

Synthetic DNA/RNA

Purification and Analysis



“Very happy with Phenomenex overall. The quality of the products and the customer service, which often goes beyond what I expect, helped me enormously with troubleshooting and method development.”

Wayne Noonan
Peter MacCallum Cancer Centre,
Australia

403 - 413

The opinions stated herein are solely those of the speaker and not necessarily those of any company or organization.

Optimized Oligo Purification and Analysis

- RPC, HPLC, prep LC, desalting, and extraction solutions
- DNA, RNA/RNAi, longmers, dye-labeled, and modified oligonucleotides
- High efficiency LC-MS protocols for characterization/QC
- Personalized technical support and customer service

Each product in the Clarity BioSolutions portfolio has been designed to efficiently and effectively purify or characterize synthetic oligonucleotides used in biological research, therapeutic development and biochemical manufacturing. Purification solutions include reversed phase HPLC (RP-HPLC), ion-exchange LC (IEX-LC), reversed phase cartridge (RPC), and desalting, while characterization solutions include high efficiency reversed phase (RP-LC-MS) columns.



Material Characteristics

Clarity Products	Particle Support	Bonded Phase	Particle Shape/Size (µm)	Pore Size (Å)	Surface Area (m ² /g)
Clarity QSP™ Cartridges	Polymer (PSDVB)	Hydrophilic polymer coating	35	500	300
Clarity Oligo-RP™ LC Columns	TWIN (silica, organic composite)	C18	3, 5, 10	110	375
Clarity RP-Desalting™ Tubes	Silica	C18	55	140	300
Clarity Oligo-MS™ LC Columns	Core-Shell	C18	1.7, 2.6, 5	100	200* (*effective)
Clarity OTX™ Extraction Plates	Polymer (surface modified PSDVB)	Mixed-mode anion exchanger	33	85	800
Clarity Oligo-XT LC Columns	Core-Shell	C18	1.7, 2.6, 5	100	200

Clarity BioSolutions Product Selection

Purification

	Clarity QSP™	Clarity Oligo-RP™ Clarity Oligo-XT	Clarity RP-Desalting™
Primary Use	High-throughput, trityl-on RPC purification	RP-HPLC purification of failure sequences from target sequences	Quick removal of salt & excess reagent
Purities	>90 %	>90 %	~70 %
Recoveries	~90 %	~70 %	~70 %
Synthesis Scale Load	Up to 50 µmol	Up to 50 µmol	Up to 1 µmol
Oligo Types	DNA, RNA/RNAi, Thioates, Dye-labeled, Modified		

Characterization / Analysis

	Clarity Oligo-RP™	Clarity Oligo-MS™ Clarity Oligo-XT	Clarity OTX™
Primary Use	RP-LC-MS analysis with optimized selectivity and sensitivity	Rapid, high efficiency RP-LC-MS analysis for QC and characterization	Extraction of oligo therapeutics from biological samples for LC-MS bioanalysis
Oligo Length	≤ 60 mer	≤ 60 mer	≤ 40 mer
Recommended Mobile Phase	TEA / HFIP	TEA/HFIP/MeOH	n/a

Clarity™ BioSolutions for Synthetic DNA/RNA

U.S. Patent No. 7, 119, 145

Clarity OTX™ Extraction Kits

Rapid Isolation of Oligo Therapeutics from Biological Samples

- > 80 % typical extraction recoveries
- No liquid-liquid extraction (LLE) required
- Suitable for a majority of therapeutic oligos, tissues, and fluids
- Optimized for LC-MS bioanalysis
- Can be automated for high-throughput

Effective Recovery

The Clarity OTX extraction solution was designed to effectively isolate a wide range of therapeutic oligonucleotides from fluids and tissues. It utilizes a mixed-mode solid phase extraction sorbent in conjunction with carefully formulated buffers to consistently deliver greater than 80 % recoveries.

Sample Preparation:

- Add an equal volume of Lysis-Loading buffer to biological fluid matrix
- Vortex briefly

Extraction Protocol

Condition: 1 mL Methanol (Vacuum ~2" Hg)

Equilibrate: 1 mL Equilibration buffer (Vacuum ~3" Hg)

Load sample: 0.4 mL - 3 mL volume (Vacuum ~3" Hg)

Vacuum: ~10" Hg for ~10 seconds to completely evacuate solution through cartridge

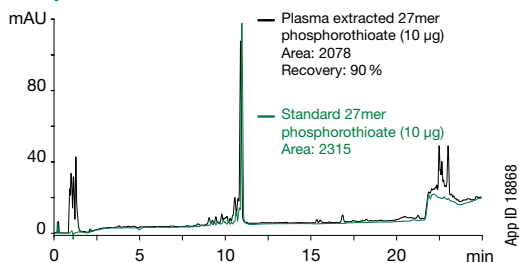
Wash: 6 mL Wash buffer (2 mL x 3) (Vacuum 3-4" Hg)

