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

393 - 405

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

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 Oligo-WAX™ LC Columns	Silica	Crosslinked polyamine (WAX)	10	360	—
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-SAX LC Columns	Polymer (surface modified PSDVB)	Hydrophilic quaternary amine	5	—	—
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 Oligo-WAX™	Clarity RP-Desalting™
Primary Use	High-throughput, trityl-on RPC purification	RP-HPLC purification of failure sequences from target sequences	Economical, high loading capacity IEX-LC prep-scale purification	Quick removal of salt & excess reagent
Purities	>90 %	>90 %	>90 %	~70 %
Recoveries	~90 %	~70 %	>90 %	~70 %
Synthesis Scale Load	Up to 50 µmol	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

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.

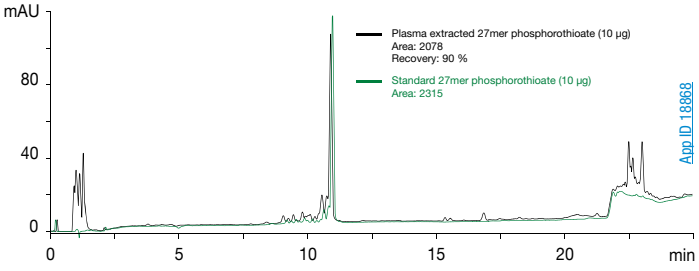
Sample Preparation:

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

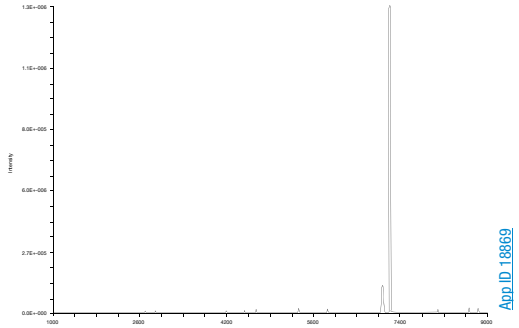
Extraction Protocol

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.

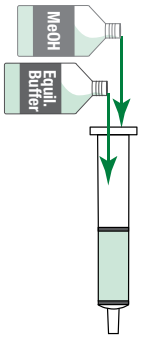
Designed for Throughput

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.

2010 R&D 100 Award Recipient

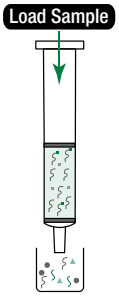
STEP 1

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



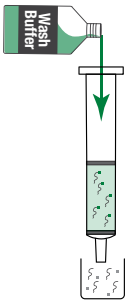
STEP 2

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.



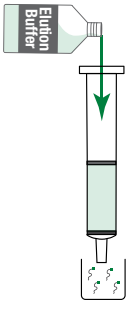
STEP 3

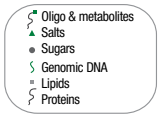
The Wash Buffer is formulated to strip off lipids and remaining proteins from the sorbent, while not disturbing the oligo therapeutics and its metabolites.



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 anlaysis.





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

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

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.

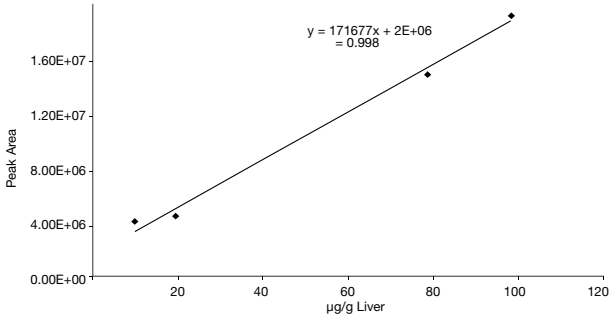
Eliminate MS Interfering Contaminants

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.

