Clarity® BioSolutions for Synthetic DNA/RNA

Clarity Oligo-RP™ HPLC Columns

Reversed Phase LC for Purification and Characterization

- **Easily separate N-1 failure sequences from target oligo with > 90 % purities**
- **Trityl-off purification of DNA, RNA, Thioates, and modified/labeled oligonucleotides**
- **Preparative dimensions and particle sizes for loads > 5 µmole**
- **Purify oligos up to 60 mer in length**
- **Excellent column for reversed phase HPLC quality control (QC) testing**

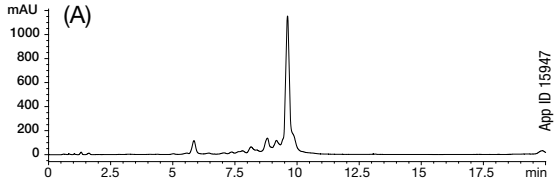
Clarity Oligo-RP has been specifically designed for the reversed phase purification of oligonucleotides with balanced hydrophobicity and polar selectivity. The media is based on composite particle TWIN™ technology. This technology gives improved selectivity and efficiency for oligonucleotides when compared to other hybrid, polymer, and silica particles found in the marketplace. It is available in 3, 5, and 10 µm particle sized beads and in a variety of dimensions.

Preparative Purification on Oligo-RP

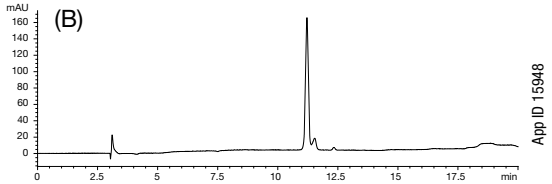
Reversed phase separation of oligonucleotides has advantages over other modes of separations such as ion-exchange. The Oligo-RP phase allows high loadability and delivers high recovery and purity, eliminating the need for extra purification steps. This is achieved through an ion-pair separation of the trityl-off oligonucleotide from failure products and other impurities.

DNA Purification (A) Preparative (B) Analytical QC

Column: Clarity 3 µm Oligo-RP C18
Dimensions: (A) 50 x 10.0 mm
(B) 50 x 4.6 mm
Part No.: (A) 00B-4441-N0
(B) 00B-4441-E0
Mobile Phase: A: 50 mM TEAA pH 7.5/ 5 % Acetonitrile
B: Methanol
Gradient: 10 % to 60 % B in 20 minutes
Flow Rate: (A) 4.7 mL/ min
(B) 1.0 mL/ min
Detection: UV @ 260 nm
Sample: 20nt DNA



A 200 µg (1 µmole) 20mer DNA sample was loaded onto a 10 mm ID Clarity Oligo-RP column. Impurities were separated from the target sequence.



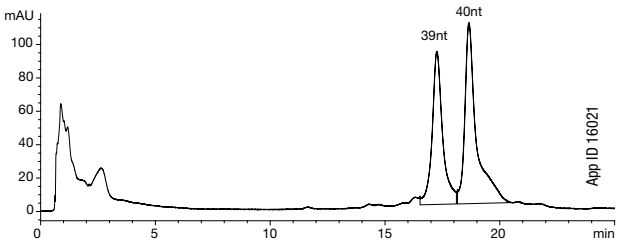
A Clarity Oligo-RP analytical column was used to verify the purity of the preparative purification. A purity of 92 % with a yield of 85 % was determined.

Separate N-1 Failure Sequences from Target N Sequence

The Oligo-RP sorbent is specifically designed to accommodate all possible interactive features of nucleosides with matching modes of reactivity to its own. The sorbent possesses hydrophobic, dipolar, π-π, and hydrogen bond donor/acceptor sites; this combination of interaction along with an ion-pairing reagent elicits a high degree of differential selectivity between nucleic acids. Thus it can recognize even the slightest changes in nucleotide sequence, such as a difference of one base (N and N-1) or substitution of one base for another.

DNA Purification of Failure N-1 from Target N Sequence

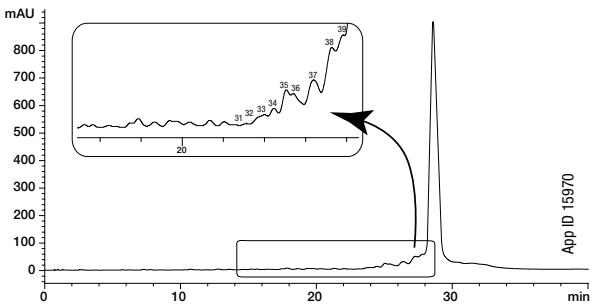
Column: Clarity 3 µm Oligo-RP C18
Dimensions: 50 x 4.6 mm
Part No.: 00B-4441-E0
Mobile Phase: A: 50 mM TEAA pH 7.5
B: Methanol
Gradient: 10 % to 45 % B in 30 minutes
Flow Rate: 1 mL/ min
Detection: UV @ 260 nm
Sample: 1. 40nt DNA with sequence
CTTCTGAACAGTTGATCTATGCACTTCAGACTTATGATCA (2.5 µg)
2. 39nt DNA with sequence
TTCTGAACAGTTGATCTATGCACTTCAGACTTATGATCA (2.5 µg)



Clarity Oligo-RP successfully separates a 40mer from a 39mer DNA oligonucleotide due to its excellent efficiency and resolving power.

Fingerprint of 40mer DNA

Column: Clarity 3 µm Oligo-RP C18
Dimensions: 50 x 4.6 mm
Part No.: 00B-4441-E0
Mobile Phase: A: 50 mM TEAA pH 7.5 / 5 % Acetonitrile
B: Methanol
Gradient: 20 % to 25 % B in 20 minutes; hold at 5 minutes @ 25 % B
Flow Rate: 1 mL/ min
Detection: UV @ 260 nm
Sample: 40nt DNA with sequence
5'-CTC CTG GGC AGT GGA TCT GCG CACTTC AGG CTC CTG GGC A-3'



Due to the high efficiency of the sorbent and ion-pairing interactions, a fingerprint of a crude 40mer DNA on Clarity Oligo-RP is produced illustrating baseline resolution of impurities from the final product.