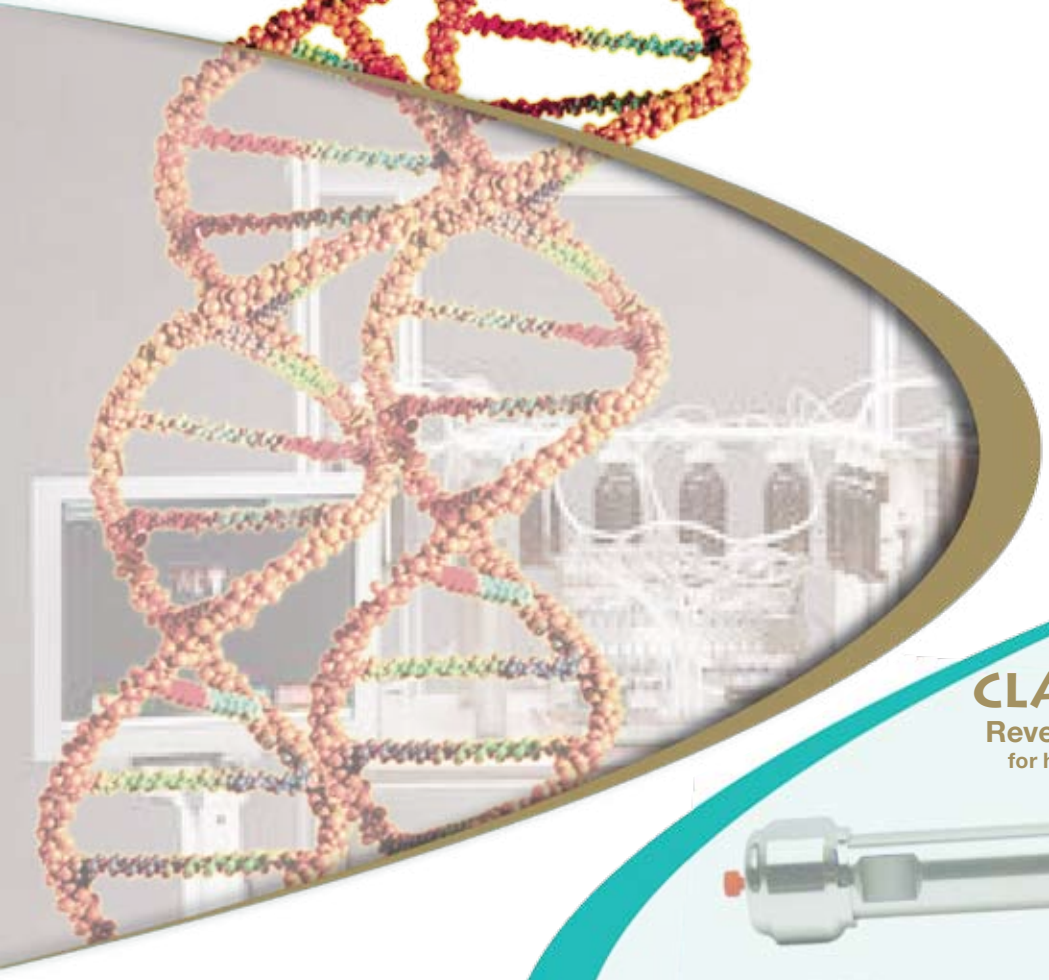

CLARITY® LC SOLUTIONS

Reversed Phase & Ion Exchange Columns

for high purity synthetic oligonucleotide purification

CLARITY® BIOSOLUTIONS patent pending

PARTNER IN PURIFICATION℠

phenomenex®

. . . breaking with tradition℠

CLARITY® LC SOLUTIONS

Partner in Purification

The number of requests for high purity oligonucleotides with synthesis scales in the µmole range (and greater) is growing and has created a need for more efficient and higher capacity purification solutions. Phenomenex would like to be your partner in purification and assist you in supplying small to large quantities of high purity synthetic DNA/RNA to your valued customers. As your purification partner, we not only provide excellent technical support and customer service, but also a novel, reliable suite of products (Clarity BioSolutions) for synthetic DNA/RNA purification. We are pleased to offer a line of LC purification solutions within the Clarity BioSolutions portfolio. Clarity Oligo-RP™ LC columns are an excellent product for those who require a high purity reversed phase solution with long lifetime, while Clarity Oligo-WAX™ LC columns are suitable for those who require an ion exchange solution with high capacity. We look forward to being a partner to companies and core labs who demand efficient, economical, and efficacious synthetic oligo purification and support.

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CLARITY® BIOSOLUTIONS patent pending

PARTNER IN PURIFICATION℠

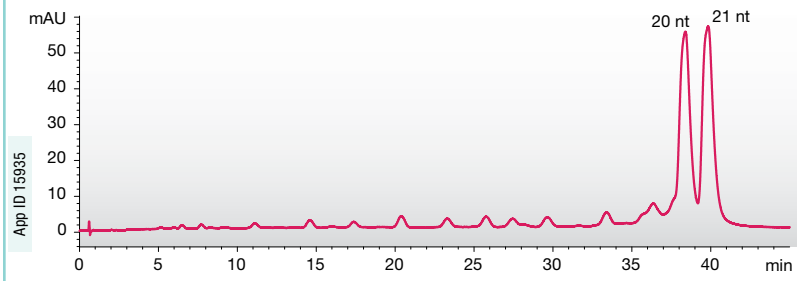
CLARITY® OLIGO-RP™

High Purity, High Efficiency Reversed Phase LC Solution

Clarity Oligo-RP LC columns have 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, in a variety of dimensions, and utilizes HPLC instrumentation for increased productivity.

