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Application Note SI-02000

Synthesis of d(T)₇₀ using StratoSpheres[™] DNA

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Introduction

The synthesis of poly–T oligomers is a valuable exercise as it reveals information about the pore structure and pore accessibility of the support during synthesis. The chromatogram is not complicated by the possibility of side reactions that can occur with the other bases, A, C and G. In this d(T)₇₀ example, the chromatograms have been normalized in order to give similar responses. In practice, the yield of product from the controlled pore glass (CPG) support (red trace) is severely compromized due to incomplete coupling reactions. There is a much larger number of capped deletion sequences eluting prior to the main peak with CPG. The main peak from the CPG support is much broader and "asymmetrical" indicating a higher proportion of "trityl on" deletion sequences. The StratoSpheres DNA dT support equals the performance of the leading commercial macroporous PS/

DVB.

Oligonucleotide Synthesis

The oligonucleotides were prepared on 0.2 μ M scale using an Applied Biosystems 392 DNA/RNA Synthesizer and standard chemistry. Following synthesis, the oligonucleotides were cleaved using ammonium hydroxide. The "DMT on" oligonucleotides were diluted with water in order to give an on-scale response during HPLC analysis.

HPLC Analysis

Column: Pellicular SAX, 4 x 250 mm Eluent: A = 25 mM Tris-HCl, 0.5% ACN, pH 8.0; B = 25 mM Tris-HCl, 0.8 M Ammonium Chloride, 0.5% ACN, pH 8.0 Gradient: 0 -100% B in 26 min Flow Rate: 1.5 mL/min Temp: 60 °C Detector: UV 260 nm



These data represent typical results. For further information, contact your local Varian Sales Office.

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