Waters Application Notebook

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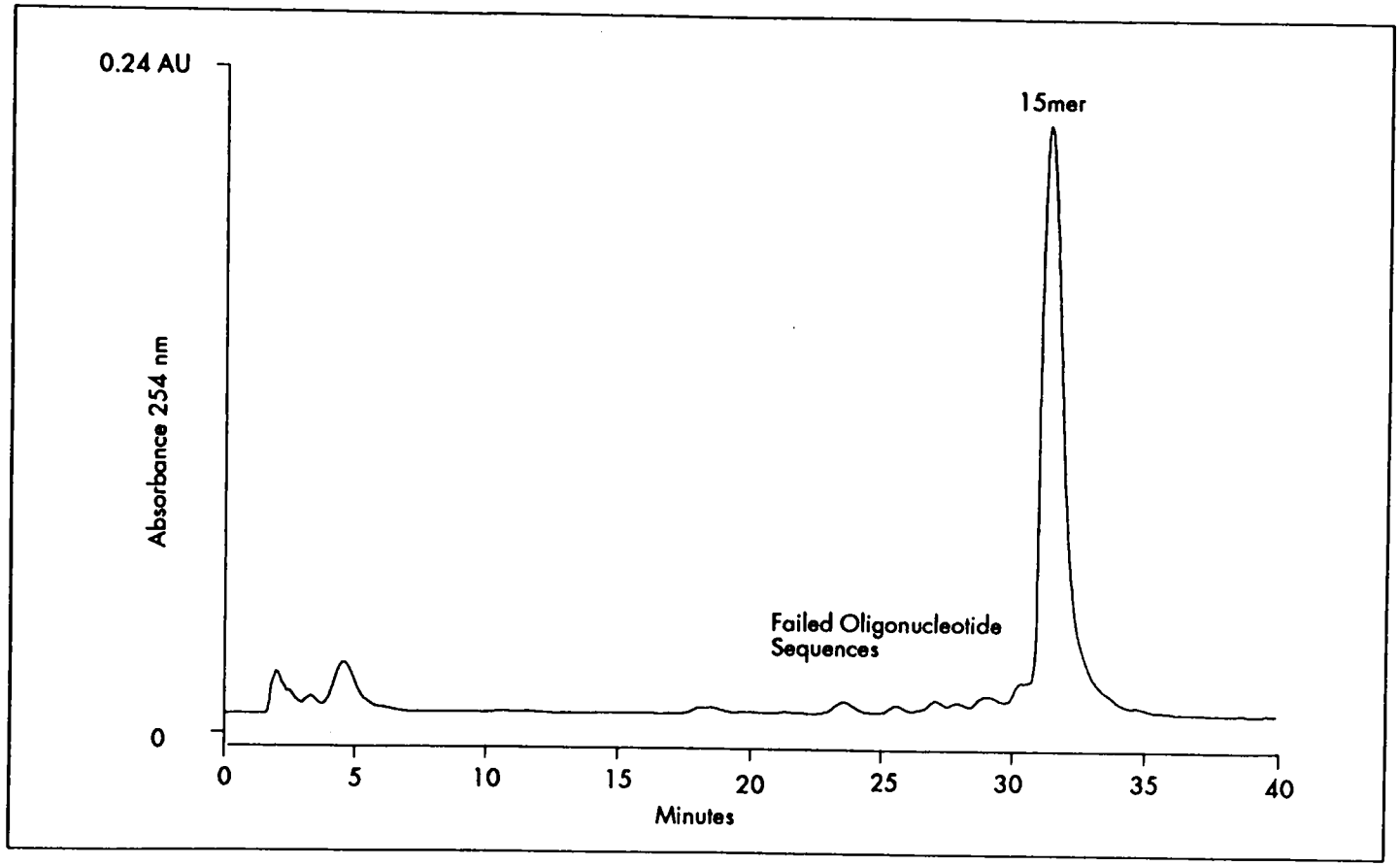
Anion Exchange Analysis of Unpurified Oligonucleotide Synthesized with the Fast Probe Protocol (FPP)



Cyclone Plus Synthesis of Oligonucleotide (15mer, random sequence); Fast Probe Protocol (FPP) 200nM

Conditions:

Sample: Oligonucleotide 15mer (random sequence)

Column: Gen-Pak™ FAX (4.6 x 100 mm)

Eluent A: 20 mM Ammonium phosphate, pH 8.0/Acetonitrile (90/10)
Eluent B: Eluent A + 1.0 M Sodium chloride

Gradient: 20 - 80% B in 60min (linear gradient)

Flow Rate: 0.5 ml/min.

Detection: 254 nm

Injection Volume: 10 µl

Sample Concentration: 0.05 O.D. 260 nm/µl

Sample Preparation: The Final DMT was removed on-line. Sample was deprotected under standard conditions (see details), dried and resuspended in Milli-Q® water.

Objective:

The objective of this application note is to demonstrate the high product yield of Millipore's DNA synthesizers, specifically the Cyclone's Fast Probe Protocol (FPP), and the ease by which it is analyzed by Millipore's separation systems.

Details:

Synthesis was done on the Cyclone™ Plus, dual column, DNA synthesizer using the 200nM Fast Probe Protocol (FPP)*. The FPP has been developed for use with amidite chemistry. The advantage of the new protocol is that cycle times and per coupling monomer consumptions are greatly reduced. The FPP reduces single column cycle time from 6 min. to 3 min. 38 sec. and dual column synthesis from 9 min. 30 sec. to 5 min.15 sec.. Also, amidite consumption per cycle has been cut in half with the FPP, from 10mg/cycle to 5mg/cycle.

The sample shown is an oligonucleotide (15mer, random sequence). The final DMT was removed on-line and the oligo was cleaved and deprotected with concentrated ammonium hydroxide (30%) for 6-8 hrs. at 55°C, dried in a SpinVac, resuspended with Milli-Q water and injected onto the Gen-Pak™ FAX column as crude product with no post synthesis purification. Based on peak area the coupling efficiency was measured to be 99.1%.

*Technical bulletin to be released

System:

The System used consisted of a Cyclone™ Plus DNA Synthesizer, 600E Multi Solvent Delivery System, 484 Tunable UV/Visible Absorbance Detector, 712 WISP, Maxima™ 820 Chromatography Data Workstation.

References:

Cyclone™ Plus Operator's Manual, "Product Isolation and Purification".