Vacuum: 10-15" Hg for ~1 minute

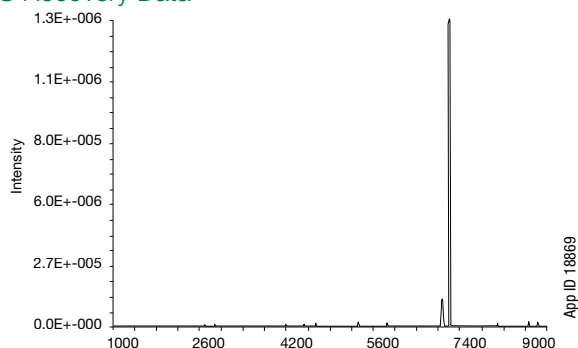
Elute: 1 mL Elution buffer (Vacuum ~3" Hg)

LC-MS Prep: Dry down or lyophilize and reconstitute in 100 µL water or aqueous buffer

UV Recovery Data



MS Recovery Data



The above illustrates the recovery of a 27mer thioate from 200 µL of human plasma. The UV data shows that 90 % recovery is achieved with the Clarity OTX extraction protocol. The MS data further demonstrates that plasma contaminants are effectively removed and complete isolation and recovery of the target is achieved.

Phenomenex

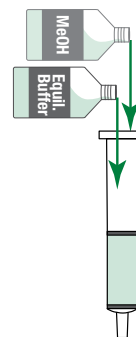
Designed for Throughput

In just 4 steps and 15 minutes, scientists can extract therapeutic oligos and their metabolites from biological samples. This is accomplished by eliminating the need for liquid-liquid extraction and providing a 96-well plate format which is amenable to parallel processing.



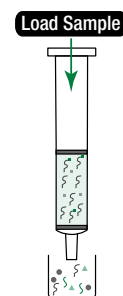
STEP 1

Preparation of SPE sorbent to selectively retain the oligo of interest and its metabolites.



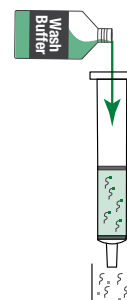
STEP 2

Salts, sugars, large proteins and genomic DNA flow through the cartridge. The oligo of interest, proteins, and lipids bind to the sorbent via a mixed-mode, weak anionic interaction.



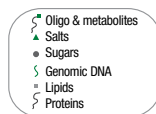
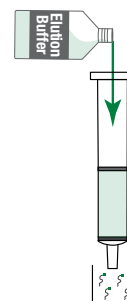
STEP 3

The Wash Buffer is formulated to strip off lipids and remaining proteins from the sorbent, while not disturbing the oligo therapeutics and its metabolites.



STEP 4

The addition of the Elution Buffer releases the target oligo therapeutic and its metabolites. The elution volume can be dried down or lyophilized and reconstituted prior to LC-MS analysis.



Request a FREE copy of the Clarity OTX User's Guide for more detailed information on the extraction protocol.

Clarity™ BioSolutions for Synthetic DNA/RNA

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Clarity OTX™ (cont'd)

Flexible Formats

To test proof of concept or for low sample volumes, Clarity OTX is available as a starter kit, which includes either a 96-well plate or 50 solid phase extraction cartridges and all the buffers (lysis-loading, equilibration, wash, and elution) required for the extraction protocol.



For labs that must process large volumes of biological samples, 96-well plates, 1 L quantities of lysis-loading buffer, and the formulations for the other three buffers are available.



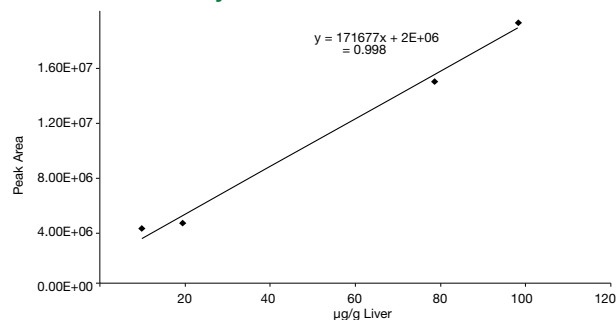
Eliminate MS Interfering Contaminants

The Clarity OTX extraction protocol effectively removes cell debris such as proteins, genomic DNA, and lipids which significantly mask the oligo therapeutics of interest. By removing these contaminants, MS noise is considerably reduced.

Excellent Linearity

Significant effort was made to develop an extraction solution that would provide good linearity and reliable quantitative results.

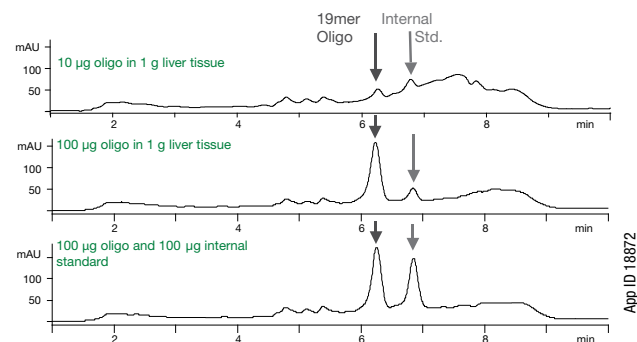
Liver Tissue Linearity Curve



From low to high concentrations of ng/mL, excellent linearity is achieved on the MS by extracting oligo therapeutics and their metabolites using the Clarity OTX methodology. Linearity for a 19mer P-S oligonucleotide in 1 g of liver tissue, based on MS peak area, was evaluated at four different oligo concentrations in liver tissue from 100 µg to 10 µg. High recovery and good linearity is seen across physiological relevant concentrations for this initial study.

