Glen Research

REPORT

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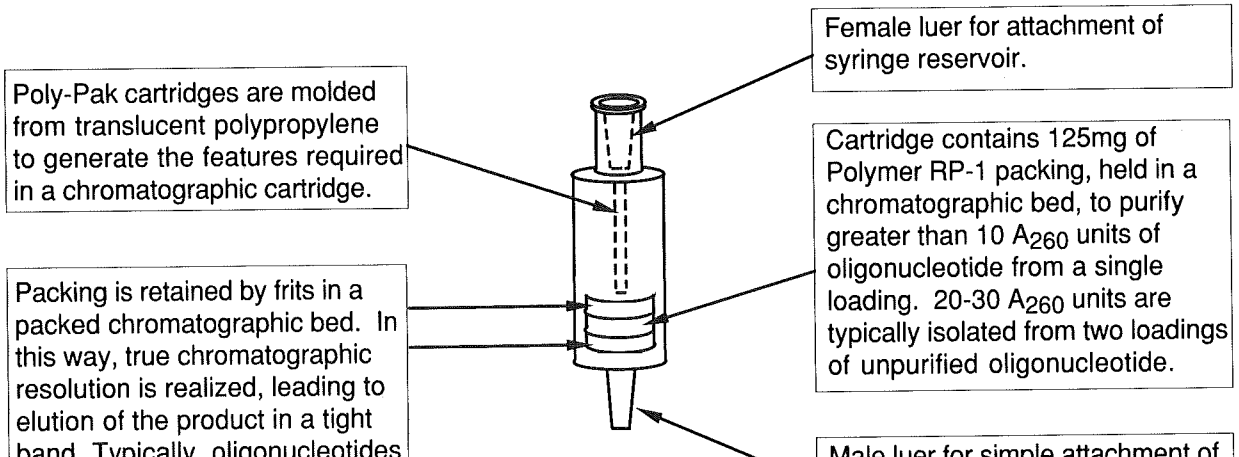
Rapid and Efficient Purification of Oligonucleotides

Reverse phase (RP) cartridges have been popular[1] for some time for rapid and inexpensive purification of synthetic oligonucleotides. The use of a polystyrene-divinylbenzene copolymer packing in cartridges or columns overcomes several disadvantages usually associated with silica gel based RP cartridges. Polystyrene is stable in the pH range 1-13, thus the ammonia deprotection solution, diluted with water, may be loaded directly onto the packing. Also, after elution of failure sequences, the dimethoxytrityl group may be removed while the oligonucleotide is support-bound. The fully deprotected product can then be eluted and isolated by lyophilization. Polystyrene packing may also be used for desalting a normal or dye-labelled oligonucleotide eluted from a gel.

The packing Glen Research has selected for use in purification cartridges is Polymer RP-1, a highly purified polystyrene resin with a particle size distribution of 12-20 micron. Glen Research Poly-Pak™ cartridges, therefore, contain 125mg of Polymer RP-1 packing retained by frits in a packed chromatographic bed. The cartridges are molded from translucent polypropylene. The

Reference: 1. K.M. Lo, S.S. Jones, N.R.Hackett,and H.G. Khorana, *Proc. Natl. Acad. Sci. USA*, 1984, **81**, 2285.



ANATOMY OF A POLY-PAK™ CARTRIDGE

Poly-Pak cartridge housing has a female luer fitting at the inlet to attach a luer-hub syringe reservoir for fast, convenient sample processing. A male luer fitting at the outlet is used to direct flow to a collection tube or to attach the cartridge to a vacuum manifold for parallel processing of multiple samples.

Poly-Pak cartridges exhibit the following attractive features:

1) The ability to prepare at least 10 A_{260} units and as much as 30 A_{260} of purified oligonucleotide. This feature is highly significant for the production of oligonucleotides destined for use in DNA amplification experiments.

2) Wash volumes are diminished to a level where the total eluent is less than 15mL. This speeds the process dramatically in comparison to other reverse phase cartridges.

3) Product oligonucleotides are eluted in 4 drops of aqueous acetonitrile, thereby minimizing the time needed for solvent evaporation.

The ability of Poly-Pak cartridges to produce high quality oligonucleotides is demonstrated in the purification of a 24-mer, as shown in the Figures to the right. Figure 1 contains the RP HPLC trace of the crude DMT-ON oligonucleotide after deprotection with ammonia. In Figure 2, the purified DMT-ON 24-mer has been eluted from the cartridge to illustrate the purity increase achieved by the cartridge. In Figure 3, The DMT group has been removed and the fully deprotected 24-mer has been isolated in 4 drops of aqueous acetonitrile.