- **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 & particle sizes for loads > 5 µmole**
- **Purify oligos up to 60 nt in length**
- **Excellent column for reversed phase HPLC quality control (QC) testing**

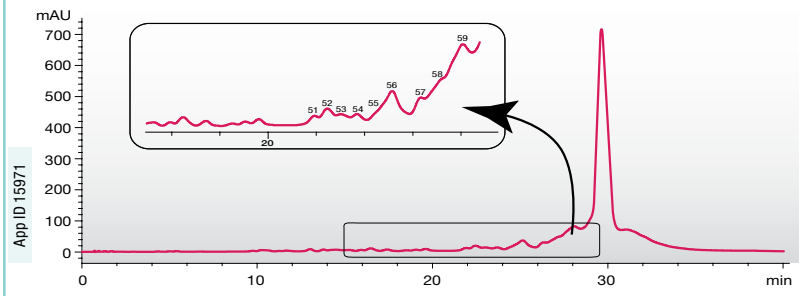
RNA Purification of Failure N-1 Sequence from Target N Sequence



Column:	Clarity 3 µm Oligo-RP C18
Dimensions:	50 x 4.6 mm
Part No.:	00B-4441-E0
Mobile Phase:	A: 10 mM TPAC B: Methanol
Gradient:	A/B (75:25) to A/B (55:45) in 80 min (50 min run time)
Flow Rate:	1 mL/min
Detection:	UV @ 260 nm
Sample:	1. 20 nt RNA with sequence CUGUAAUCUCUUGUCUATT (2.5 µg) 2. 21 nt RNA with sequence UCUGUAAUCUCUUGUCUATT (2.5 µg)

Excellent selectivity characteristics of Clarity Oligo-RP allow baseline separation of failure N-1 sequences from target N sequences.

Resolution Achieved Between 60 nt Impurities with Similar Sequences



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:	60 nt DNA with sequence 5'-CTC CTG GGC CGT GGC TCT GCG CAC TTC AGG AAA CTG GGC ACT CCT GGG CAG TGG ATC TGC-3'

The high efficiency and selectivity of the sorbent as well as ion-pairing interactions produce a fingerprint of a crude 60 nt DNA on Clarity Oligo-RP illustrating resolution of impurities in the final product. Oligo-RP can recognize even the slightest changes in a nucleotide sequence. (numbers on each peak represent the sequence length of the impurity present)

TWIN™ Technology – Engineered for DNA/RNA Purification

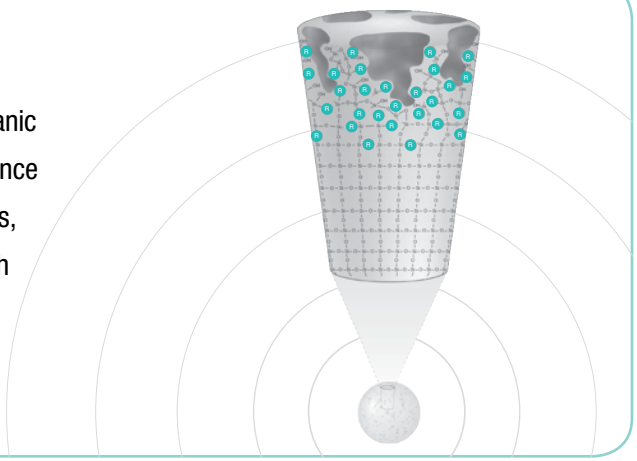
The Oligo-RP media is based on the TWIN technology used to manufacture the patent pending, state-of-the-art Gemini® material, but has been tailored to purify synthetic, as well as natural, DNA & RNA based on the chemical characteristics of these molecules. This solution is able to recognize minute differences in interaction features of two oligonucleotides with very closely resembling structures (for example N/N-1 sequences). In addition, this recognition ability enables Clarity Oligo-RP to provide better resolution between such close pairs of sequences both on the analytical and preparative scale.

- **Long column lifetime due to extended pH stability & mechanical strength**
- **Excellent efficiency enables separation of oligos with similar chemistries**
- **Improved oligo selectivity over hybrid, polymer, and silica particles**

Manufacturing of TWIN Media

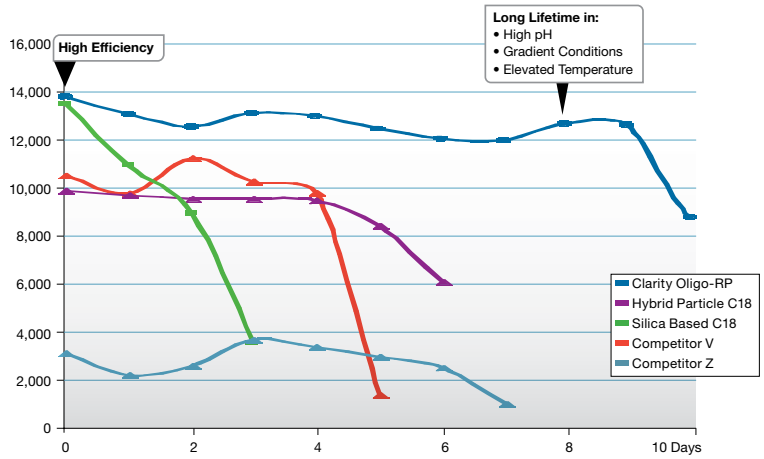
TWIN™ Technology

During the final stage of silica manufacturing a unique silica-organic layer is grafted to create a completely new composite particle. Since the internal base silica is unaltered by this manufacturing process, the particle retains its mechanical strength and rigidity along with excellent efficiency, while the silica-organic shell protects the particle from chemical attack.



