

Oligonucleotide Purification

Selecting the Best Purification Method for Your Oligo

When ordering their oligos from Sigma-Genosys, many people wonder how pure is pure enough. This technical bulletin will help you select the best purification option for your oligo, depending upon the oligo type and its intended application.

In DNA synthesis, each nucleotide is coupled sequentially to the growing chain. In each coupling cycle, a small percentage of the oligo chains will not be extended, resulting in a mixture of full length product and truncated sequences.

After the oligo is cleaved from the support and the protecting groups are removed, the purification separates the full length product from the truncated sequences. In general, the purity required for a specific application depends on the potential problems from the presence of truncated oligomers. For some applications, it is crucial that only the full length (n) oligo be present. For others, the presence of shorter oligos (n-1, n-2, ...) will not affect the experimental results.

Purification of Modified Oligos

Oligos that have been modified with biotin, fluorescein, 6-FAM, HEX, and TET can be purified by any of the methods listed below. For oligos that have been modified with the ABI dyes, Texas Red, and any Molecular Probes dyes, we recommend purification by HPLC or PAGE. PAGE is recommended for 5'-phosphorylated oligos or for any application that is sensitive to truncated sequences.

Desalting

At Sigma-Genosys, every oligo is desalted. Desalting removes residual by-products from the synthesis, cleavage, and deprotection procedures.

For many applications (including PCR), desalting is acceptable for oligos ≤ 35 bases; the overwhelming abundance of full length oligo outweighs any contributions from shorter oligos. For all oligos > 35 bases in length, it is recommended that an additional method of purification be considered. Desalted oligos are generally shipped within 24 hours.

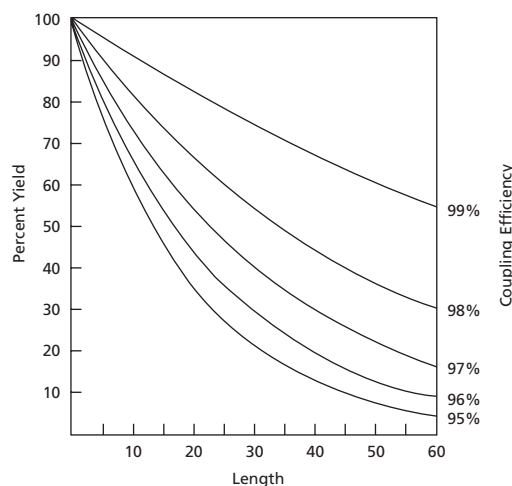


Figure 1. For any synthesis, the overall yield of full length product will depend on the average coupling efficiency and the total number of coupling cycles. For instance, a 20-mer synthesized with a 98% coupling efficiency will result in a crude mixture with 68% full length product.



Reverse-Phase Cartridge Purification (RP1)

Separation on a reverse-phase cartridge offers the next level of purity (typically 80-95%). The level of purity for RP1 is almost equivalent to that provided by HPLC and the recovery is often higher for 0.05 and 0.2 μmol synthesis scales.

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). Because the differences in hydrophobicity between the full length-DMT product and non-DMT truncated sequences are reduced as the oligo length is increased, cartridge purification is usually not recommended for oligos >50 bases.

For most high resolution applications, cartridge purification will provide the necessary purity level. This procedure is very rapid and efficient, hence oligos ≤ 35 bases can generally be shipped within 48 hours (2 working days). Applications requiring single base resolution may call for additional purification by PAGE.

Reverse-Phase HPLC

Reverse-phase HPLC operates on the same principle as a reverse-phase cartridge, but typically yields a product of 90-97% purity. The capacity and resolving properties of HPLC columns are also much greater than cartridge devices, so HPLC is the method of choice for purifying larger quantities of oligos (i.e. $\geq 1 \mu\text{mol}$). As with cartridges, reverse-phase HPLC is usually not recommended for purifying oligos longer than 50 bases. HPLC-purified oligos are generally shipped in 3-5 working days.

PAGE

With the excellent resolution of PAGE, a 95-99% purity can be achieved. Yields from PAGE are lower than from other methods due to the relatively inefficient extraction of oligos from the gel. The basis of the separation is charge and molecular weight. In most cases, full length (n) oligos can be separated from oligos only one base shorter (n-1). PAGE is the recommended technique for purifying oligos >50 bases long. PAGE purified oligos are generally shipped in 3-4 working days.

Gel Filtration

For antisense studies, all phosphorothioate backbone modified oligos (also referred to as s-oligos) receive a final desalting by gel filtration. Gel filtration is also recommended for any oligo to be used in cell culture or *in vivo* studies. Gel filtration prevents cytotoxic effects from trace synthesis by-products or trace solvents which may carry over from purification.

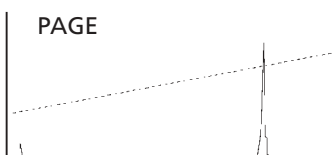
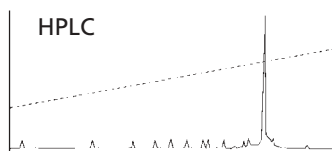
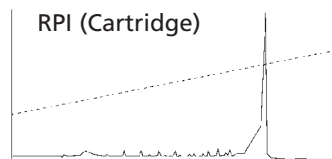


Figure 2. Ion exchange HPLC analysis of a desalted 20-mer that was purified by cartridge, HPLC, and PAGE.



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