oligonucleotides are routinely synthesized via solid-phase techniques using automated instrumentation. Because the coupling efficiency in each synthesis step is not 100%, failure sequences often contaminate the desired product. Furthermore, the amount of impurities is directly proportional to the length of the oligonucleotide synthesized. For many investigations, product purification is required to assure the specificity of the oligonucleotide for the target RNA or DNA. Conventionally, polyacrylamide gel electrophoresis has been used; however, this technique has limitations. Only 5 to 10 $O.D._{260}$ units of product can be purified in any single gel separation. Furthermore, post-electrophoresis recovery techniques must be employed which can result in poor product recovery as well as gel matrix contamination. High performance liquid chromatography (HPLC) is an alternative to electrophoresis techniques that offers a choice of separation chemistries and a flexibility of isolation scale.

Figure 1: Large Scale Reverse Phase HPLC Purification of Phosphorothioated Oligonucleotide.



The optimized separation is directly scaled-up from an analytical to a preparative cartridge column.

Reverse phase HPLC purifies milligrams to grams of oligonucleotides.

Upon completion of the DNA synthesis, the oligonucleotide product can be removed from the synthesis support using procedures which maintain the presence of the hydrophobic dimethoxytrityl (DMT) blocking group on the product. The shorter failure sequences lack the DMT group. Reverse phase chromatography takes advantage of a hydrophobic interaction between the DMT group and the column packing to separate the oligonucleotide product from the failure sequences. Volatile eluents are used that facilitate easy product recovery via lyophilization. Waters Bondapak™ $HC_{18}HA$ packings are an excellent selection for oligonucleotide separations that consistently yield quantitative recoveries. Available in a choice of column geometries, the Bondapak $HC_{18}HA$ column best for your isolation can be selected. For methods optimization and scale up, Waters unique Radial-Pak™ and PrepPak® cartridge columns offer an

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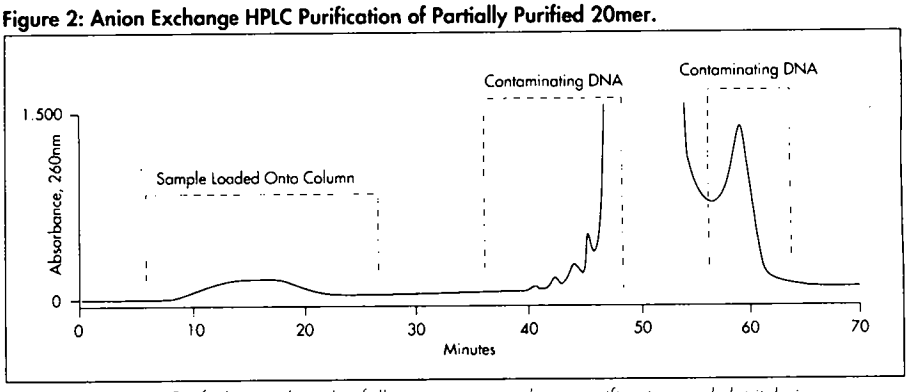
economical alternctive to stainless steel columns. For example, as much as 63 mg of a synthesis reaction mixture containing a phosphorothioated 20mer can be processed under these conditions. Because the same Bondapak $HC_{18}HA$ packing is available in larger cartridge column formats, it is possible to predictably scale up the separation to process multigram quantities. Figure 1 illustrates the separation achieved for 6.3 g synthesis reaction mixture using a PrepPak® 47 mm I.D. x 300 mm cartridge column. This process resulted in the quantitative recovery of the 20mer synthesis product possessing >90% homogeneity.

Figure 2: Anion Exchange HPLC Purification of Partially Purified 20mer.



Ion exchange HPLC of oligonucleotides following reverse phase purification and detritylation improves product homogeneity. Detection performed at 310 nm (A) and 260 nm (B).

Ion Exchange HPLC.

Product homogeneity in excess of 99% can be obtained if anion exchange HPLC is used after a reverse phase purification step. Millipore offers a family of resin based ion exchange packings for high resolution oligonucleotide separations and predictable, high capacity large scale performance. Application of a 15 µm strong anion exchange chemistry to the further purification of a detritylated phosphorothioated 20mer illustrates the potential for this multi-step process (Figure 2).

Waters™ HPLC Systems For Oligonucleotide Purification.

Millipore offers a choice of systems for purifying synthetic oligonucleotides. Coupled with one of Waters high performance detectors, a system may be designed that meets the unique needs of your laboratory.

Waters 600 Multisolvent Delivery System is a versatile HPLC system for both analysis and purification. Its flow rate range accommodates a wide selection of columns and packings.

Waters Delta Prep™ 4000 Preparative Chromatography System lets you develop methods, perform large scale purification, and check fraction purity with the same system.

The Millennium 2010 Chromatography Manager provides comprehensive documentation of all analysis steps, including method set-up and storage, sample identifications and offers flexible reporting capabilities for easier compliance with regulatory guidelines.

Fast, efficient synthesis of DNA and RNA

To select the automated synthesizer best suited to your research requirements, you should consider the synthesis scale, capability for multi-column (parallel) syntheses, and the availability of pre-programmed and user-programmable protocols. You must also consider the kind of nucleic acids to be synthesized since the monomers, reagents and protocols employed will vary. Millipore offers a complete range of instruments and chemicals for DNA and RNA synthesis.

For example, the 8800 Large Scale DNA Synthesizer supports standard ß-cyanoethyl phosphoramidite, H-phosphonate and Beucage chemistries for large scale syntheses. The synthesis scale range of the 8800, recently improved to 30 - 600 µmoles, is the highest available and is continually being extended to accommodate even larger syntheses. Low monomer/CPG ratios ensure economic production of homogeneous nucleic acid probes or anti-sense oligonucleotides.

Millipore developed and patented the ß-cyanoethyl phosphoramidite chemistry for DNA synthesis. Our innovations continue with the development of Expedite™ monomers, a novel new chemistry that reduces deprotection time from 8 hours to 15 minutes. A complete range of chemicals and reagents for DNA and RNA synthesis are manufactured at our U.S., Germany and Japan facilities.

Ordering Information:	**Part No.**
Bondapak $HC_{18}HA$ PrepPak Cartridge 37-55 µm, 125 Å, 47 mm x 300 mm	38570
Protein-Pak Q 15HR Column, 15 µm, 1000 Å	
10 mm x 100 mm	37663
20 mm x 100 mm	37664
50 mm x 100 mm	37665