TWIN™ Technology – Engineered for DNA/RNA Purification *(cont'd)*

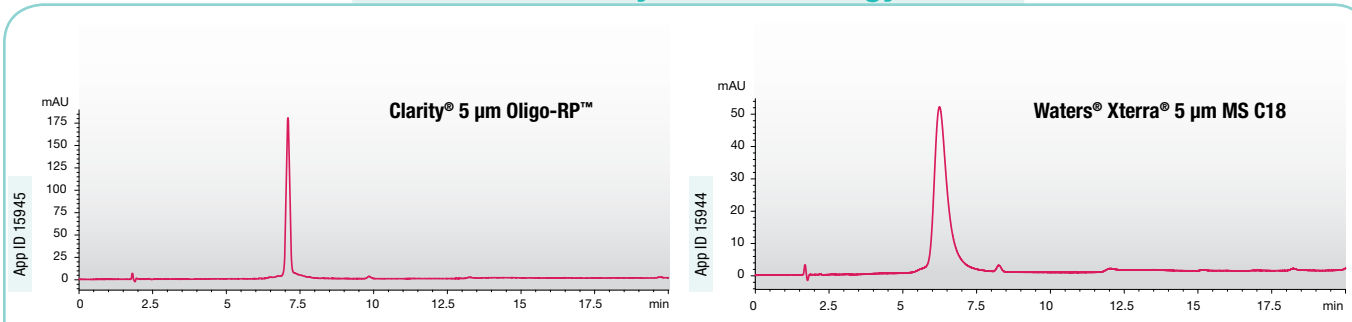
Extended Lifetime and High Efficiency



Mobile Phase:	A: 10 mM Ammonium Bicarbonate, pH 10.0 B: 90:10 Acetonitrile/Buffer
Gradient:	0 % to 100 % B in 10 min; hold at 100 % B for 7 min; re-equilibrate at 0 % B for 3 min
Flow Rate:	1.0 mL/min
Temperature:	50 ˚C
Detection:	UV @ 254 nm
Sample:	1. Amitryptyline 2. Prednisolone

The unique engineering of Clarity Oligo-RP provides stability, high efficiency, and increased column lifetime compared to other commercial HPLC columns.

TWIN vs Hybrid Technology



The state-of-the-art composite TWIN™ technology used to manufacture Clarity Oligo-RP gives it advantages over typical hybrid technology, such as improved peak shape and efficiency.

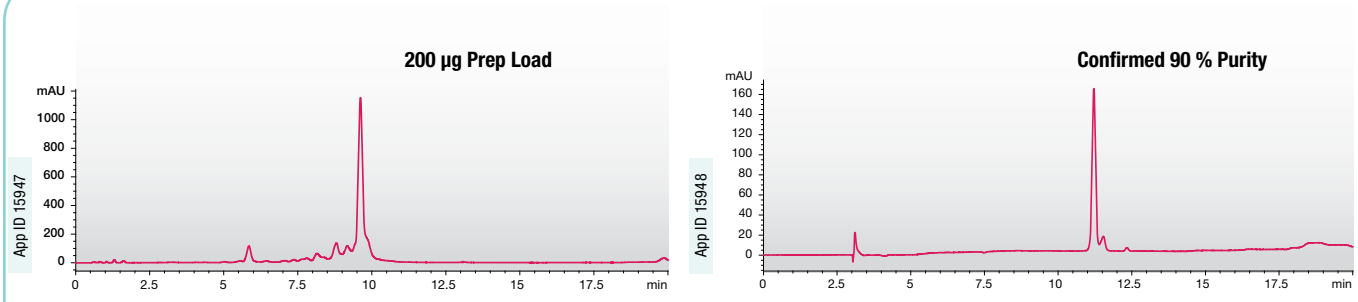
Dimensions:	150 x 4.6 mm
Part No.:	00F-4442-E0
Mobile Phase:	A: 50 mM TEAA, pH 7.5 / 5 % Acetonitrile B: Methanol
Gradient:	A/B (90:10) to A/B (40:60) in 20 min
Flow Rate:	1 mL/min
Detection:	UV @ 260 nm
Sample:	20 nt DNA with sequence ACGTCATGTCGAGATCATCG (1.5 µg)

Advantages of Utilizing Reversed Phase HPLC

Reversed phase separation of oligonucleotides has advantages over other modes of separations such as ion exchange and PAGE. The Oligo-RP phase allows high loadability and delivers high recovery and purity, eliminating the need for extra purification steps such as desalting. Because reversed phase HPLC is an extremely high-resolution technique, Oligo-RP columns are easily able to separate the target oligonucleotide from the many impurities in the mixture, such as the N-1 failure sequence. In addition, this one phase can separate both DNA and RNA chemistries and modified oligos.