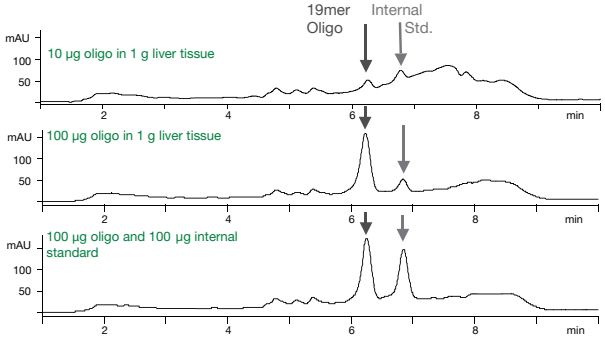
Liver Tissue Linearity Curve



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 100µg to 10µg. 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 10µg 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 (60 mL) Equilibration buffer (250 mL) Wash buffer (350 mL) Elution buffer (60 mL)	ea
KS0-9253	Clarity OTX Starter Kit- 96-Well Plate	100 mg/ 96-well plate (x1) Lysis-loading buffer (60 mL) Equilibration buffer (250 mL) Wash buffer (350 mL) Elution buffer (60 mL)	ea
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

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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**

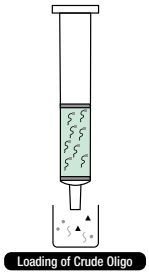
The Quick, Simple, Pure (QSP) Protocol

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

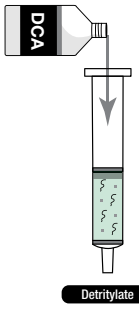
STEP 1

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



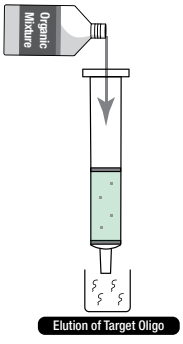
STEP 2

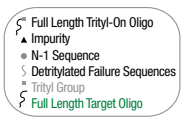
Detritylate
Less than 2 % depurination observed. A faint orange band will appear at top half of cartridge indicating DMT retention.



STEP 3

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





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.

2008 R&D 100 Award Recipient

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.

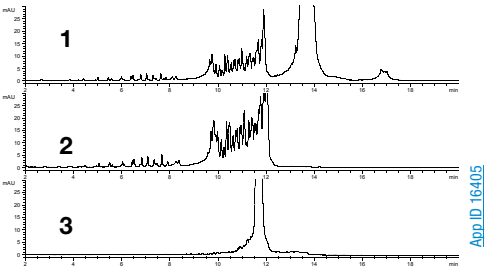
Request a FREE copy of Technical NoteTN-0008, Avoiding Depurination During Trityl-on Purification for more information.

Clarity QSP™ *(cont'd)*

High Purity, High Yield DNA and RNA

53nt DNA Purification

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



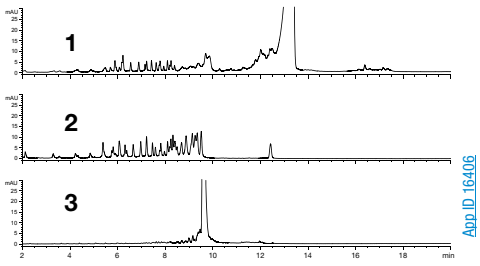
1. Crude Trityl-on
2. Load fraction
3. Detritylated final elution

OD_{260}

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)



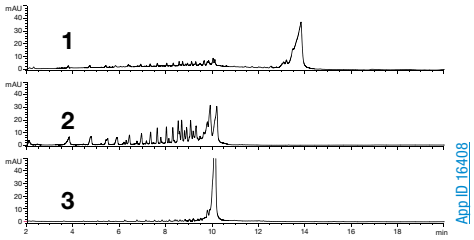
1. Crude Trityl-on
2. Load fraction
3. Detritylated final elution

OD_{260}

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

OD_{260}

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 Plates & Cartridges

Part No.	Description		Unit
Formats			
8E-S102-DGB	Clarity QSP 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 NoteTN-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

