



Oligonucleotide Purification

Selecting the Best Purification Method

This technical bulletin will help you select the best purification option for your oligo, depending upon the oligo type and intended application.

In DNA synthesis, each oligonucleotide is coupled sequentially to the growing chain in a 3' to 5' direction. In each coupling cycle, a small percentage of the oligo chain will not be extended, resulting in a mixture of full-length product (n) and truncated sequences (**Figure 1**).

Figure 1

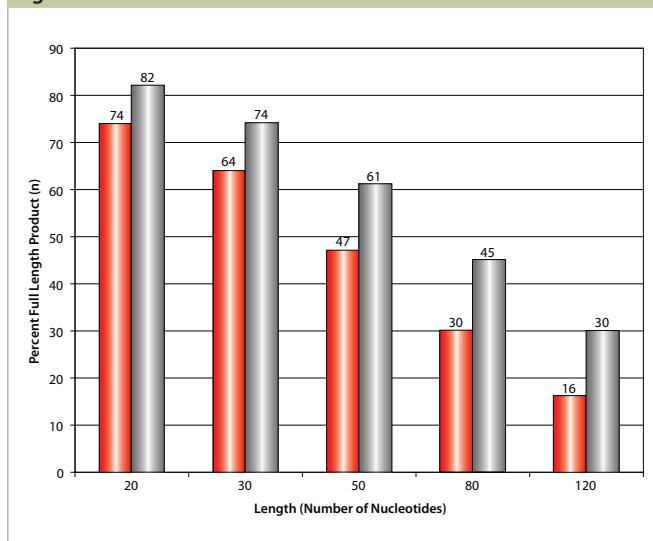


Figure 1. Effect of coupling efficiency on the amount of full length product (n) as a function of the length. Coupling Efficiency of 98.5% (red) and 99% (gray) per nucleotide addition.

Upon synthesis completion, the oligo is cleaved from the support and the protecting groups are removed. The purification step separates the full-length product from the truncated sequences. In general, the purity required for a specific application depends on the potential problems that the presence of truncated oligomers may cause. For some applications, it is crucial that the majority of the full-length (n) oligo be present. For others, the small presence of shorter oligos (n-1, n-2, ...) will not affect the experimental results. The efficiency of a given purification is thus determined by its ability to remove such truncated oligomers, although the efficacy will depend on the nature of the oligo (DNA, RNA, modified, etc.)

Purification of Modified Oligos

Oligos that include common modifications (such as biotin, fluorescein, primary amines) can be produced at any of the purification levels described below. For oligos containing more complex structures (such as ROX™, Texas Red®, Dual Labeled Probes, Molecular Beacons, etc.), HPLC purification is recommended. This level of purification provides the optimal conditions for the isolation of the full-length product due to the hydrophobicity imparted by the fluorescent labels and the ability to select a unique absorbance.

Desalting

Every oligo manufactured by Sigma is desalted. The desalting procedure removes residual by-products from the synthesis, cleavage and deprotection procedures.

For many applications, including PCR, desalting is acceptable for oligos less than or equal to 35 bases in length, as the overwhelming abundance of full-length oligo outweighs any contributions from shorter products. Oligos greater than 35 bases in length, may require an additional method of purification such as Reverse-Phase Cartridge Purification (RP1).

Reverse-Phase Cartridge Purification (RP1)

Separation on a reverse-phase cartridge offers the next level of purity. The basis of the separation is the difference in hydrophobicity between full-length product (which contains a 5'-DMT group) and truncated sequences (without DMT groups). While the full-length DMT oligo is retained on the column, the truncated sequences are washed off. After cleaving the DMT on the cartridge, the expected product is recovered.

As the oligo length increases, the proportion of uncapped products (truncated sequences bearing the DMT) tends to increase. These impurities will not be removed by RP1 and thus for longer oligos, HPLC or PAGE is recommended. Oligos modified with certain dyes at the 5'-end (for example Cyanine® dyes or WellRED dyes), are compatible with RP1 due to the increased lipophilicity imparted by the dye molecule.