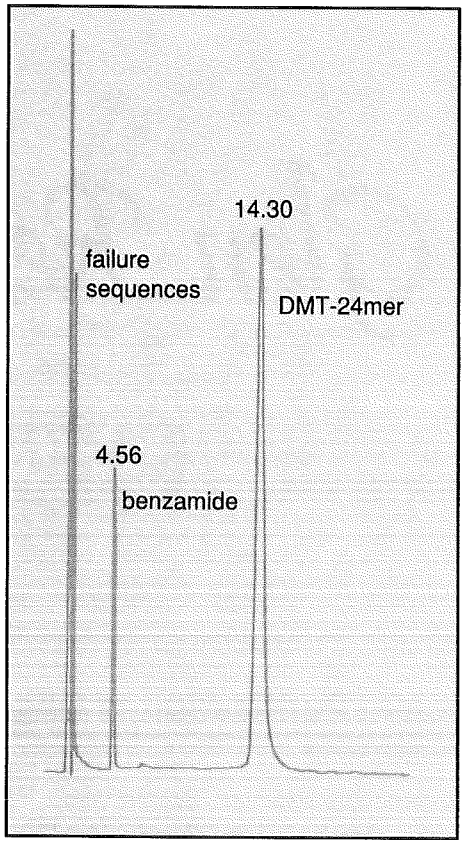


Figure 1: RP HPLC of crude 24mer with DMT-ON following the conditions shown in Table 1.

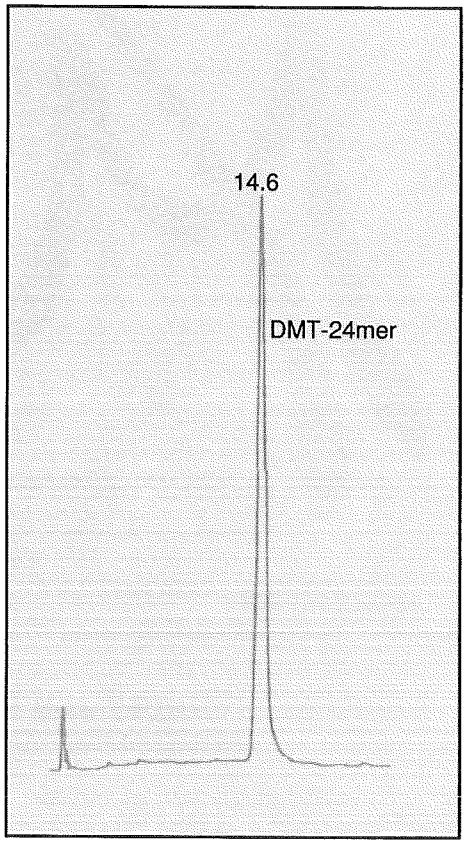


Figure 2: RP HPLC of Poly-Pak purified 24mer with DMT-ON prior to deblocking with TFA following the conditions shown in Table 1.

Time	0.1M TEAA(%)	Acetonitrile(%)
0	90	10
10	84	16
40	84	16
50	90	10
60	90	10

Column: Spherisorb ODS-2 (150X4.6mm)
Flow Rate: 1mL/min.
Detector: UV at 254nm

Table 1: RP HPLC conditions for DMT-ON Chromatography.

Time	0.1M TEAA(%)	Acetonitrile(%)
0	93	7
50	83	17
60	93	7
65	93	7

Column: Spherisorb ODS-2 (150X4.6mm)
Flow Rate: 1mL/min.
Detector: UV at 254nm

Table 2: RP HPLC conditions for DMT-OFF Chromatography

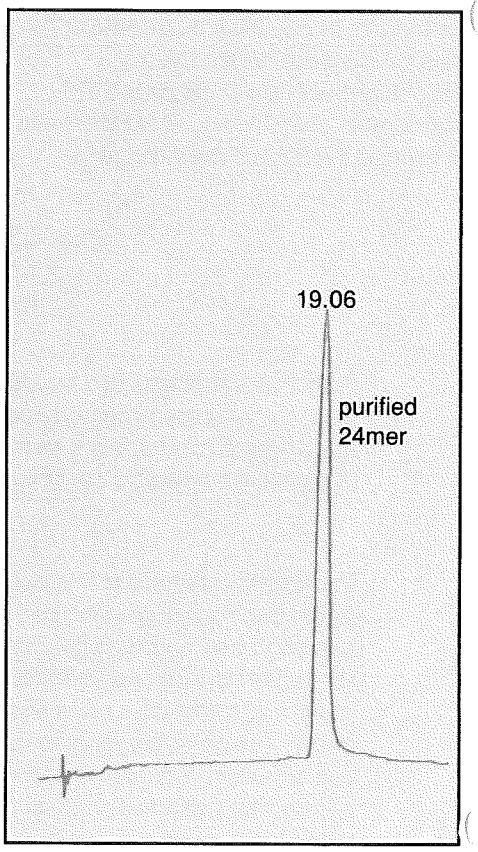


Figure 3: RP HPLC of purified 24mer with DMT-OFF following the conditions shown in Table 2.