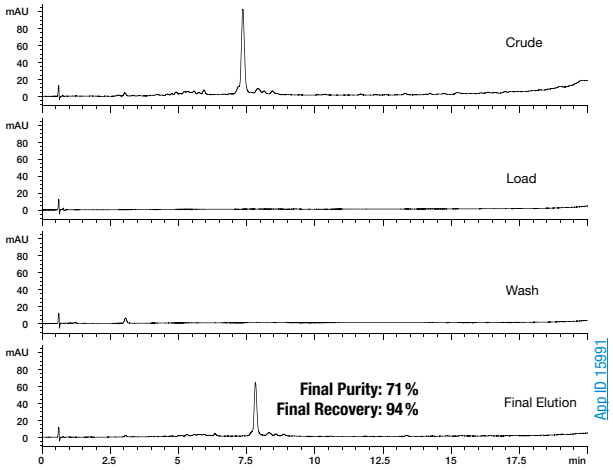
Quick, Simple Removal of Salt and Reagent

- **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™, Oligo-WAX™, 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

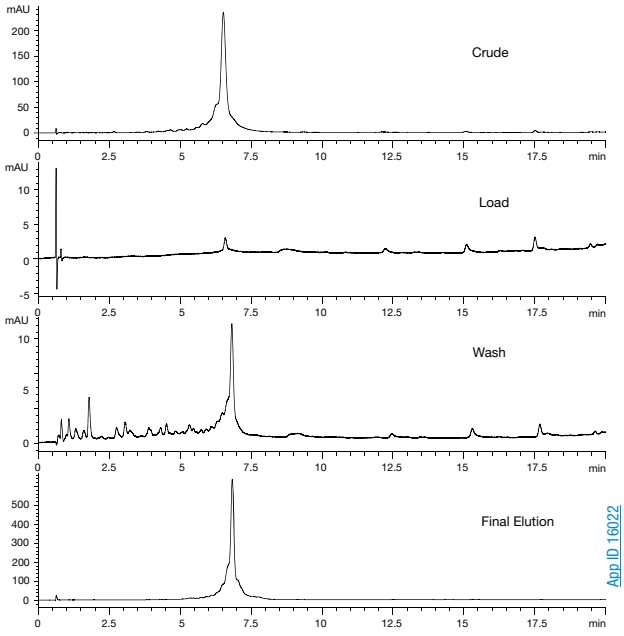
Column: Clarity 3 µm Oligo-RP C18
Dimensions: 50 x 4.6 mm
Part No.: 00B-4441-E0
Mobile Phase: A: 50 mM TEAA, pH 7.5 / 5 % Acetonitrile
B: Methanol
Gradient: A/B (90:10) to A/B (40:60) in 20 min
Flow Rate: 1 mL/ min
Detection: UV @ 260 nm
Sample: 25nt DNA oligonucleotide



A quencher-labeled sample of DNA (25nt) with the sequence FAMTTGACTTAGACTTAGA-CTTAGTTT 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-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
Sample: 40nt DNA



Ordering Information

Clarity RP-Desalting Tubes

	200 mg/3 mL*	500 mg/3 mL**
Phase	**50/box**	**50/box**
C18	8B-S041-FBJ	8B-S041-HBJ

Clarity RP-Desalting Well Plates*

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

** For 200 µmol synthesis*
*** For 1 µmol synthesis*

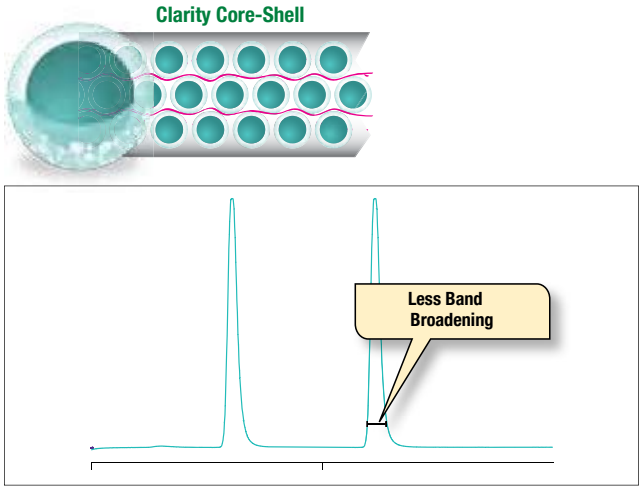
For more information on the Clarity products please contact your Phenomenex technical consultant.