- **Recognize minute differences in oligonucleotides such as N and N-1 sequences**
- **Desalting not required after HPLC analysis**
- **Base line separation/collection of desired peak at preparative scale (5 µmole and greater)**
- **Compatible with MS friendly buffers**
- **3 µm material available for improved efficiency and resolution**

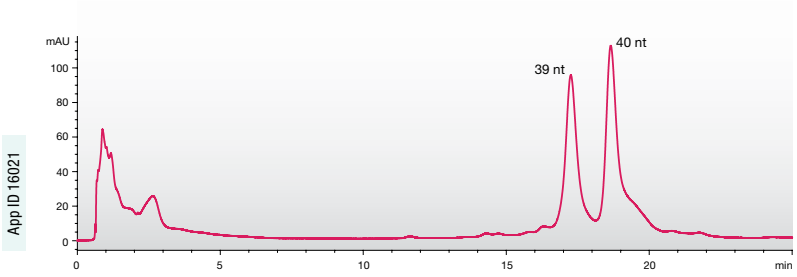
High Purity Preparative DNA Purification



Baseline separation of impurities from the target peak minimizes loss of yield while maintaining high purity and enables the desired oligo to be collected in one fraction rather than the typical practice of collecting multiple fractions and analyzing each for purity. Larger diameters of Clarity Oligo-RP accommodate much higher loading capacities compared to analytical dimension while still producing a highly purified product.

Column:	Clarity 3 µm Oligo-RP C18
Dimensions:	50 x 10 mm (50 x 4.6 mm – analytical analysis)
Part No.:	00B-4441-N0 (00B-4441-E0 – analytical analysis)
Mobile Phase:	A: 50 mM TEAA, pH 7.5 / 5 % Acetonitrile B: Methanol
Gradient:	A/B (90:10) to A/B (40:60) in 20 min
Flow Rate:	1 mL/min
Detection:	UV @ 260 nm
Sample:	DNA with sequence ACGTCATGTCGAGATCATCG (200 µg)

40 nt DNA Purification from 39 nt Impurity



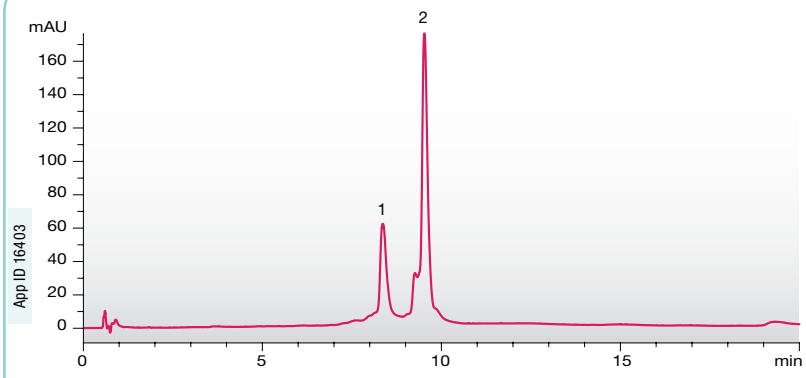
Column:	Clarity 3 µm Oligo-RP
Dimensions:	50 x 4.6 mm ID
Part No.:	00B-4441-E0
Mobile Phase:	A: 50 mM TEAA B: Methanol
Gradient:	A/B (90:10) to A/B (55:45) in 30 min
Flow Rate:	1 mL/min
Detection:	UV-Vis Abs. - Variable Wave. @ 260 nm
Temperature:	ambient
Sample:	1. DNA 40 nt (GC: 37 %) 2. DNA 39 nt (GC: 35 %)

The combination of reversed phase conditions with an Oligo-RP column can recognize even the slightest changes in a nucleotide sequence, such as a substitution of one base by another or a difference of one base as seen in this application.

Clarity Oligo-RP Reversed Phase Applications

Oligo-RP can be used for a variety of oligonucleotide analyses and purifications including RNA, DNA, modified oligos, labeled sequences, and many more. If you have specific questions on whether Oligo-RP is the right product for you, please contact Phenomenex and we will assist you in selecting the correct solution.

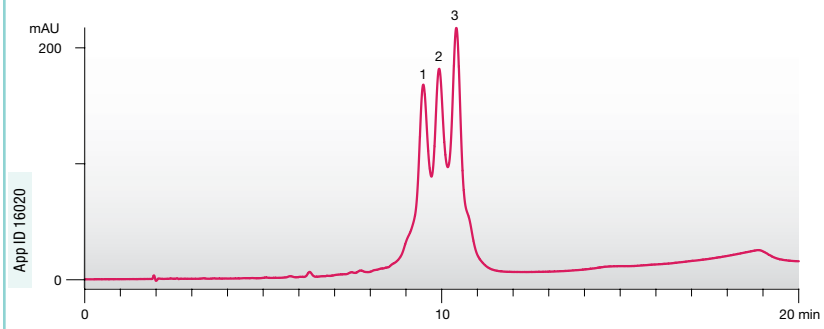
Separate ssDNA from dsDNA



Column: Clarity 3 µm Oligo-RP
Dimensions: 50 x 4.6 mm ID
Part No.: 00B-4441-E0
Mobile Phase: A: 50 mM TEAA
B: MeOH
Gradient: A/B (90:10) to A/B (40:60) in 20 min
Flow Rate: 1 mL/min
Detection: UV-Vis Abs. - Variable Wave.
Temperature: ambient
Sample: 1. ss DNA
2. ds DNA

Clarity Oligo-RP is capable of baseline separating single stranded from double stranded DNA due to its unique selectivity.