Reverse-Phase HPLC (RP-HPLC)

Reverse-Phase High Performance Liquid Chromatography (RP-HPLC) operates on the same principle as a reverse-phase cartridge. However, the higher resolution allows for higher purity levels. HPLC is an efficient purification method for oligos with fluorophores, as their intrinsic lipophilicity provides excellent separation of product from its contaminants. Furthermore, RP-HPLC is a method of choice for larger scales due to the capacity and resolving properties of the column. The resolution based on lipophilicity will decrease with the length of the oligo. Therefore, RP-HPLC is usually not recommended for purifying products longer than 50 bases. Although longer oligos (up to 80 bases) can be purified using this method, the purity and yields may be adversely affected.

PAGE

The basis of the PAGE separation is charge over molecular weight, leading to excellent size resolution, resulting in purity levels of 95–99% full-length product. Yields from PAGE are lower than from other methods due to the complex procedure required for extracting oligos from the gel and the removal of the vast majority of truncated products. This technique is recommended when a highly purified product is required. PAGE is the recommended purification for longer oligos (≥ 50 bases).

Anion-Exchange HPLC

Anion-Exchange separation is based on the number of phosphate groups in the molecule. The Anion-Exchange purification method involves the use of a salt-gradient elution on a quaternary ammonium stationary phase column or a similar structure. The resolution is excellent for the purification of smaller quantities. This technique can be coupled with purification by RP-HPLC, adding a second dimension to the separation process. Anion-Exchange HPLC is limited by length (usually up to 40mers). The longer the oligonucleotide the lower the resolution on the Anion-Exchange HPLC column and thus the purity of the target oligo.

Figure 2 displays an IE-HPLC analysis of a 20mer sequence, indicating the purity gains by each of the standard purification methods (excluding IE-HPLC purification). A summary of the recommended purifications by application is shown in **Table 1**.

Our Technical Service Team can assist in determining what purification method is best suited for your specific application.

Figure 2

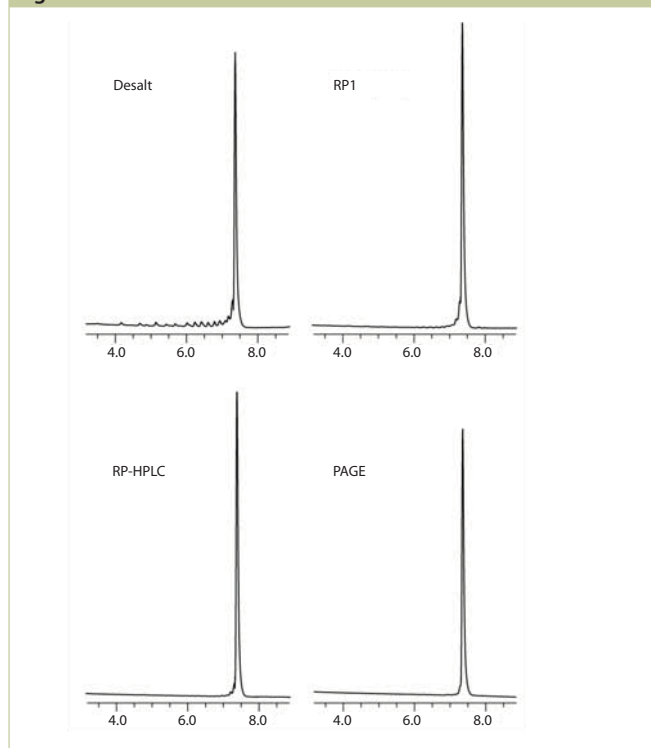


Figure 2. IE-HPLC analysis of a 20mer sequence purified by the different methods.

Table 1

Application	Desalted	RP1	RP-HPLC	PAGE
Standard PCR and RT-PCR	X	X		
Sequencing	X	X		
QPCR probes			X	
Cloning			X	X
Microarrays	X	X	X	X
siRNA screening	X	X		
Antisense			X	X
Bioengineering			X	X
Crystallography, NMR			X	X
In-vivo applications			X	

Table 1. Recommended purification method by application.