Detect Low Dosage Levels

Due to the typical 85 % and greater recoveries of the parent oligonucleotide therapeutic and its metabolites and the elimination of interfering compounds, detection in low sensitivity ranges is possible when using a sensitive MS.



UV chromatograms of oligonucleotide extracted from liver tissue using Clarity OTX. The 19mer extracted phosphorothioate oligonucleotide was spiked with 10 µg of a oligonucleotide internal standard before analysis. The top two chromatograms represent different levels of the incubated P-S oligo. The bottom chromatogram is an external standard of equal amounts of the 19mer oligo and internal standard. Note the high recovery of the oligonucleotide and low level of plasma contaminants from the incubated samples.

Ordering Information

Clarity OTX			
Part No.	Description		Unit
KS0-8494	Clarity OTX Starter Kit-Tubes	Includes: 100 mg/3 mL cartridges (x50) Lysis-loading buffer (100 mL) Equilibration buffer (250 mL) Wash buffer (350 mL) Elution buffer (100 mL)	ea
KS0-9253	Clarity OTX Starter Kit-96-Well Plate	100 mg/ 96-well plate (x1) Lysis-loading buffer (100 mL) Equilibration buffer (250 mL) Wash buffer (350 mL) Elution buffer (100 mL)	ea
8M-S103-4GA	Clarity OTX Microelution Well Plate	2 mg/ well	1/box
8E-S103-CGA	Clarity OTX Well Plate	25 mg/ well	1/box
8E-S103-EGA	Clarity OTX Well Plate	100 mg/ well	1/box
8B-S103-EBJ	Clarity OTX Cartridge	100 mg/3 mL	50/box
8B-S103-HCH	Clarity OTX Cartridge	500 mg/6 mL	30/box
AL0-8579	Clarity OTX Lysis-Loading Buffer V2.0	1 L	ea



Contact your local sales representative for additional part numbers.

Clarity™ BioSolutions for Synthetic DNA/RNA

U.S. Patent No. 7, 119, 145

Clarity QSP™ Cartridges and 96-Well Plates

High-throughput, RPC Purification

- > 90 % typical purities & recoveries for RNA & DNA
- For oligos 10 – 100 mer
- Simple 3-step process for trityl-on oligos
- Cost-effective solution for high purity
- Purification without using ion-pairing agents

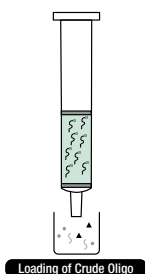
The Quick, Simple, Pure (QSP) Protocol

Following the easy, step-by-step QSP protocol anyone can deliver high purity RNA and DNA. The process includes brief sample preparation followed by 3 simple steps to isolate the oligo of interest from impurities and failure sequences. The QSP sorbent and loading buffers have been engineered to work synergistically with crude synthetic mixtures to produce greater than 90 % recoveries and purities in less than 20 minutes.

Pre-treatment: Trityl-on oligo sample preparation. Mix equal volume of loading buffer with cleavage/deprotection solution

STEP 1

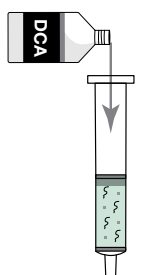
Load crude oligo cocktail
All trityl-off impurities flow directly through; no wash required.



Loading of Crude Oligo

STEP 2

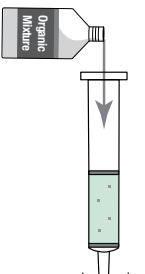
Detritylate
Less than 2 % depurination observed.
A faint orange band will appear at top half of cartridge indicating DMT retention.



Detritylate

STEP 3

Elute target oligo
pH buffered solutions used to maintain safe pH for oligo; select elution buffer based on downstream requirements.



Elution of Target Oligo

- Full Length Trityl-On Oligo
- Impurity
- N-1 Sequence
- Detritylated Failure Sequences
- Trityl Group
- Full Length Target Oligo

Dual-Component System

Two components, loading buffer and SPE cartridge or 96-well plate, are required for Clarity QSP purification. Various loading buffers have been formulated specifically for DNA and RNA chemistries so that one-step loading in synthetic cocktails is permissible and no ion-pairing reagents are required. Multiple SPE formats are available to suit a wide range of synthesis scales and automation requirements. 96-well plates are of a standard footprint and should fit most commercial vacuum manifolds and liquid handling robots.

Loading Buffers

- DNA: for all DNA and RNA-TOM chemistries
- RNA-TBDMS: for RNA-TBDMS and 2' modified RNA chemistries



SPE Formats

- 60 mg/ 3 mL cartridges: < 200 nmol scale
- 150 mg/ 3 mL cartridges: < 1 µmol scale
- 5 g/ 60 mL cartridges: 5 – 25 µmol scale



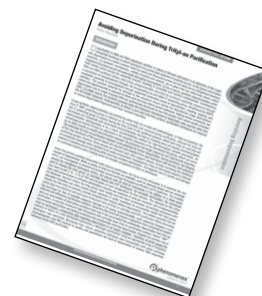
- 50 mg/ 96-well plate: 200 nmol scale per well



96-well plate

Negligible Depurination

Significant effort was made during the development of Clarity QSP to minimize the causes of depurination. The lower acid concentrations and limited exposure times within the protocol generate less than 2 % depurination.



