

CLARITY® BIOSOLUTIONS FOR DNA/RNA PURIFICATION

OLIGONUCLEOTIDE PURIFICATION PERFECTED

- Clarity BioSolutions is an extensive product portfolio for synthetic oligonucleotide purification and analysis
- Purify DNA, RNA (including siRNA), phosphorothioates, and modified oligos (dyes, quenchers, etc.)
- Recoveries and purities ranging from 70-95 % depending on product used

Specifications for a finished synthetic oligonucleotide vary based on its intended use (for example PCR primer vs. a sequence for antisense studies). A purification strategy is developed dependent upon many factors such as purity and yield requirements, synthesis scales, oligo length and modifications. Clarity BioSolutions offers several strategies to suit your purification needs.



Material Characteristics

Clarity Products	Particle Support	Bonded Phase	Particle Shape/Size (µm)	Pore Size (Å)	Surface Area (m ² /g)
Clarity Desalting Tubes	Silica	C18	55	140	300
Clarity Oligo-RP HPLC Columns	TWIN (silica, organic composite)	C18	3, 5, 10	110	375



Which Clarity BioSolutions Product to Use?

One or more of the Clarity BioSolutions products may be suitable in the lab.

	Clarity® Oligo-RP™ HPLC Columns	Clarity® Desalting Tubes
Purity	95 % ± 2 %	70 % ± 5 %
Recovery Yield	70 % ± 10 %	80 % ± 10 %
Time (min)	15 – 30 minutes**	15 minutes
Oligo Type	DNA, RNA including siRNA, and Phosphorothioates. All forms of oligos with or without dyes, quenchers, and modified bases	DNA, RNA, and oligos with or without dyes, quenchers, and modified bases
Oligo Length*	1 – 60 bases	1 – 60 bases
Synthesis Scale Load***	≥ 50 nmole	≤ 1 µmole
Trityl-on vs. Trityl-off	Trityl-off	Trityl-off

* The longer the oligonucleotide, the less of your synthesis you can load

** Time range is dependent on dimension of HPLC column

*** Load varies based on dimensions of purification product



Note: Purity values based upon reversed phase (RP) chromatography analysis

Clarity is a registered trademark of Phenomenex, Inc.

CLARITY[®] BIOSOLUTIONS FOR DNA/RNA PURIFICATION

Clarity[®] Oligo-RP[™] HPLC Columns

- Reversed phase HPLC for high purity synthetic oligonucleotides
- Easily separate N-1 failure sequences from target with 95 % purities
- Preparative dimensions for loads >1 μ mole
- Purify oligos up to 60mer in length

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 hybrid particles found in the marketplace. It is available in 3, 5, and 10 μ m particle sized beads and in a variety of dimensions.

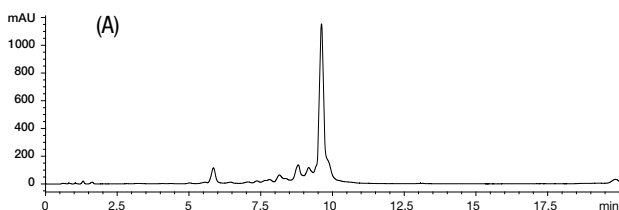
Preparative Purification on Oligo-RP

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.

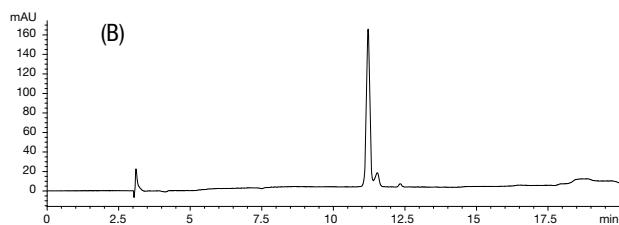
DNA Purification (A) Preparative (B) Analytical QC

App ID 15947 & 15948

Column: Clarity 3 μ m 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 μ g (1 μ mole) 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.

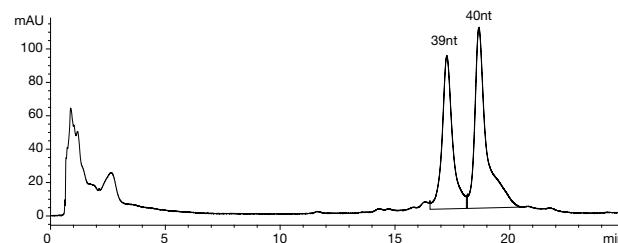
Separate N-1 Failure Sequences from Target N Sequence

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

App ID 16021

Column: Clarity 3 μ m 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 μ g)
2. 39nt DNA with sequence
TTCTGAACAGTTGATCTATGCACTTCAGACTTATGATCA (2.5 μ g)

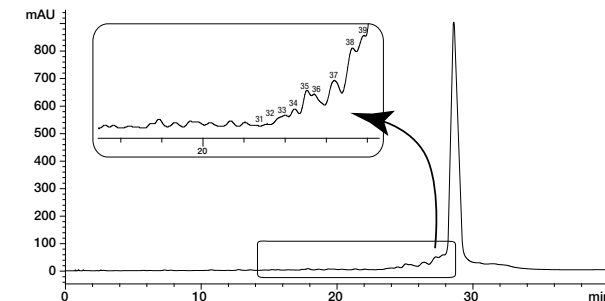


Clarity Oligo-RP successfully separates a 40mer from a 39mer DNA oligonucleotide due to its excellent efficiency and resolving power.

