Essentials in bioresearch

94-0497

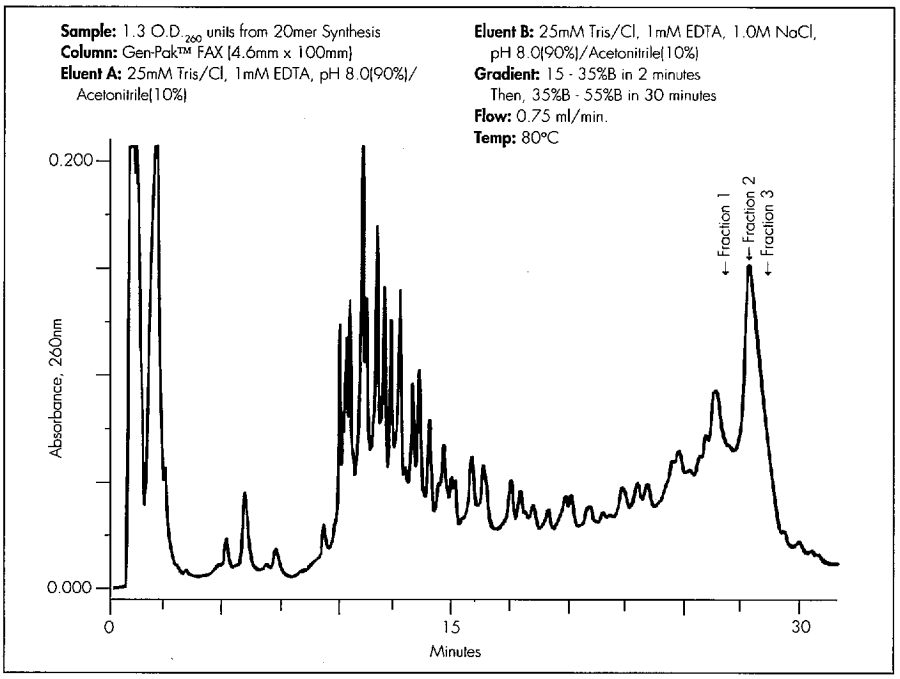
Instrumentation, Chemistries, and Expertise for Bioseparations

Rapid, High Resolution Analysis and Purification of Synthetic Oligonucleotides

Single-step HPLC isolations.
In contrast to gel electrophoresis, HPLC techniques allow you to separate and directly collect the oligonucleotide product. Additional manipulations, such as DNA visualization and extraction, are not required and gel matrix contamination is avoided. As a result, mass recoveries in excess of 95% are routinely achieved by ion exchange chromatography. Waters Gen-Pak™ FAX column is capable of purifying up to 5 to 20 $O.D._{260}$ units of crude synthetic oligonucleotide depending upon product size. Single nucleotide resolution is easily obtained for oligomers up to 20 bases in length (Figure 1). Polyacrylamide gel electrophoresis analysis of collected fractions confirms this resolution (Figure 2). Chromatography at 80°C can further enhance resolution since oligonucleotide secondary structure interactions are minimized under these conditions.

Rapid analysis monitors synthesizer performance.
Even when synthesis of a particular oligonucleotide is routine and repetitive, it is useful to have a rapid analysis technique that allows you to monitor synthesizer performance. In Figure 3, the analysis of two synthesis mixtures representing identical 89mer sequences produced on different days is illustrated. It is apparent that the two synthesis products differ substantially. Were you to use sample B in subsequent applications, it is likely that your results would be compromised. A simple, 40-minute chromatographic analysis assures that a high quality oligonucleotide has been produced and saves the time and expense of repeating experiments.

Figure 1: Anion-Exchange HPLC Purification of a Detritylated 20mer



Anion exchange chromatography using Waters Gen-Pak FAX column provides single nucleotide resolution of detritylated samples up to 20 nucleotides in length.

Figure 2: Autoradiogram of Fractions Collected from Gen-Pak FAX Purification of 20mer Oligonucleotide

Polyacrylamide gel electrophoresis of fractions collected from anion exchange HPLC purification confirm the resolving power of Waters Gen-Pak FAX chemistry.

Waters. The absolute essential for bioresearch.

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Waters
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Versatile column chemistry.

Waters unique Gen-Pak FAX packing consists of a microparticulate, resin-based material bonded with an anion exchange moiety and has been specifically developed for a broad range of nucleic acid separations. In addition to synthetic oligonucleotide separations, the Gen-Pak FAX chemistry can be applied to high resolution separations of plasmid DNA from cell lysates and DNA fragments generated from restriction enzyme digestions or from the polymerase chain reaction††. Each application has been thoroughly documented to assist you in applying HPLC to your particular samples.