Request a FREE copy of Technical Note [TN-0008](#), Avoiding Depurination During Trityl-on Purification for more information.



Clarity™ BioSolutions for Synthetic DNA/RNA

U.S. Patent No. 7, 119, 145

Clarity QSP™ (cont'd)

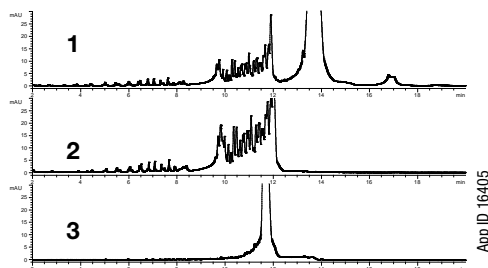
High Purity, High Yield DNA and RNA

Clarity QSP is a next generation trityl-on purification solution that was specifically designed to complement contemporary synthetic processes and consistently deliver high purities and recoveries for all types of synthetic DNA and RNA sequences. Complete discrimi-

nation between full-length trityl-on sequences from impurities is guaranteed. The final elution step delivers concentrated, full-length sequences in a stable media suitable for in vivo applications and downstream analysis conducive for MS, NMR, CE, and HPLC.

53nt DNA Purification

Sequence: ACAGTCGTACAGTCATATATTACTATTCACTGTCTACTGCAGTCGTTATCTAT
Synthesis Scale: 200 nmol
Format: 50 mg / 1 mL



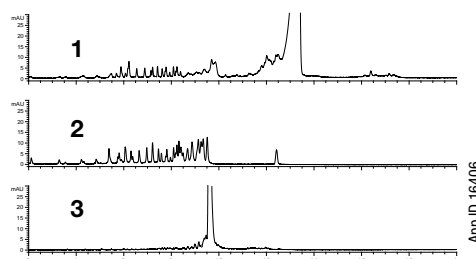
1. Crude Trityl-on
2. Load fraction
3. Detritylated final elution

OD₂₆₀

Crude Trityl-on	Load Fraction	Detritylated Final Elution	Recovery	Purity (Peak area)
39.7	6.51	29.6	89 %	93 %

High-Throughput DNA Purification

Sequence: GTGGATCTGCGCACTTCAGGCTCCTGGGCT
Synthesis Scale: 200 nmol
Format: 96-Well Plate (50 mg / well)



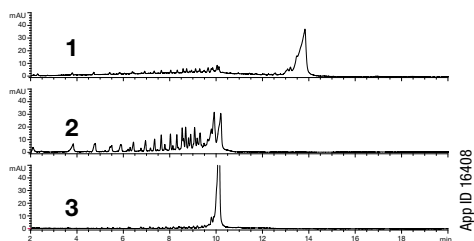
1. Crude Trityl-on
2. Load fraction
3. Detritylated final elution

OD₂₆₀

Crude Trityl-on	Load Fraction	Detritylated Final Elution	Recovery	Purity (Peak area)
28.3	5.3	20.8	90.3 %	92 %

Crude 27nt RNA Purification (TBDMS Chemistry)

Sequence: Proprietary
Synthesis Scale: 1 µmol
Format: 150 mg / 3 mL



1. Crude Trityl-on
2. Load fraction
3. Detritylated final elution

OD₂₆₀

Crude Trityl-on	Load Fraction	Detritylated Final Elution	Recovery	Purity (Peak area)
33.4	9.22	22.9	94 %	84 %

Ordering Information

Clarity QSP™ Well Plate & Cartridges

Part No.	Description	Unit
Formats		
8E-S102-DGB	Clarity QSP 96-Well Plate	50 mg/well 1/box
8B-S102-UBJ	Clarity QSP Cartridge	60 mg/3 mL 50/box
8B-S102-SBJ	Clarity QSP Cartridge	150 mg/3 mL 50/box
8B-S042-LFF	Clarity QSP Cartridge	5 g/60 mL 16/box

Buffers*

AL0-8280	Clarity QSP DNA Loading Buffer	1 L	ea
AL0-8282	Clarity QSP RNA-TBDMS Loading Buffer	1 L	ea

* RNA-TOM loading buffer available upon request



For more information on the Clarity QSP protocol, depurination, or applications, please request a copy of the Clarity QSP User's Manual.



Request Technical Note TN-0015 Comparing Performance of High-Throughput, Trityl-on RNA/DNA Purification Products to see the benefits of using Clarity QSP over other trityl-on solutions.



Clarity RP-Desalting™ Tubes and Well Plates

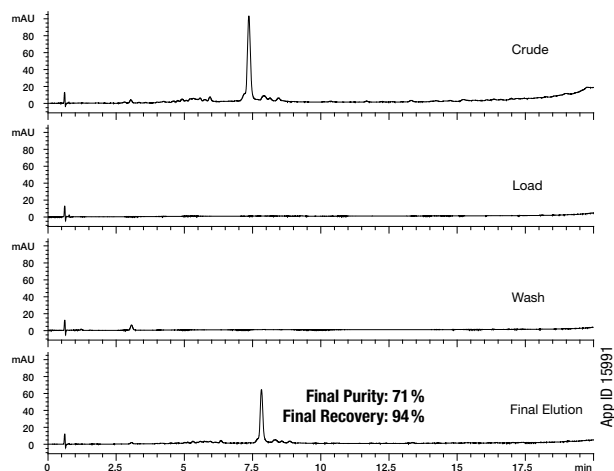
Quick, Simple Removal of Salt and Reagent

