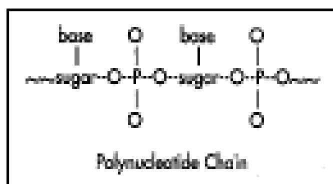
APPLICATION NOTE
1195-003

HPLC SEPARATION GUIDE

November '95

Separation of Nucleotides

In every living cell there are found nucleoproteins: substances made up of proteins combined with natural polymer of another kind, the nucleic acids. The backbone of the nucleic acid molecule consists of a polyester chain (called a polynucleotide chain). The ester is derived from phosphoric acid (the acid portion) and a sugar (the alcohol portion). A base-sugar unit is called a *nucleoside*; a base-sugar-phosphoric acid unit is called a nucleotide.



High performance liquid chromatography (HPLC) had been widely applied to the separation of nucleobases, nucleosides, and nucleotides. The two most commonly employed separation techniques involve either ion-exchange or reversed-phase chromatography. Separation of nucleotides is often compounded by multifunctional and hydrophobic interactions with the stationary phase.

We report here a reversed-phase HPLC method that results in high resolution of Cy3dUTP triphosphate and Cy3dUDP diphosphate (Figure 1). Both Cy3dUTP and Cy3dUDP consist of tri and diphosphate nucleotides that have been covalently bonded to an organic dye (Cy3-OSu). Cy3dUTP and Cy3dUDP can be represented by the organic structure shown in Figure 2. The ability to isolate or identify these closely related organic compounds (or other labeled biomolecules of similar structure) is the basis for their utility as chromosome probes.

Figure 1.

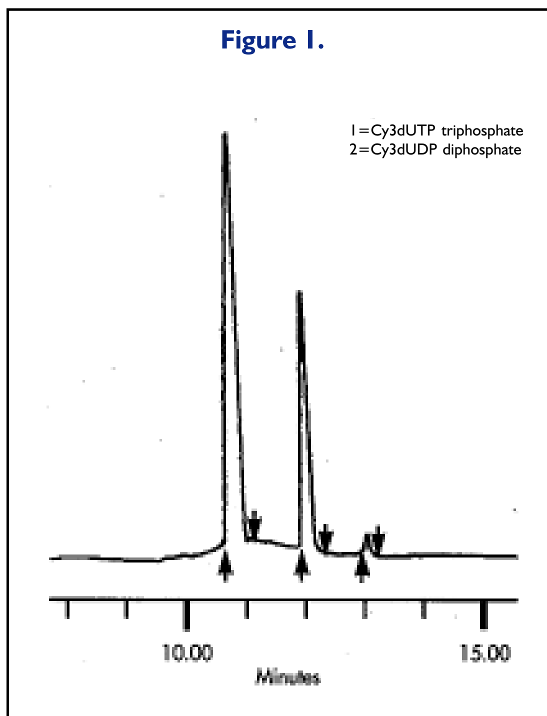
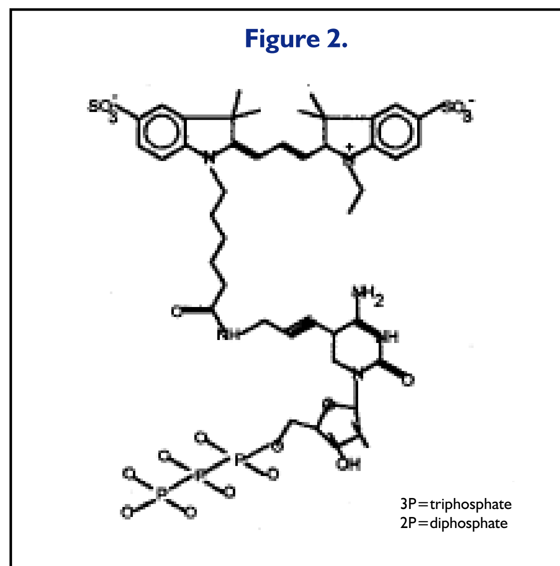


Figure 2.



Column Specifications:

Particle:	Silica, 3 μm
Pore Size:	60Å
Surface Area:	500 m^2/g
% Carbon:	24%
pH range:	1-12

Chromatographic Conditions (Figure 1)

Solutes: Cy3dUTP and Cy3dUDP
Column: SMT **0D-5-60**
Mobile Phase: A=ACN
B=50mm Phosphate buffer (pH 7)
(Linear gradient 10-40% A in 20 min)
Flow: 1 mL/min
Detector: UV/Visible-550nm

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SEPARATION METHODS TECHNOLOGIES
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