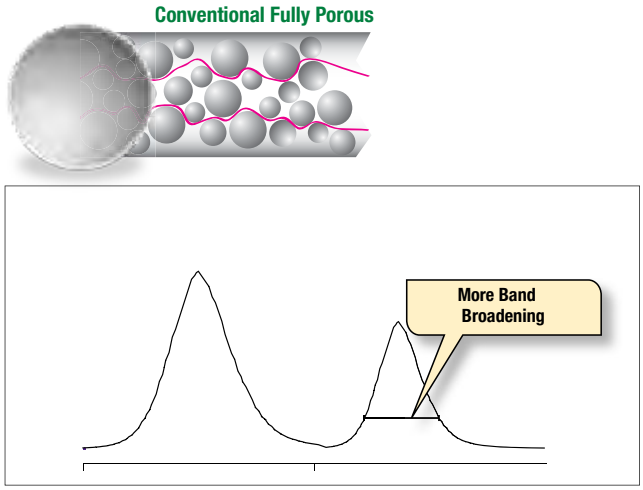
U.S. Patent Nos. 7, 563, 367 and 8, 658, 038 and foreign counterparts.

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.



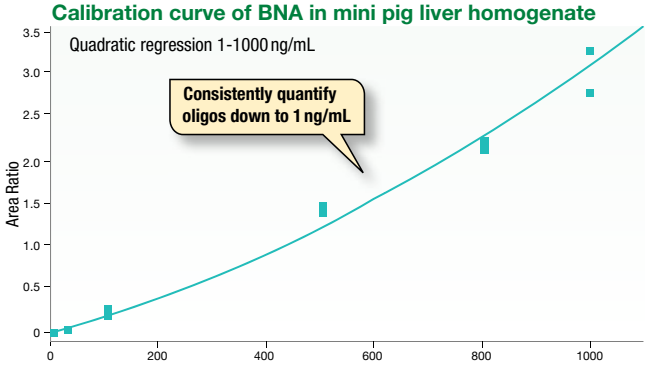


Fully Porous		Clarity Core-Shell	Average Efficiency Gain with Clarity*
5 µm	VS	5 µm	90 % Higher
3 µm	VS	2.6 µm	85 % Higher

Fully Porous		Clarity Core-Shell	Average Efficiency Gain with Clarity*
1.7 µm	VS	1.7 µm	20 % Higher

* May not be representative of all applications

Sensitive, Reliable Analysis



LC-MS-MS Conditions:

Column: Clarity 5 µm Oligo-XT
Dimensions: 50 x 2.1 mm
Part No.: 00B-4745-AN
HPLC system: Shimadzu® Nexera® X2 UHPLC
Mobile Phase: A: 1.0 % HFIP & 0.1 % DIEA with 10 µM EDTA in Methanol
B: 1.0 % HFIP & 0.1 % DIEA with 10 µM EDTA in Methanol Water (50:50 v/v)

Gradient:

Time (min)	% B
0.5	30
2.5	60
3	100
3.5	100
4	30
5	30

Flow Rate: 500 µL/min
Inj. Volume: 10 µL
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**
- **2.6 µm particles deliver increased efficiency at reduced backpressures**
- **Easily transfer quantitative LC-MS methods to any system with 2.6 µm particles**
- **1.7 µm particles boost performance of existing sub-2 µm methods**

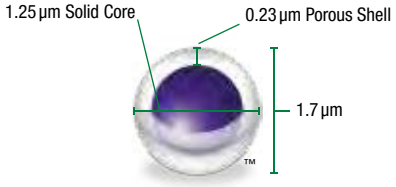
Clarity Oligo-MS, C18 columns have been engineered for the MS characterization of synthetic DNA and RNA samples. This media is based on core-shell technology which generates extremely high efficiencies due to the innovative particle design. This increase in efficiency improves the resolution between 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.