- 70 % typical purity by removing salt and excess reagent
- 80 % typical recovery of target oligo
- For cleanup of trityl-off DNA and RNA sequences
- Removes salt prior to MS analysis
- Also in a high-throughput 96-well plate format

Clarity QSP™, and Oligo-RP™ can be used to yield highly purified target oligonucleotides (> 85 % purity) from a synthesis mixture. For simple desalting and reagent removal of a trityl-off synthetic oligonucleotide, Clarity RP-Desalting tubes can be used. Clarity RP-Desalting tubes are a poly-functional silica-based C18 sorbent that provides a high capacity, fast and effective desalting process.

Desalting of Dye-Labeled DNA

Column: Clarity 3 µm Oligo-RP C18
Dimensions: 50 x 4.6 mm
Part No.: 00B-4441-EQ
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: 25nt DNA oligonucleotide

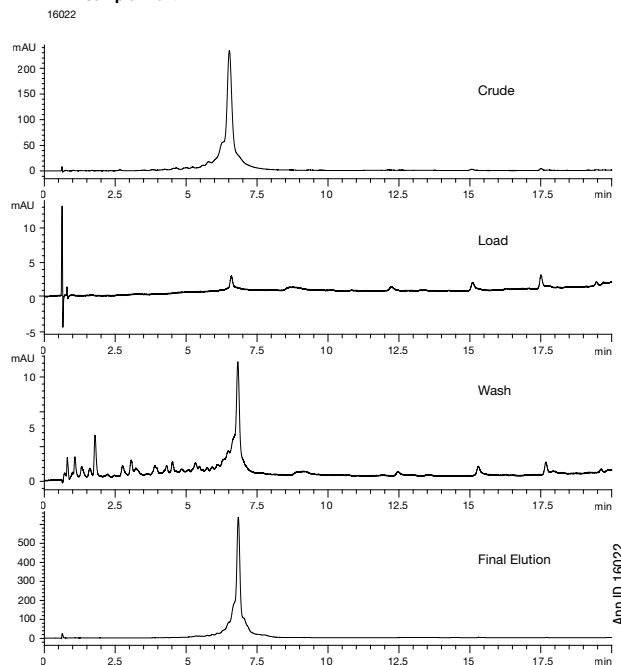


A quencher-labeled sample of DNA (25nt) with the sequence FAMTTGACTTAGCTTAGA-CTAGTTT was desalted using Clarity RP-Desalting tubes in the 200 mg/3 mL format. Collection fractions were then analyzed for purity and recovery using the above protocol.



Crude DNA Desalting

Column: Clarity 3 µm Oligo-RP C18
Dimensions: 50 x 4.6 mm
Part No.: 00B-4441-EQ
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
Sample: 40nt DNA



Ordering Information

Clarity RP-Desalting Tubes

Part No.	Description	Unit
8B-S041-FBJ	Clarity RP-Desalting Cartridge 200 mg/3 mL(50/box)*	ea
8B-S041-HBJ	Clarity RP-Desalting Cartridge 500 mg/3 mL(50/box)**	ea

Clarity RP-Desalting Well Plates

Part No.	Description	Unit
8E-S041-SGA	Clarity RP-Desalting 150 mg/well*	ea



For more information on the Clarity products please contact your Phenomenex technical consultant.

* For 200 µmol synthesis
 ** For 1 µmol synthesis

Clarity™ BioSolutions for Synthetic DNA/RNA

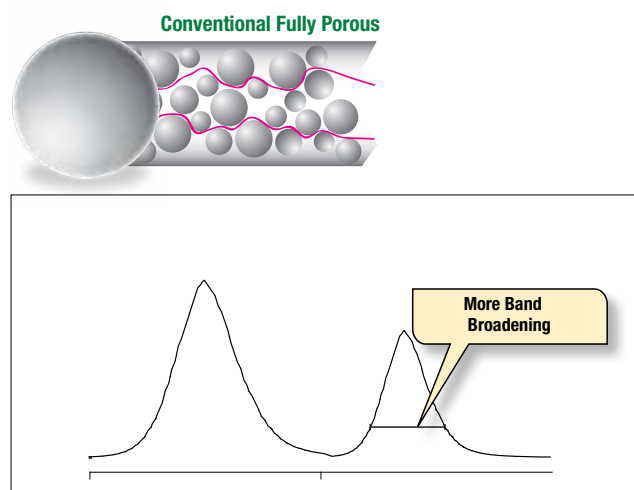
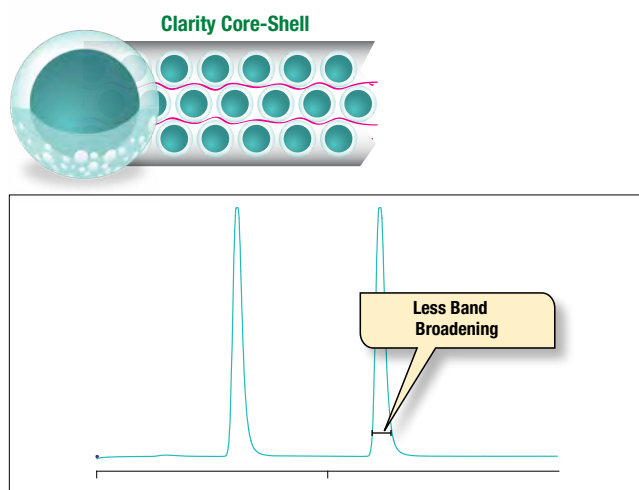
U.S. Patent Nos. 7, 563, 367 and 8, 658, 038 and foreign counterparts.