ORDERING INFORMATION

Item	Catalog No.	Pack	Price ($)
Poly-Pak Cartridge	60-1100-01	each	8.00
	60-1100-10	10/Pk	80.00
Starter Kit of Wash Solvents *(Sufficient for use with up to 10 cartridges)*	60-4900-01	1 Kit	60.00
Vacuum Manifold (12-way)	60-9000-01	each	475.00

Purification of Labelled Oligonucleotides Using Poly-Pak Cartridges

Amino-modification is an important technique for the preparation of labelled oligonucleotides used in DNA sequencing and amplification, and in the production of diagnostic probes. Currently, the most popular method for the attachment of an amino group to a synthetic oligonucleotide is the use of a 5'-amino-modifier containing the monomethoxytrityl (MMT) protecting group. These modifiers are specifically designed to take advantage of reverse phase purification techniques, including cartridge purification.

Figure 1 shows a 15-mer that has been amino-modified with a 6 carbon spacer. The oligonucleotide has been purified using a Poly-Pak cartridge following the standard trityl-ON procedure. This purified product is now ready for reaction with a suitable labelling compound, for example biotin or fluorescein.

The amino-modified 15-mer, shown in igure 1, was reacted with fluorescein isothiocyanate (FITC). The reaction mixture was desalted and purified on a Poly-Pak cartridge and eluted in aqueous acetonitrile to yield the product of quality shown in Figure 2. Using a similar technique, highly purified biotin labelled sequences have been rapidly isolated.

Recently, 3'-Amino-Modifier C3-CPG, as described on Page 4, has gained popularity for the direct synthesis of oligonucleotides labelled at the 3'-terminus. Again, the intermediate amino-modified oligonucleotide can be purified on a Poly-Pak cartridge using the DMT-ON procedure. After labelling at the 3'-terminus, the product can be rapidly desalted and purified to a high quality as described above.

Glen Research is now happy to offer kits for modifying and labelling ligonucleotides at the 3'- or 5'-terminus with biotin-XX-NHS ester or FITC. The 5'-labelling kits contain our Amino-Modifier C6 and sufficient materials for labelling up to ten 1 µmole syntheses. The 3'-labelling kits contain four 1 µmole synthesis columns containing 3'-Amino-Modifier C3-CPG along with the necessary solvents and buffers.

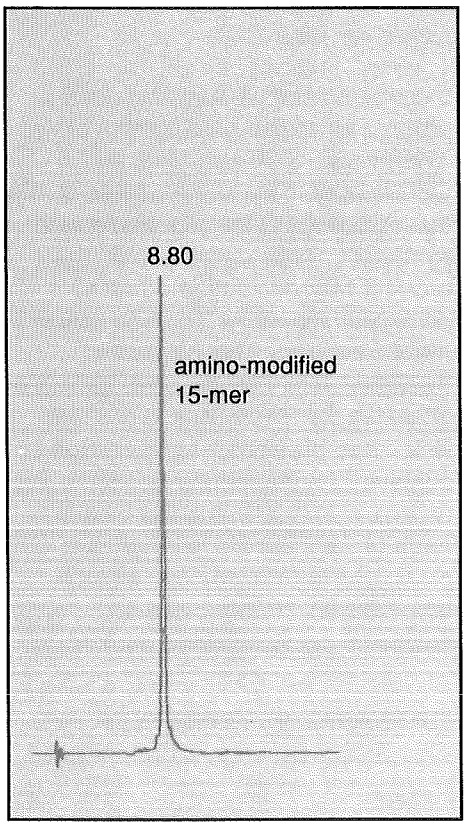


Figure 1: RP HPLC of Poly-Pak purified amino-modified 15-mer following the conditions shown in Table 1.

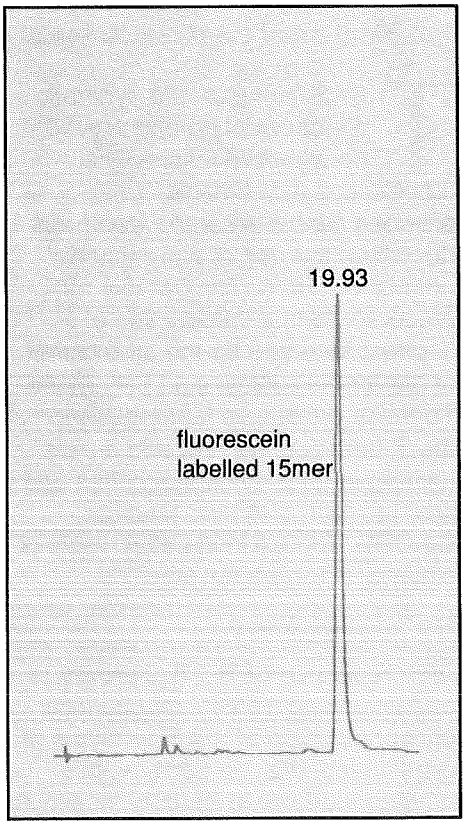


Figure 2: RP HPLC of Poly-Pak purified fluorescein labelled 15-mer following the conditions shown in Table 1.