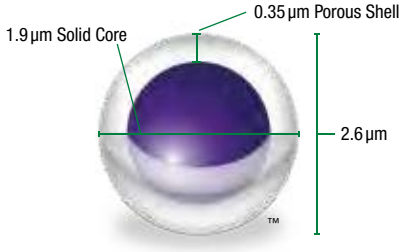
1.7 µm Core-Shell Particle

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



2.6 µm Core-Shell Particle

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

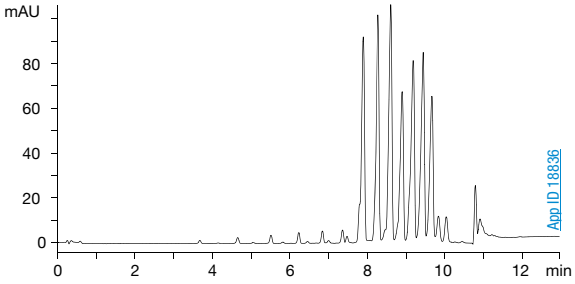


Achieve Baseline Resolution of N-1 and N+1 Oligo from Target

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 2.6 µm 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)

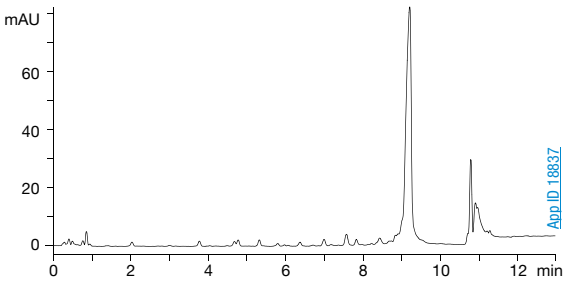


Rapid Separation of Complex Oligo Samples

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 2.6 µm 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**

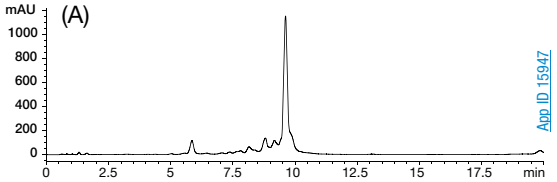
Clarity Oligo-RP has been specifically designed for the reversed phase purification of oligonucleotides with balanced hydrophobicity and polar selectivity. The media is based on composite particle TWIN™ technology. This technology gives improved selectivity and efficiency for oligonucleotides when compared 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

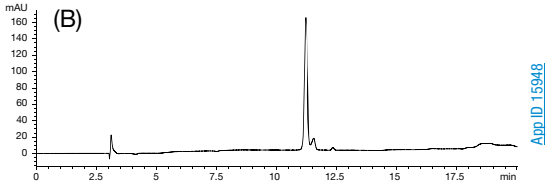
Reversed phase separation of oligonucleotides has advantages over other modes of separations such as ion-exchange. The Oligo-RP phase allows high loadability and delivers high recovery and purity, eliminating the need for extra purification steps. This is achieved through an ion-pair separation of the 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-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 µmol) 20mer DNA sample was loaded onto a 10 mm ID Clarity Oligo-RP column. Impurities were separated from the target sequence.



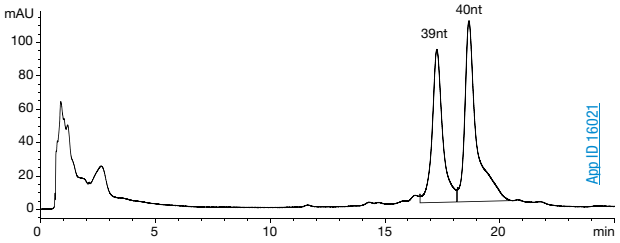
A Clarity Oligo-RP analytical column was used to verify the purity of the preparative purification. A purity of 92 % with a yield of 85 % was determined.