Clarity Oligo-XT Core-Shell LC Columns

A Sensitive Solution for Oligo Characterization and Bioanalysis

Unlike traditional fully porous oligo columns, Clarity Oligo-XT relies on the power of core-shell technology to provide extremely high efficiencies for both low and high oligo concentrations. Because the Clarity Oligo-XT particle is not fully porous, analytes spend less time diffusing into and out of the pores as they travel through

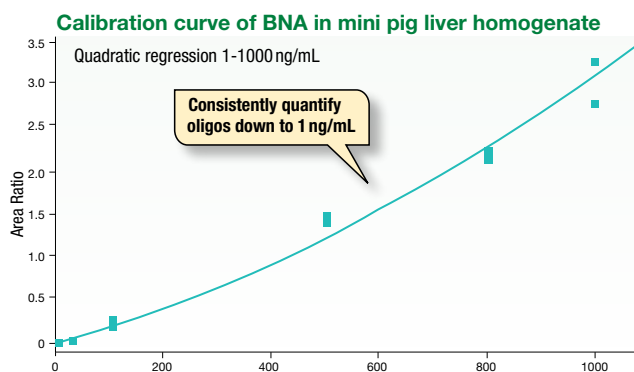
the column, resulting in less band broadening for higher peak efficiencies, making Clarity Oligo-XT a great choice for analyses that require sensitivity such as oligo characterization and oligo analysis from bioanalytical samples.



Fully Porous		Clarity Core-Shell	Average Efficiency Gain with Clarity*
5 μ m	VS	5 μ m	90 % Higher
3 μ m	VS	2.6 μ m	85 % Higher
1.7 μ m	VS	1.7 μ m	20 % Higher

* May not be representative of all applications

Sensitive, Reliable Analysis



LC-MS/MS Conditions:

Column: Clarity 5 μ m Oligo-XT	Gradient:	Time (min)	% B
Dimensions: 50 x 2.1 mm		0.5	30
Part No.: 00B-4745-AN		2.5	60
HPLC system: Shimadzu® Nexera® X2 UHPLC		3	100
Mobile Phase: A: 1.0 % HFIP & 0.1 % DIEA with 10 μ M EDTA in Methanol		3.5	100
B: 1.0 % HFIP & 0.1 % DIEA with 10 μ M EDTA in Methanol/ Water (50:50 v/v)		4	30
		5	30
	Flow Rate:	500 μ L/min	
	Inj. Volume:	10 μ L	
	Temperature:	40 °C	
	Detection:	Thermo Q Exactive™ Hybrid Quadrupole-Orbitrap™ Mass Spectrometer, HESI, negative polarity	

Clarity Oligo-MS™ LC Columns

Rapid and Efficient LC-MS Separation for QC and Characterization

- Core-shell particle technology provides improved speed, resolution, and sensitivity
- 2.6µm particles deliver increased efficiency at reduced backpressures
- Easily transfer quantitative LC-MS methods to any system with 2.6µm particles
- 1.7µm particles boost performance of existing sub-2µm methods

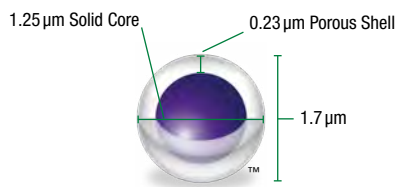
Clarity Oligo-MS, C18 columns have been engineered for the MS characterization of synthetic DNA and RNA samples. This media is based on core-shell technology which generates extremely high efficiencies due to the innovative particle design. This increase in efficiency improves the resolution between critical oligo sequences, gives higher sensitivity for easier MS quantitation, and allows for a decrease in column length for higher throughput.

Core-Shell Technology for Synthetic DNA/RNA Analysis

Clarity Oligo-MS media is not fully porous like traditional particles used for the analysis of oligonucleotides. It is a core-shell particle technology which uses a sol-gel processing technique to grow a homogeneous porous shell onto a solid core. This highly optimized process combined with uniform particle size distribution produces a column that generates extremely high plate counts.