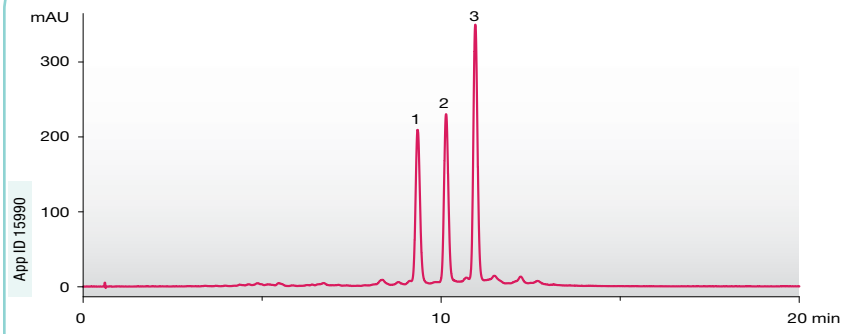
RP-HPLC Purification of 40 nt Oligos with Varying GC Content



Column: Clarity 3 µm Oligo-RP
Dimensions: 50 x 4.6 mm ID
Part No.: 00B-4441-E0
Mobile Phase: A: 50 mM TEAA / 5 % Acetonitrile
B: Methanol
Gradient: A/B (90:10) to A/B (40:60) in 20 min
Flow Rate: 1 mL/min
Detection: UV @ 260 nm
Temperature: ambient
Sample: 1. DNA 40 nt (GC: 65 %)
2. DNA 40 nt (GC: 50 %)
3. DNA 40 nt (GC: 37 %)

C & G rich compounds were separated from non C & G rich ones. This was possible due to the fact that the sorbent possesses a tailored mixture of hydrophobic, dipolar, pi-pi, and hydrogen bonding characteristics.

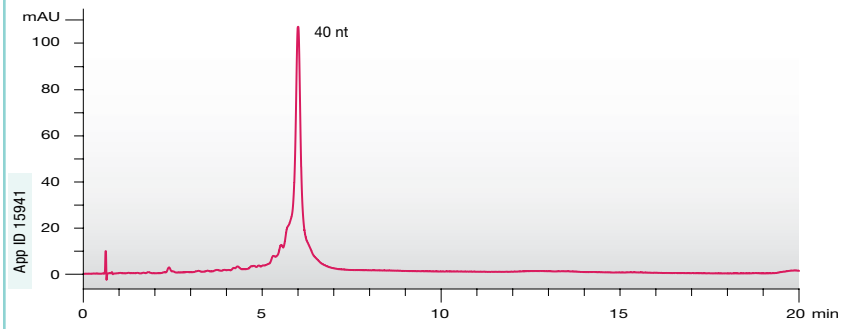
Dye-labeled DNA Purification



Column: Clarity 3 µm Oligo-RP
Dimensions: 50 x 4.6 mm ID
Part No.: 00B-4441-E0
Mobile Phase: A: 50 mM TEAA / 5 % Acetonitrile
B: Methanol
Gradient: A/B (90:10) to A/B (60:40) in 20 min
Flow Rate: 1 mL/min
Detection: UV @ 260 nm
Temperature: ambient
Sample: 1. FAM-DNA (25 nt) GC% 72 %
2. FAM Labeled DNA (25 nt) GC% 56 %
3. FAM Labeled DNA (25 nt) GC% 20 %

Oligo-RP delivers high purity labeled oligos such as those with a FAM label. Baseline separation is achieved ensuring good yields and purity.

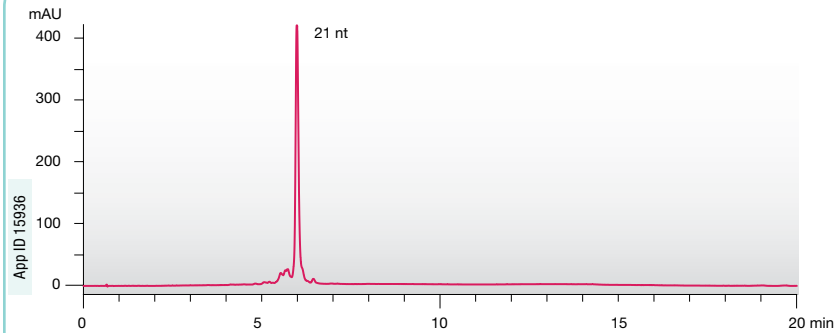
40 nt DNA Purification



Column: Clarity 3 µm Oligo-RP
Dimensions: 50 x 4.6 mm ID
Part No.: 00B-4441-E0
Mobile Phase: A: 50 mM TEAA / 5 % Acetonitrile
B: Methanol
Gradient: A/B (90:10) to A/B (40:60) in 20 min
Flow Rate: 1 mL/min
Detection: UV @ 260 nm
Temperature: ambient
Sample: 1. DNA 40 nt

40 nt purifications are sometimes difficult on a reversed phase column. Oligo-RP, however, can purify lengths up to 60 nt and sometimes longer depending on the sequence.