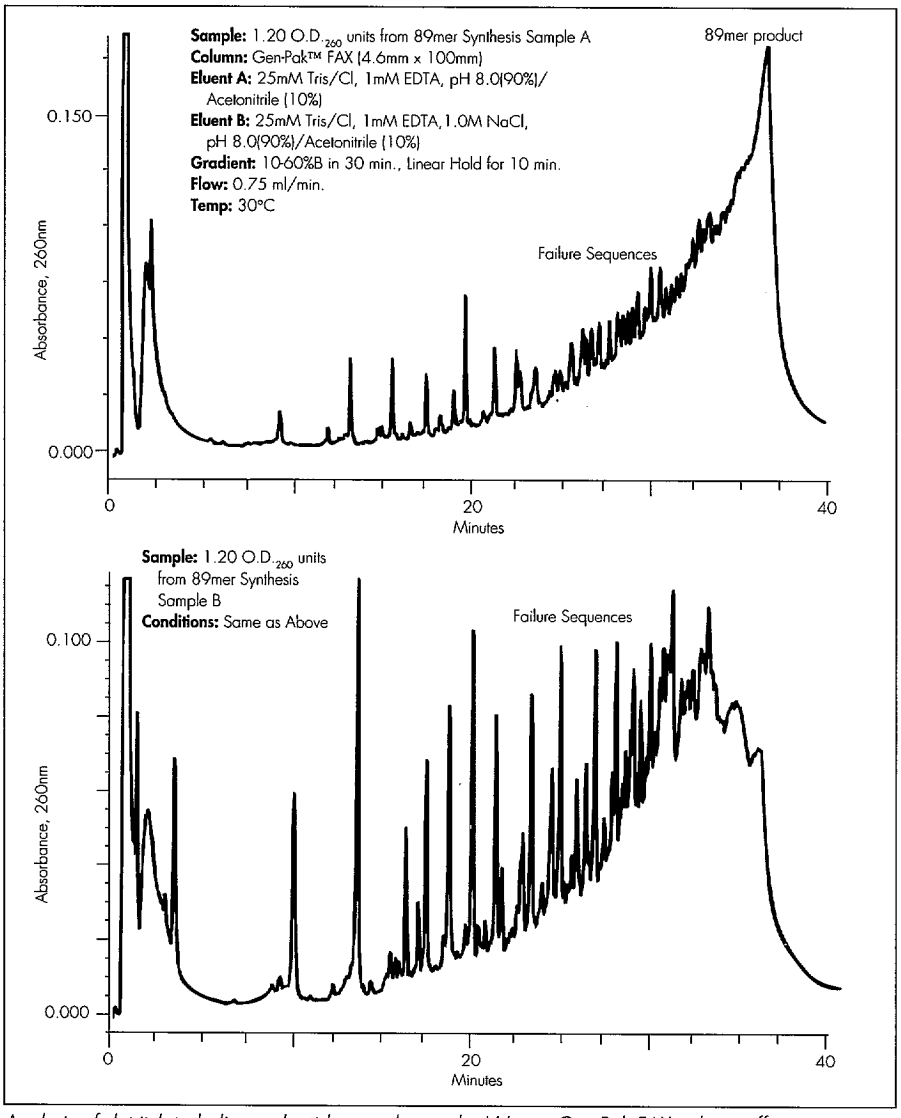
Fast, efficient synthesis of DNA and RNA

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 Cyclone™ Plus Synthesizer is optimized for the production of PCR or sequencing primers, probes, linkers and longer fragments, The power of the Cyclone Plus system lies in its combination of simplicity, speed and performance. Synthesize DNA at scales of 0.05, 0.2, 1.0 and 15 µmoles using ß-cyanoethyl phosphoramidite chemistry in a single or dual column format. Each synthesis protocol is available on preprogrammed cartridges for easy implementation.

- 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.

Figure 3: Anion Exchange HPLC Analysis of Two Detritylated Synthetic Oligonucleotides Possessing the Same Sequence but Synthesized on Different Days.



Analysis of detritylated oligonucleotide samples on the Waters Gen-Pak FAX column offers a convenient means to confirm product quality prior to sample utilization. For demanding applications, oligonucleotide products can be directly isolated from the smaller truncated sequences without use of tedious electrophoretic techniques.

A complete range of chemicals and reagents for DNA and RNA synthesis are manufactured at our U.S., Germany and Japan facilities.

References.

1) Vornham, A.V. and J. Kerschner. 1986. Purification of small oligonucleotides by polyacrylamide gel electrophoresis and transfer to diethylaminoethyl paper.Anal. Biochem. 152:221-225.

Ordering Information:

Item	Part No.
Gen-Pak FAX (4.6mm x 100mm)	15490
In-line precolumn filter kit recommended for use with Gen-Pak FAX	84560
Temperature Control System	38039
Automated DNA/RNA Synthesizer and Chemicals	*
Oligonucleolide Purification HPLC System	*

* Contact your local Waters technical representative to configure the optimal systems for your laboratory.
††The PCR process is covered by U.S. patents issued to Cetus Corporation.

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