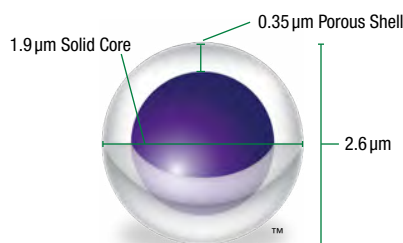
1.7µm Core-Shell Particle

- Reduced diffusion path maximizes efficiency
- Increased efficiencies compared to traditional fully porous sub-2µm columns. Typical operating backpressures > 400 bar



2.6µm Core-Shell Particle

- Reduced diffusion path maximizes efficiency
- Ultra-high performance on any system with Clarity Oligo-MS 2.6µm columns

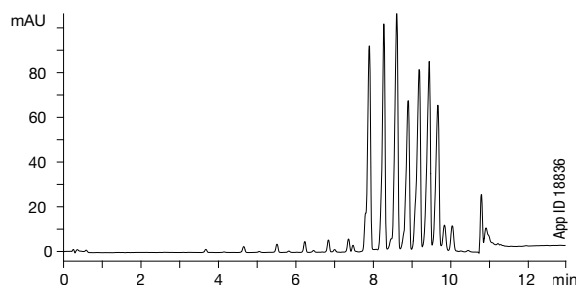


Achieve Baseline Resolution of N-1 and N+1 Oligo from Target

The high plate counts generated by the Clarity Oligo-MS material produce extremely high efficiencies and thus excellent resolution between oligonucleotides of similar length and structure. Scientists can achieve baseline resolution between synthetic oligonucleotides with just one base difference allowing easier quantitation.

Poly dT Standard (12-18mer)

Column: Clarity 2.6µm Oligo-MS C18
Dimensions: 50 x 2.1 mm
Part No.: [00B-4479-AN](#)
Mobile Phase: A: 100 mM HFIP / 4 mM TEA / 2% Methanol
 B: 100 mM HFIP / 4 mM TEA / 98% Methanol
Gradient: A/B (95:5) to A/B (80:20) in 10 min
Flow Rate: 0.5 mL/min
Temperature: 50 °C
Detection: UV @ 260 nm (22 °C)
Injection Volume: 20 µL
Sample: Poly dT (12-18)

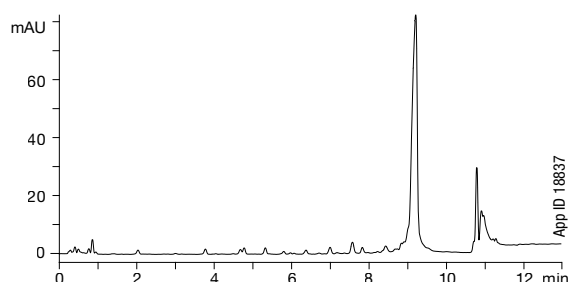


Rapid Separation of Complex Oligo Samples

Due to the high resolving power of Clarity Oligo-MS columns, high-throughput methods for the separation of complex synthetic mixtures can be developed. Using short (50 mm length) columns, impurities are separated from the peak of interest in less than 12 minutes.

Crude DNA 30mer

Column: Clarity 2.6µm Oligo-MS C18
Dimensions: 50 x 2.1 mm
Part No.: [00B-4479-AN](#)
Mobile Phase: A: 100 mM HFIP / 4 mM TEA / 2% Methanol
 B: 100 mM HFIP / 4 mM TEA / 98% Methanol
Gradient: A/B (95:5) to A/B (80:20) in 10 min
Flow Rate: 0.5 mL/min
Temperature: 50 °C
Detection: UV @ 260 nm (22 °C)
Injection Volume: 20 µL
Sample: Crude DNA 30mer



Clarity Oligo-RP™ LC 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 μmol
- 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.

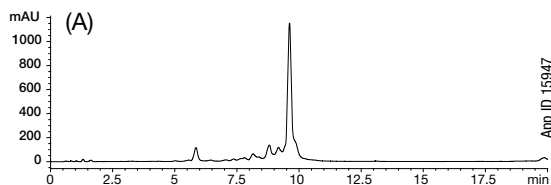
Preparative Purification on Oligo-RP

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.

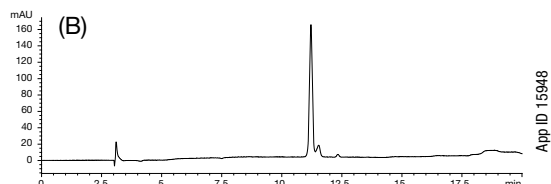
DNA Purification

(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-NO
 (B) 00B-4441-EO
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 μmol) 20mer DNA sample was loaded onto a 10 mm ID Clarity Oligo-RP column. Impurities were separated from the target sequence.



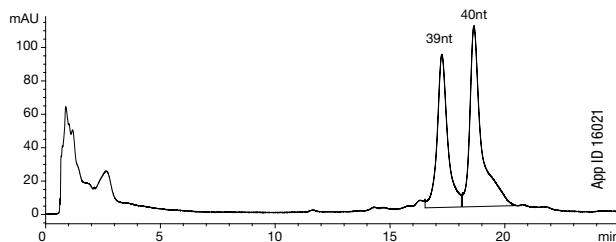
A Clarity Oligo-RP analytical column was used to verify the purity of the preparative purification. A purity of 92 % with a yield of 85 % was determined.

