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Essentials in biore

Rapid Purification and Quantitation of Polymerase Chain Reaction* Products.

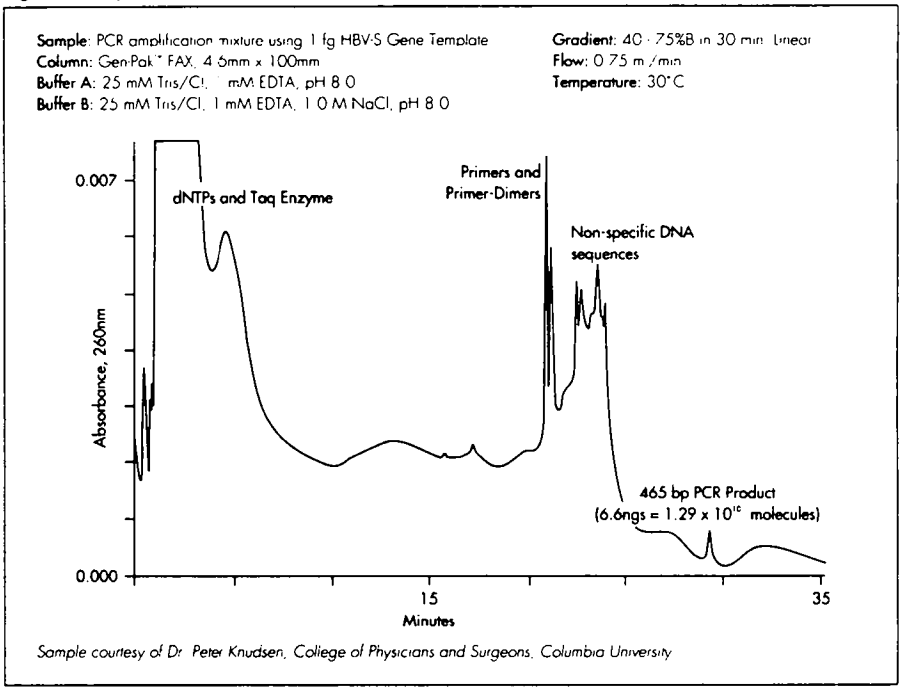
Alternative to multi-step electrophoretic procedures.

The polymerase chain reaction (PCR) is a recently developed procedure for the *in-vitro* amplification of DNA sequences that has gained widespread acceptance. Utilizing this technique, one can obtain a selective enrichment of a specific DNA sequence by factors greater than 10^5[1]. For many research and diagnostic applications, it is necessary to purify as well as quantitate these DNA fragments. Conventionally, this is accomplished using a multi-step electrophoretic procedure[2]. Visualization of the target DNA can then be accomplished by staining with a DNA intercalating reagent such as ethidium bromide. Although a minimum of 1 to 5 ng of DNA can then be detected by ultraviolet illumination, this technique is only semi-quantitative[3]. Furthermore, isolation of the PCR product requires additional gel manipulation and extraction techniques. In comparison, High Performance Liquid Chromatography (HPLC) enables one to purify and quantitate PCR products using a simple, single step procedure.

Optimize PCR amplifications and quantitate products using HPLC.

PCR amplification conditions (melting, annealing, and extension temperatures as well as the concentrations of primer, DNA template and DNA polymerase enzyme) must be optimized in order to obtain amplified PCR products in high yields[4]. High performance anion exchange chromatography using the Waters Gen-Pak™ FAX Column is ideally suited for use in post-PCR amplification analysis. Figure 1 illustrates the analysis of a PCR mixture containing a 465 base pair product following a 40 cycle amplification of 1 femtogram of the Hepatitis B Virus S-gene template. The HPLC analysis clearly indicates a low product yield suggesting non-optimal reaction conditions. Sub-nanogram sensitivity via on-line 260 nm monitoring is easily achieved thereby eliminating the need for ethidium bromide staining. Furthermore, the use of a simple calibration curve using DNA standards of known concentration allows calculation of the actual number of DNA molecules present in the sample.

Figure 1: Rapid Analysis of a PCR Amplification Mixture.



Rapid analysis of a PCR amplification mixture is achieved by anion exchange chromatography on the Waters Gen-Pak FAX Column. High sensitivity on-line detection enables direct quantitation of the 465 base pair product produced from amplification of 1 femtogram of Hepatitis B Virus S-gene template.

*See U.S. Patent No. 4683202 to Cetus Corporation

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Direct recovery of PCR amplified DNA.