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

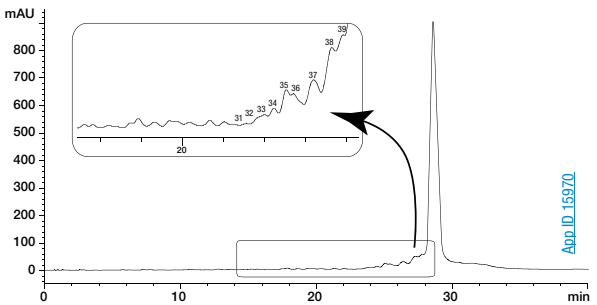
Column: Clarity 3 µm Oligo-RP C18
Dimensions: 50 x 4.6 mm
Part No.: 00B-4441-E0
Mobile Phase: A: 50 mM TEAA pH 7.5
B: Methanol
Gradient: 10 % to 45 **% B** in 30 minutes
Flow Rate: 1 mL/ min
Detection: UV @ 260 nm
Sample: 1. 40nt DNA with sequence
CTTCTGAACAGTTGATCTATGCACTTCAGACTTATGATCA (2.5 µg)
2.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

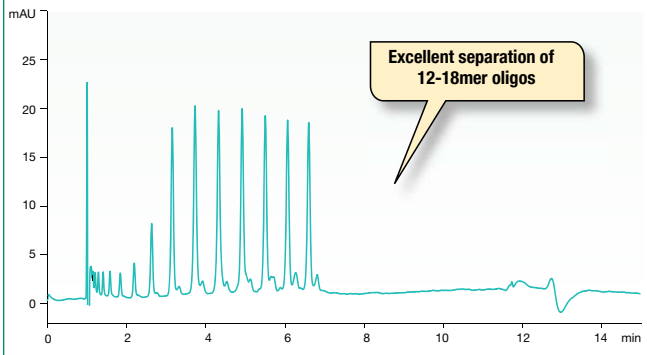
Clarity Oligo-SAX LC Columns

High Resolution Oligo Characterization

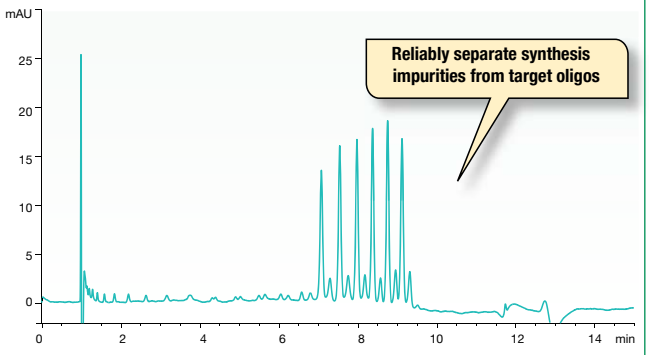
The characterization of synthetic oligos is important in the drug development process, and one common technique used is strong anion-exchange liquid chromatography. This high resolution technique is preferred when extensive characterization (i.e. LC-MS) is not necessary. Another valuable benefit is that n-1 failure sequences can still be separated without the use of an ion-pair reagent. Clarity Oligo-SAX strong anion-exchange columns allow analysts to reliably characterize a variety of different sized synthetic oligos while providing excellent separation of oligos and synthesis impurities.