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

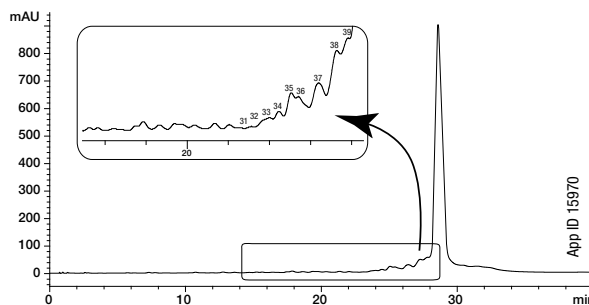
Column: Clarity 3 μm Oligo-RP C18
Dimensions: 50 x 4.6 mm
Part No.: 00B-4441-EO
Mobile Phase: A: 50 mM TEAA pH 7.5
 B: Methanol
Gradient: 10 % to 45 % B in 30 minutes
Flow Rate: 1 mL / min
Detection: UV @ 260 nm
Sample: 1. 40nt DNA with sequence
 CTTCTGAACAGTTGATCTATGCACCTTCAGACTTATGATCA (2.5 μg)
 2.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

Clarity Oligo-XT, Oligo-MS™, and Oligo-RP™ LC Columns

Ordering Information

Minibore Columns (mm)				SecurityGuard™ Cartridges (mm)	SecurityGuard™ ULTRA Cartridges†
Phase	50 x 2.0	100 x 2.0	150 x 2.0	4 x 2.0*	—
3 µm Oligo-RP C18	00B-4441-B0	00D-4441-B0	00F-4441-B0	/10pk AJ0-8134	—
5 µm Oligo-RP C18	—	—	00F-4442-B0	/10pk AJ0-8134	—
Phase	50 x 2.1	100 x 2.1	150 x 2.1	—	2.1
1.7 µm Oligo-MS C18	00B-4480-AN	00D-4480-AN	—	—	/3pk AJ0-9068
2.6 µm Oligo-MS C18	00B-4479-AN	00D-4479-AN	00F-4479-AN	—	/3pk AJ0-9068
1.7 µm Oligo-XT	00B-4747-AN	00D-4747-AN	—	—	/3pk AJ0-9515
2.6 µm Oligo-XT	00B-4746-AN	00D-4746-AN	—	—	/3pk AJ0-9515
5 µm Oligo-XT	00B-4745-AN	—	—	—	/3pk AJ0-9515

for ID: 2.0-3.0 mm

for 2.1 mm ID



Find additional LC columns for oligonucleotide analysis in our Biozen portfolio

See pp. 210 - 230

Analytical Columns (mm)					SecurityGuard™ Cartridges (mm)	SecurityGuard™ ULTRA Cartridges†
Phase	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	4 x 3.0*	4.6
2.6 µm Oligo-MS C18	00B-4479-E0	00D-4479-E0	—	—	—	/3pk AJ0-9066
2.6 µm Oligo-XT	00B-4746-E0	00D-4746-E0	—	—	—	/3pk AJ0-9514
3 µm Oligo-RP C18	00B-4441-E0	00D-4441-E0	00F-4441-E0	—	/10pk AJ0-8135	—
5 µm Oligo-RP C18	00B-4442-E0	—	00F-4442-E0	00G-4442-E0	/10pk AJ0-8135	—
5 µm Oligo-XT	—	—	00F-4745-E0	—	—	/3pk AJ0-9514
10 µm Oligo-RP C18	—	—	00F-4445-E0	00G-4445-E0	/10pk AJ0-8135	—

for ID: 3.2-8.0 mm

for 4.6 mm ID

Semi-Prep Columns (mm)					SecurityGuard™ Cartridges (mm)
Phase	50 x 10.0	100 x 10.0	150 x 10.0	250 x 10.0	10 x 10†
3 µm Oligo-RP C18	00B-4441-N0	—	—	—	/3pk AJ0-8136
5 µm Oligo-RP C18	00B-4442-N0	00D-4442-N0	00F-4442-N0	00G-4442-N0	/3pk AJ0-8136
5 µm Oligo-XT	00B-4745-N0	00D-4745-N0	00F-4745-N0	—	/3pk AJ0-9516
10 µm Oligo-RP C18	—	—	00F-4445-N0	00G-4445-N0	/3pk AJ0-8136

for ID: 9-16 mm



For more about SecurityGuard ULTRA and cartridge holder ordering information, see p. 335.

Increase lab safety with HPLC / UHPLC solvent protection, see SecurityCAP™ products on pp. 417-418



For Column Heater, see p. 416

Axia™ Packed Preparative Columns (mm)					SecurityGuard Cartridges (mm)	
Phase	100 x 21.2	150 x 21.2	250 x 21.2	150 x 30	15 x 21.2**	15 x 30.0*
5 µm Oligo-RP C18	00D-4442-P0-AX	—	00G-4442-P0-AX	—	/ea AJ0-8210	/ea AJ0-8310
5 µm Oligo-XT	00D-4745-P0-AX	00F-4745-P0-AX	00G-4745-P0-AX	00F-4745-U0-AX	/ea AJ0-9517	/ea AJ0-9518
10 µm Oligo-RP C18	—	00F-4445-P0-AX	00G-4445-P0-AX	00F-4445-U0-AX	/ea AJ0-8210	/ea AJ0-8310

for ID: 18-29 mm

30-49 mm

*SecurityGuard™ Analytical Cartridges require universal holder Part No.: [KJ0-4282](#)

†SemiPrep SecurityGuard Cartridges require holder, Part No.: [AJ0-9281](#)

**PREP SecurityGuard Cartridges require holder, Part No.: [AJ0-8223](#)

◆PREP SecurityGuard Cartridges require holder, Part No.: [AJ0-8277](#)

†SecurityGuard ULTRA cartridges require holder, Part No.: [AJ0-9000](#)