Compared to electrophoretic techniques, purification of PCR amplified DNA by ion exchange chromatography on the Waters Gen-Pak™ FAX Column is rapid, direct and easily automated. As seen in the optimized PCR amplification of the same 465 base-pair DNA segment within the Hepatitis B Virus S-gene (Figure 2), single-step isolation of the PCR product from the contaminants contained in the mixture is possible. Because ethidium bromide is not used, no subsequent purification steps are required. Furthermore, samples are recovered in a Tris/EDTA/NaCl buffer and are ready for subsequent use.

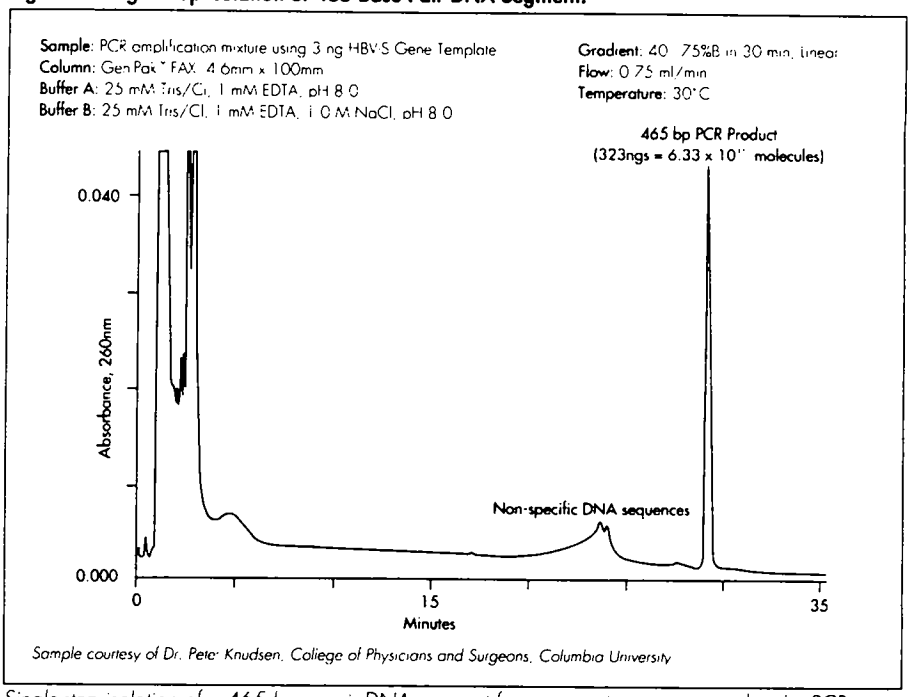
Versatile column chemistry.

The analysis and purification of DNA restriction fragments, synthetic oligonucleotides and plasmids is made possible by the Waters Gen-Pak FAX Column, a polymer-based, anion exchange packing material. In each case, simple easily implemented protocols have been developed to aid you in applying this chromatographic technology to your research. These techniques offer an alternative to gel electrophoretic, centrifugation and ultrafiltration techniques currently used for molecular biology applications.

Increased utilization of instrumentation.

In addition to applications involving PCR products, Millipore, an established leader in chromatographic chemistries and instrumentation, offers a comprehensive assortment of documented purification techniques for proteins, peptides, amino acids and other biomolecules. Thus, the versatility of HPLC instrumentation makes chromatography a cost effective purification and analysis tool in todays molecular biology laboratory.

Figure 2: Single-Step Isolation of 465 Base Pair DNA Segment.



Sample courtesy of Dr. Peter Knudsen, College of Physicians and Surgeons, Columbia University

Single-step isolation of a 465 base pair DNA segment from contaminants contained in the PCR amplification mixture is easily accomplished on the Waters Gen-Pak FAX Column. The sample is recovered in a Tris/EDTA/NaCl buffer ready for subsequent.

Summary.

Compared with gel electrophoresis, HPLC methodologies provide a rapid and easily automated technique for the analysis, quantitation and collection of microgram to sub-nanogram levels of PCR amplified DNA. These characteristics are ideal for those applications where precise quantitation of PCR products is desired. In addition, because of the non-destructive nature of this technique, one can simultaneously purify the desired PCR amplification product for subsequent use. The utilization of this chromatographic approach will prove to be of significant value to researchers involved in the analysis and purification of DNA fragments generated from the polymerase chain reaction.

References

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Ordering Information	Part No.
Waters Gen-Pak FAX Column (4.6mm x 100mm)	15490
Waters Temperature Control System	38039
Waters DNA Purification HPLC System	*

*Contact your local Waters technical representative to configure the optimal system for your laboratory.