Fingerprint of 40mer DNA

App ID 15970

Column: Clarity 3 μ m 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.



See p. 302 for Ordering Information.

CLARITY® BIOSOLUTIONS FOR DNA/RNA PURIFICATION

Clarity® Oligo-RP™ HPLC Columns

(cont'd)

ORDERING INFORMATION

*SecurityGuard™ Analytical Cartridges require universal holder Part No.: KJO-4282

3 µm Minibore & Analytical Columns (mm)				SecurityGuard™ Cartridges		
	50 x 2.0	100 x 2.0	50 x 4.6	100 x 4.6	4 x 2.0* mm	4 x 3.0* mm
Phase					/10pk	/10pk
C18	00B-4441-B0	00D-4441-B0	00B-4441-E0	00D-4441-E0	AJO-8134	AJO-8135
					for ID: 2.0-3.0 mm 3.2-.8.0 mm	

‡Semi-prep SecurityGuard™ Cartridges require holder, Part No.: AJO-7220

3 µm Semi-Prep Columns (mm)		SecurityGuard™ Cartridges
	50 x 10.0	10 x 10 mm†
Phase		/3pk
C18	00B-4441-N0	AJO-8136
		for ID: 9-16 mm

*SecurityGuard™ Analytical Cartridges require universal holder Part No.: KJO-4282

5 µm Analytical Columns (mm)		SecurityGuard™ Cartridges	
	50 x 4.6	150 x 4.6 mm	4 x 3.0 mm*
Phase			/10pk
C18	00B-4442-E0	00F-4442-E0	AJO-8135
			for ID: 3.2-.8.0 mm

‡Semi-prep SecurityGuard™ Cartridges require holder, Part No.: AJO-7220

**PREP SecurityGuard™ Cartridges require holder, Part No.: AJO-8223

5 µm Semi-Prep and Prep Columns (mm)					SecurityGuard™ Cartridges
	50 x 10.0	100 x 10.0	250 x 21.2	10 x 10 mm†	15 x 21.2 mm**
Phase				/3pk	/ea
C18	00B-4442-N0	00D-4442-N0	00G-4442-P0	AJO-8136	AJO-8210
				for ID: 9-16 mm	18-30 mm

Clarity® Desalting Tubes

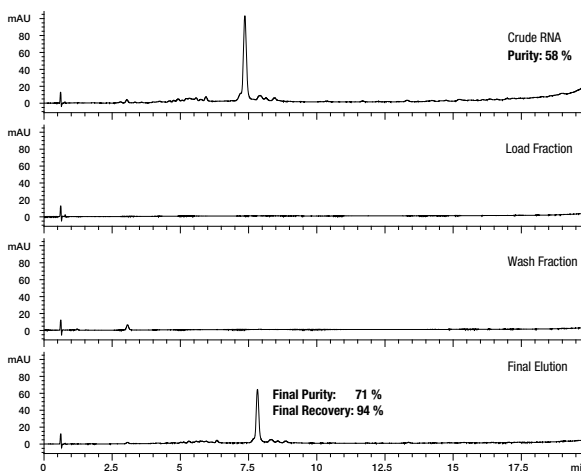
- 70 % typical purity from removal of salt & excess reagent
- Removes salt prior to MS analysis or post IEX chromatography
- Economical, disposable tubes

Clarity® Oligo-RP™ can be used to yield highly purified target oligonucleotides (> 85 % purity) from a synthesis mixture. However, some applications (example – PCR primers) do not require this degree of purity. For simple desalting of a trityl-off synthetic oligonucleotide, Clarity desalting tubes can be used. Clarity desalting tubes are a poly-functional silica-based C18 sorbent that provides a high capacity, fast and effective desalting process.



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, 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 FAM - TTTGACTTAGACTTAGACTTAGTTT was desalted using Clarity Desalting Tubes in the 200 mg/3 mL format. Collection fractions were then analyzed for purity and recovery using the above protocol.

Clarity Desalting Tubes

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

* For 200 µmole synthesis
** For 1 µmole synthesis



Evaluate Clarity® BioSolutions in your lab for 45 days, if you are not completely satisfied return it for a FULL REFUND.



NOTE – for more information on the Clarity products please contact your technical consultant to request a FREE copy of one of the many Clarity Technical Notes.