21 nt RNA Purification



Column: Clarity 3 µm Oligo-RP
Dimensions: 50 x 4.6 mm ID
Part No.: 00B-4441-E0
Mobile Phase: A: 50 mM TEAA / 5 % Acetonitrile
B: Methanol
Gradient: A/B (90:10) to A/B (40:60) in 20 min
Flow Rate: 1 mL/min
Detection: UV @ 260 nm
Temperature: ambient
Sample: 1. RNA 21 nt

RNA, due to the fact that it is more polar, is more of a challenge to separate on a RP-HPLC columns than DNA. The dipolar and hydrogen binding aspect of Oligo-RP makes it an excellent purification tool for RNA sequences such as the 21 nt in this application.

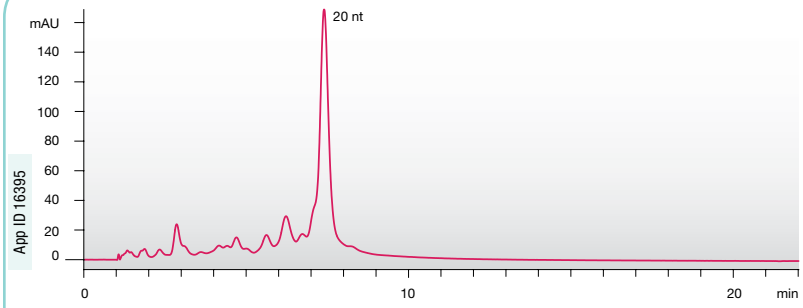
CLARITY® OLIGO-WAX™

High Purity, High Loadability Preparative Ion Exchange LC Solution

Clarity Oligo-WAX LC columns were designed with the synthetic DNA/RNA preparative chromatographer in mind. In preparative chromatography, it is imperative that not only high purities are achieved but also that the media has high capacity and efficiency to produce high yields. Oligo-WAX is an advantageous combination of purity, capacity, mechanical strength, and efficiency.

- **Columns amenable to HPLC and FPLC systems**
- **High capacity media**
- **Increased efficiency versus other IEX media**
- **High purity silica-based media**

20 nt DNA Purification



Column:	Clarity 10 µm Oligo-WAX
Dimensions:	150 x 4.6 mm ID
Part No.:	00F-4451-E0
Mobile Phase:	A: 20 mM Tris pH 8.0 / 10 % Acetonitrile B: 20 mM Tris pH 8.0 / 10 % Acetonitrile / 1.2 M NaCl
Gradient:	100 % A to 100 % B in 20 min
Flow Rate:	2.2 mL/min
Detection:	UV-Vis Abs. - Diode Array 260 nm
Temperature:	ambient
Sample:	1. Desalted DNA 20 nt

After desalting, a 75 µg mixture of a synthesized 20 nt ssDNA (ACG TCA TGT CGA GAT CAT CG) was loaded onto a Clarity Oligo-WAX column to purify it from closely eluting impurities. Note the good separation, due to the high efficiency and selectivity of the silica-based weak anion exchanger, of the full-length oligo from N-1 impurities and other contaminants.

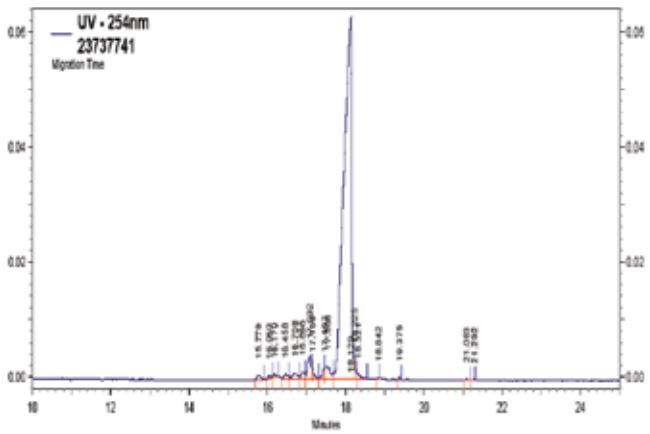
Oligo-WAX Technology – Tailored for Preparative Purification

The majority of synthetic oligo preparative purifications are performed using a strong anion exchanger bonded to a 10 or 15 µm polymer backbone. Polymer backbones are amenable to clean in place protocols, while strong anion exchangers have a wide effective pH range. To date, these technologies have been satisfactory for prep purifications and will continue to be. However, due to the fact that Clarity Oligo-WAX is a crosslinked weak anion exchanger bonded to a 10 µm high purity silica, this technology offers a few advantages that aren't available with typically used purification products.

- **Higher loading capacity due to very high density ligand**
- **Improved peak efficiency & flow characteristics due to pore morphology and spherical shape of the silica matrix**
- **Better fractionation of closely eluting compounds resulting in higher purity**
- **Increased productivity by running at higher flower rates and pressures**

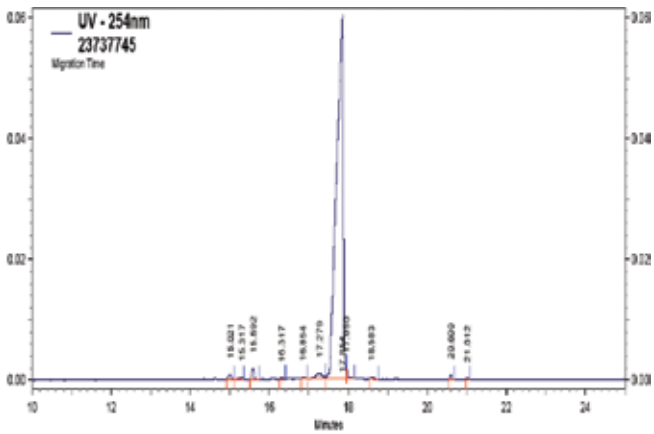
CE Purity Analysis of Ion Exchange Purification