A Sensitive Separation

Poly (dT) 12-18mer



Poly (dT) 19-24mer



Conditions for both separations:

Column: Clarity 5 µm Oligo-SAX
Dimensions: 250 x 4.6 mm
Part No.: 00G-4749-E0
Mobile Phase: A: 20 mM Tris, pH 8.0
B: 20 mM Tris + 1.25 M NaCl, pH 8.0

Gradient:

Time (min)	% B
0	35
10	49.4
10.1	75
10.1	75

Flow Rate: 1.6 mL/ min
Temperature: Ambient
LC System: Agilent® 1200
Detection: UV-Vis @ 260 nm
Sample: As noted above

Clarity Oligo-WAX™ LC Columns

High Purity, High Loadability Preparative Ion-Exchange Purification

- **Excellent efficiency column results in > 90 % purities due to good fractionation of closely eluting compounds**
- **High loading capacity due to very high density ligand**
- **Increase productivity by running at higher flow rates and pressures**
- **Columns amenable to HPLC and FPLC systems**

Clarity Oligo-WAX LC columns were designed with the synthetic DNA/RNA preparative chromatographer in mind. Oligo-WAX is an advantageous combination of purity, capacity, mechanical strength, cost, and efficiency.

Tailored for Preparative Purification

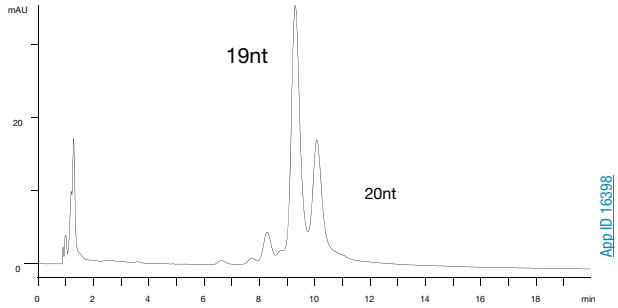
The majority of synthetic oligo preparative purifications are performed using a strong anion exchanger bonded to a 10 or 15 µm polymer backbone. Polymer backbones are amenable to clean in place protocols and strong anion exchangers have a wide effective pH range. To date, these technologies have been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

Purify Failure Sequences and Contaminants from Target Sequence

Ion-exchange is an excellent separation mode for purifying contaminants and failure sequences from target sequences. Clarity Oligo-WAX, due to its increased efficiency compared to other ion-exchange columns, has the ability to recognize minute charge differences in nucleotide sequences such as failure sequences or base substitutions.

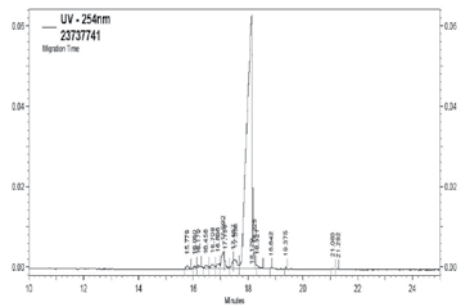
DNA Purification of N-1 Sequence from Target N Sequence

Column: Clarity 10 µm Oligo-WAX
Dimensions: 150 x 4.6 mm
Part No.: 00F-4451-E0
Mobile Phase: A: Water
B: Acetonitrile
C: 100 mM Tris, pH 8
D: 2 M Sodium chloride
Gradient: A/B/C/D (70:10:20:0) to (10:10:20:60) in 20 min
Flow Rate: 2.2 mL/min
Detection: UV-Vis Abs.-Diode Array (ambient)
Sample: Depurinated A & G and 20mer DNA



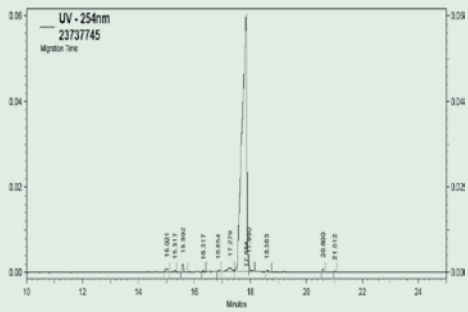
CE Purity Analysis of Ion-Exchange Purification

GE Healthcare SOURCE™ 15Q 150 x 10 mm (self-packed)



- 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 mm (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 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.

guarantee

If analytical Clarity LC products do not provide at least an equivalent separation as compared to a competing product of the same particle size, similar phase and dimensions, return the column with comparative data within 45 days for a FULL REFUND.