Time	0.1M TEAA(%)	Acetonitrile(%)
0	93	7
20	75	25
30	75	25
35	93	7
40	93	7

Column: Spherisorb ODS-2 (150X4.6mm)
Flow Rate: 1mL/min.
Detector: UV at 254nm

Table 1: RP HPLC conditions for Amino-Modified Oligonucleotides.

ORDERING INFORMATION

Item	Catalog No.	Pack	Price ($)
Biotin-XX-NHS Ester 5'-Labelling Kit	50-1956-01	each	195.00
FITC 5'-Labelling Kit	50-1966-01	each	195.00
(Above 5'-Labelling Kits contain 5'-Amino-Modifier C6 (100 µmoles), labelling reagents as shown and all solvents and buffers required for up to ten 1 µmole syntheses.)			
Biotin-XX-NHS Ester 3'-Labelling Kit	50-2956-01	each	195.00
FITC 3'-Labelling Kit	50-2966-01	each	195.00
(Above 3'-Labelling Kits contain 3'-Amino-Modifier C3-CPG (Pack of 4 µmole columns), labelling reagents as shown and all solvents and buffers required for up to four 1 µmole syntheses.)			

Modification Update

Modification of the 3'-Terminus of Oligonucleotides

The 3'-terminus of a synthetic oligonucleotide has proved to be attractive for chemical modification experiments. In a previous *Glen Research Report*, we described the use of a protected ribonucleoside support for use as a precursor for the introduction of a 3'-phosphate and for the attachment of carrier molecules, e.g., poly-lysine. To these processes for modification of the 3'-terminus, we now add a new technique for 3'-phosphorylation and a new product, 3'-Amino-Modifier C3-CPG, for use in the attachment of biotin or a fluorescent label.

3'-Phosphorylation

Glen Research CPR-1 has proved to be fast and convenient for chemical phosphorylation of the 5'-terminus of oligonucleotides. In addition, this reagent has proved its utility for simple phosphorylation of the 3'-terminus. It is introduced as the first addition to any nucleoside support, followed by normal synthesis of the target oligonucleotide. After the standard ammonia deprotection, the linkage decomposes and is ß-eliminated from the target molecule, leaving a phosphate group at the 3'-terminus.

3'-Amino-Modifier C3-CPG

Glen Research offers a variety of reagents for amino-modification of the 5'-terminus and we now introduce a product for amino-modification of the 3'-terminus. 3'-Amino-Modifier C3-CPG is used in the same way as normal nucleoside CPG for oligonucleotide synthesis. After deprotection, the product oligonucleotide contains a primary amine with a 3 carbon spacer at the 3'-terminus. This product is therefore useful for the introduction of a label at the 3'-terminus; a process which had previously been difficult to achieve. An interesting benefit of this approach is that during the development of, for example, a diagnostic probe, the 5'-terminus can also be labelled with ^{32}P to provide an additional highly sensitive marker.

Alternative 5'-Amino-Modifier C6

An alternative to the use of the 5'-Amino-Modifier C6, in which the primary amine is protected with the acid-labile MMT group, is the use of the analogue with the primary amine protected with the base-labile trifluoroacetyl (TFA) group. While it is desirable in most cases to use the MMT group to purify the intermediate amino-modified oligonucleotide by a RP technique, it may be occasionally attractive to use the TFA-analogue in which the protecting group is removed during the ammonia deprotection step. Using the latter strategy, the crude intermediate is reacted with biotin or a fluorescent label to give a product which must be purified by RP HPLC.

ORDERING INFORMATION

Item	Catalog No.	Pack	Price($)
Chemical Phosphorylation			
CPR-1	10-1900-90	100 µmole	60.00
	10-1900-02	0.25g	200.00
3'-Amino-Modifier			
3'-Amino-Modifier C3-CPG *(1 µmole columns)*	20-2950-41	Pack/4	125.00
5'-Amino-Modifier			
5'-Amino-Modifier C6-TFA	10-1916-02	0.25g	75.00

Glen Research

Glen Research Corporation
P.O. Box 1047
Herndon, VA 22070
(800) 327-GLEN, (703) 437-6191

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