GE™ Healthcare SOURCE™ 15Q 150 x 10



• Final Purity = 88.8 %, N-1 = 2.0 % • Final amount = 205.9 OD's
• Recovery of full-length product = 28.9 % • Conductivity = 200 µS/cm

Clarity Oligo-WAX 10 µm 150 x 10 (pre-packed)



• Final Purity = 95.1 %, N-1 = 1.3 % • Final amount = 188.9 OD's
• Recovery of full-length product = 28.4 % • Conductivity = 151 µS/cm

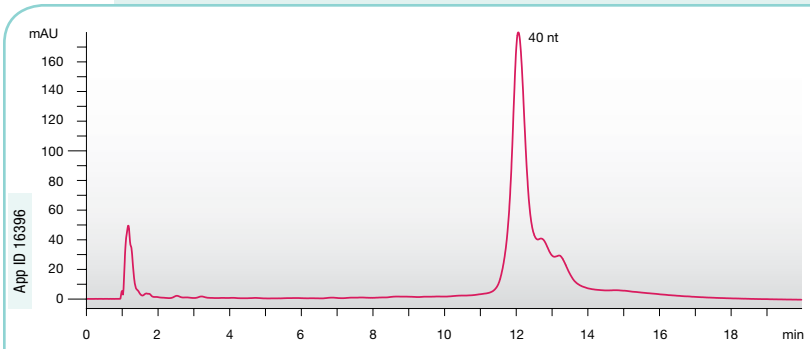
Two purification runs were performed on each column with fractional QC being taken after each run. Passing fractions from the two purification runs were combined into one pooled lot for each column. That pooled lot was then divided equally and run through a Clarity desalting tube. Final OD's and QC were taken after desalting, including ESI, CE, and conductivity. The purity and resolution of Clarity Oligo-WAX was considerably better than SOURCE 15Q. Though SOURCE had a slightly higher recovery of full length oligo, it was not be a wide enough margin to offset the purity advantage.

Data courtesy of a large, Iowa-based oligo manufacturer.
Comparative separations may not be representative of all applications.

Clarity Oligo-WAX Ion Exchange Applications

Oligo-WAX is recommended for preparative purifications of synthetic RNA and DNA. If you have specific questions on whether Oligo-WAX is the right product for you or how it differs from commercial SAX columns on the market, please contact Phenomenex and we will be pleased to assist you.

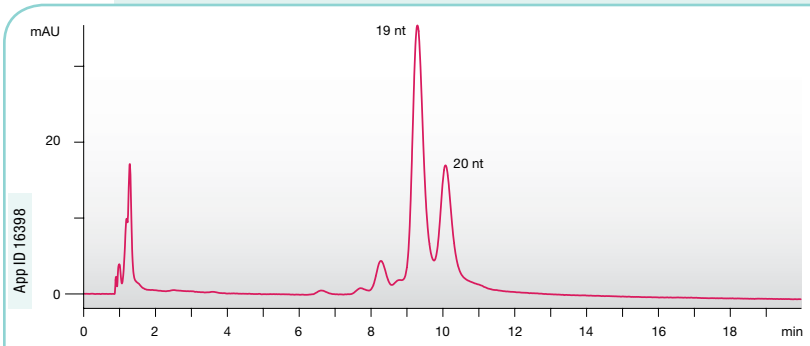
Purification of Long Oligo Sequences



Column: Clarity 10 µm Oligo-WAX
Dimensions: 150 x 4.6 mm ID
Part No.: 00F-4451-E0
Mobile Phase: A: 20 mM Tris pH 8.0 / 10 % Acetonitrile
B: 20 mM Tris pH 8.0 / 10 % Acetonitrile / 1.2 M NaCl
Gradient: 100 % A to 100 % B in 20 min
Flow Rate: 2.2 mL/min
Detection: UV-Vis Abs. - Diode Array 260 nm
Temperature: ambient
Sample: 1. DNA Purified 40 nt

100 µg for a 40 nt ssDNA (CTC CTG GGC AGT GGA TCT GCG CAC TTC AGG CTC CTG GCG A) was purified using Clarity Oligo-WAX. In purifying longer sequences, such as this 40 nt DNA, the results produced from commonly used ion exchange columns are typically broad peaks. However, the efficiency of the Oligo-WAX column generates sharp peaks, which are excellent for better purification of closely eluting contaminants.

DNA Purification of Hydrolyzed N-1 from N Sequence



Column: Clarity 10 µm Oligo-WAX
Dimensions: 150 x 4.6 mm ID
Part No.: 00F-4451-E0
Mobile Phase: A: 20 mM Tris pH 8.0 / 10 % Acetonitrile
B: 20 mM Tris pH 8.0 / 10 % Acetonitrile / 1.2 M NaCl
Gradient: 100 % A to 100 % B in 20 min
Flow Rate: 2.2 mL/min
Detection: UV-Vis Abs. - Diode Array 260 nm
Sample: 1. Depurinated A & G & 20 nt DNA

To test the separation efficiency of the Oligo-WAX column, a mixture of a 20 nt synthesized DNA (ACTCGGCTTCCTCCTCCTCTT) was exposed to polyamine hydrolysis to generate a 19 nt contaminant. This mixture was run on the Oligo-WAX column. Note the near baseline separation between the almost identical 19 nt and 20 nt.

