

Oligonucleotides

Oligonucleotides of DNA and RNA are important for therapeutic, diagnostic, and research uses. Obtaining these high purity oligonucleotides requires purification steps to remove impurities generated during the synthesis.

Cartridge Desalting with Reverse Phase

Oligonucleotide 'cartridge desalting' is a crude reverse phase separation that removes small molecules, salts, solvents, and other byproducts of the oligonucleotide ("oligo") synthesis. It works by adsorption of the hydrophobic DMT-on sequences to the hydrophobic resin, allowing the more hydrophilic impurities to pass through. By changing the buffer, the DMT-on sequences can be eluted without the impurities.

DuPont™ AmberChrom™ CG chromatography resins are an excellent option for cartridge desalting. They are available in a variety of pore sizes to match the target length of your sequence. We recommend starting with AmberChrom™ CG161 for shorter oligo sequences < 30-mer and AmberChrom™ CG300 for medium and longer oligo sequences.

Reverse Phase HPLC Purification

Reverse phase HPLC purification is required for high purity oligos used in therapeutic, diagnostics, and select research applications. In this process, the chromatographic separation removes chemically-similar failure sequences (e.g. N-1, etc.) from the target oligonucleotide sequence. These separations can be done with the DMT protecting group 'on' or 'off'.

DuPont™ AmberChrom™ reverse phase chromatography resins XT20 and XT30 are known to be very effective in both the DMT-on and DMT-off purification of DNA and RNA oligonucleotides. This performance is why AmberChrom™ chromatography resins have been the go-to resins for preparative scale oligonucleotide purification for applications such as diagnostics, primers, and therapeutics for 10+ years.

Unlike reverse phase silica, the AmberChrom™ polymeric resins are stable across the extremes of pH (1-14), resistant to pressures up to 60 bars and temperatures up to 60°C, and are inert to common buffers and solvents. The robustness of the AmberChrom™ polymeric matrix and the selectivity of the resin design offer reliable separations and a long lifetime for all stages of product development, from benchtop scale to commercial manufacturing.

DMT-on Purification

The DMT protecting group provides the oligo with a hydrophobic nature that attracts it to the hydrophobic resin. Through proper selection of process conditions and the mobile phase, the target DMT-on sequence is separated from other closely related DMT-on impurities through differential partitioning between the mobile phase and the resin.

DMT-off Purification

Some process developers prefer to deprotect the oligonucleotide and remove the DMT group prior to purification. In this instance, the reverse phase separation requires an ion pairing agent in the mobile phase. The ion pairing agent is a molecule that has both hydrophobic and cationic features that will allow the anionic oligonucleotide to adsorb to the hydrophobic resin. Again, through proper selection of process conditions and the mobile phase, the target DMT-off sequence is separated from closely related DMT-off impurities through differential partitioning between the mobile phase and the resin.

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