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

Ordering Information sds

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***	**—**
				/10pk	—
3 µm Oligo-RP C18	00B-4441-B0	00D-4441-B0	00F-4441-B0	AJ0-8134	—
				/10pk	—
5 µm Oligo-RP C18	—	—	00F-4442-B0	AJ0-8134	—
Phase	**50 x 2.1**	**100 x 2.1**	**150 x 2.1**	**—**	**2.1**
				—	/3pk
1.7 µm Oligo-MS C18	00B-4480-AN	00D-4480-AN	—	—	AJ0-9068
				—	/3pk
2.6 µm Oligo-MS C18	00B-4479-AN	00D-4479-AN	00F-4479-AN	—	AJ0-9068
				—	/3pk
1.7 µm Oligo-XT	00B-4747-AN	00D-4747-AN	—	—	AJ0-9515
				—	/3pk
2.6 µm Oligo-XT	00B-4746-AN	00D-4746-AN	—	—	AJ0-9515
				—	/3pk
5 µm Oligo-XT	00B-4745-AN	—	—	—	AJ0-9515
				for ID: 2.0-3.0 mm	for 2.1 mm ID

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**
					—	/3pk
2.6 µm Oligo-MS C18	00B-4479-E0	00D-4479-E0	—	—	—	AJ0-9066
					—	/3pk
2.6 µm Oligo-XT	00B-4746-E0	00D-4746-E0	—	—	—	AJ0-9514
					/10pk	—
3 µm Oligo-RP C18	00B-4441-E0	00D-4441-E0	00F-4441-E0	—	AJ0-8135	—
					/10pk	—
5 µm Oligo-RP C18	00B-4442-E0	—	00F-4442-E0	00G-4442-E0	AJ0-8135	—
					—	/3pk
5 µm Oligo-XT	—	—	00F-4745-E0	—	—	AJ0-9514
					—	—
5 µm Oligo-SAX	00B-4749-E0	00D-4749-E0	00F-4749-E0	00G-4749-E0	—	—
					/10pk	—
10 µm Oligo-RP C18	—	—	00F-4445-E0	00G-4445-E0	AJ0-8135	—
					/10pk	—
10 µm Oligo-WAX	—	00D-4451-E0	00F-4451-E0	—	AJ0-8324	—
					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‡**
					/3pk
3 µm Oligo-RP C18	00B-4441-N0	—	—	—	AJ0-8136
					/3pk
5 µm Oligo-RP C18	00B-4442-N0	00D-4442-N0	00F-4442-N0	00G-4442-N0	AJ0-8136
					/3pk
5 µm Oligo-XT	00B-4745-N0	00D-4745-N0	00F-4745-N0	—	AJ0-9516
					/3pk
10 µm Oligo-RP C18	—	—	00F-4445-N0	00G-4445-N0	AJ0-8136
					/3pk
10 µm Oligo-WAX	—	—	00F-4451-N0	00G-4451-N0	AJ0-8325
					for ID: 9-16 mm

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♦**
					/ea	/ea
5 µm Oligo-RP C18	00D-4442-P0-AX	—	00G-4442-P0-AX	—	AJ0-8210	AJ0-8310
					/ea	/ea
5 µm Oligo-XT	00D-4745-P0-AX	00F-4745-P0-AX	00G-4745-P0-AX	00F-4745-U0-AX	AJ0-9517	AJ0-9518
					/ea	/ea
10 µm Oligo-RP C18	—	00F-4445-P0-AX	00G-4445-P0-AX	00F-4445-U0-AX	AJ0-8210	AJ0-8310
					/ea	—
10 µm Oligo-WAX	—	—	00G-4451-P0-AX	—	AJ0-8639	—
					for ID: 18-29 mm	30-49 mm

For more about SecurityGuard ULTRA and cartridge holder ordering information, see p. 326.

For Column Heater, see p. 408

*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