Reversed phase & ion exchange high purity synthetic oligonucleotide purification

Material Characteristics

	Clarity® Oligo-RP™	Clarity® Oligo-WAX™
Particle Support	TWIN™ (silica organic composite)	High Purity Silica
Bonded Phase	C18	Crosslinked polyamine (WAX)
Particle Size	3, 5, and 10 µm	10 µm
Pore Size	110 Å	360 Å
Surface Area	375 m^2/g	160 m^2/g
pH stability	1.0 – 12.0	1.5 – 11.0
pH Effective Range	1.0 – 12.0	4.0 – 8.5 (Amine ligand deprotonates at pH 9)

Ordering Information

3 µm Columns (mm)

Phases	50 x 1.0	100 x 1.0	50 x 2.0	100 x 2.0	150 x 2.0
Oligo-RP	00B-4441-A0	00D-4441-A0	00B-4441-B0	00D-4441-B0	00F-4441-B0

Phases	50 x 4.6	100 x 4.6	150 x 4.6	50 x 10.0
Oligo-RP	00B-4441-E0	00D-4441-E0	00F-4441-E0	00B-4441-N0

5 µm Columns (mm)

Phases	50 x 1.0	150 x 2.0	50 x 4.6	150 x 4.6	250 x 4.6
Oligo-RP	00B-4442-A0	00F-4442-B0	00B-4442-E0	00F-4442-E0	00G-4442-E0

Phases	50 x 10.0	100 x 10.0	250 x 10.0	250 x 21.2	250 x 30.0
Oligo-RP	00B-4442-N0	00D-4442-N0	00G-4442-N0	00G-4442-P0	00G-4442-U0

10 µm Columns (mm)

Phases	100 x 4.6	150 x 4.6	150 x 10.0	250 x 10.0
Oligo-RP	–	00F-4445-E0	00F-4445-N0	–
Oligo-WAX	00D-4451-E0	00F-4451-E0	00F-4451-N0	00G-4451-N0

Phases	250 x 21.2	150 x 30.0	150 x 50.0	250 x 50.0
Oligo-RP	00G-4445-P0	00F-4445-U0	00F-4445-V0	00G-4445-V0
Oligo-WAX	–	00F-4451-U0	–	–

GUARD CARTRIDGE SYSTEM

Part No.	Description		Unit	For ID
AJ0-8134	SecurityGuard™ Cartridges	Clarity Oligo-RP 4 x 2.0 mm*	10/Pk	2.0 - 3.0 mm
AJ0-8135	SecurityGuard Cartridges	Clarity Oligo-RP 4 x 3.0 mm*	10/Pk	3.2 - 8.0 mm
AJ0-8136	SecurityGuard Semi-Prep Cartridges	Clarity Oligo-RP 10 x 10 mm‡	3/Pk	9 - 16 mm
AJ0-8210	SecurityGuard PREP Cartridge	Clarity Oligo-RP 15 x 21.2 mm ID**	Ea	18 - 29 mm
AJ0-8310	SecurityGuard PREP Cartridge	Clarity Oligo-RP 15 x 30 mm ID**	Ea	30 - 49 mm
AJ0-8324	SecurityGuard Cartridges	Clarity Oligo-WAX 4 x 3.0 mm*	10/Pk	3.2 - 8.0 mm
AJ0-8325	SecurityGuard Semi-Prep Cartridges	Clarity Oligo-WAX 10 x 10.0 mm‡	3/Pk	9 - 16 mm

**SecurityGuard Analytical Cartidges require holder, Part No.: KJ0-4282*
‡SecurityGuard Analytical Cartidges require holder, Part No.: AJ0-7220
***SecurityGuard Analytical Cartidges require holder, Part No.: AJ0-8223*

Clarity BioSolutions Portfolio

Phenomenex also offers other reliable products for synthetic DNA/RNA purification. Clarity® QSP™ is an excellent product for those who require a high-throughput, high purity solution. Clarity Desalting Tubes are a poly-functional silica-based C18 sorbent that provides a high capacity, fast and effective desalting process. For more information, please visit www.phenomenex.com/clarity.

Clarity QSP 96-Well Plates

- **For high-throughput, parallel processing of purified oligos**
- **Load 0.2 µmole synthesis scale per well**
- **Purify 96 crude oligo samples in ~45 minutes**
- **Easily amenable to automated liquid handling system**

Clarity QSP Cartridge

- **50 mg/ 1 mL for purifying 0.2 µmole synthesis scale or less**
- **150 mg/ 3 mL for purifying up to 1.0 µmole scale**
- **5 g/ 60 mL for large scale purifications up to 50 µmole**
- **Purify crude oligo samples in ~8 minutes**
- **Use either vacuum or positive pressure systems**

Clarity Desalting Tubes

- **For the removal of salt and excess reagent**
- **Provides moderate purification of synthetic oligos**

guarantee

Evaluate Clarity® Biosolutions in your lab for 45 days, if you are not completely satisfied return it for a full refund.

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