
CLARITY® BIOSOLUTIONS patent pending

# CLARITY® QSP™ CARTRIDGES AND 96-WELL PLATES

HIGH-THROUGHPUT SYNTHETIC DNA/RNA PURIFICATION

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QUICK SIMPLE PURE


## Partners in Purification

The increase in nucleotide demand has fostered a pressing need for more efficient and efficacious purification platforms. Downstream users of your oligonucleotides depend on you to quickly provide them high quality product for their research and/or product commercialization efforts. To meet these demands, Phenomenex wants to be your partner in purification and assist you in providing superior synthetic oligonucleotides and maintaining high customer satisfaction. As your purification partner, Phenomenex not only provides excellent technical support and customer service, but also a novel, reliable suite of products (Clarity BioSolutions) for synthetic DNA/RNA purification. Based on partner feedback, we are happy to introduce the latest edition to the Clarity BioSolutions portfolio, Clarity QSP. Clarity QSP is an excellent product for those who require a high-throughput, high purity solution. 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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# Introducing Clarity QSP - Quick, Simple, Pure

Developed to be a versatile, gentle, and extremely effective trityl-on purification process, Clarity QSP delivers near impurity-free, concentrated full-length sequences in a stable media suitable for *in-vivo* applications and downstream analysis conducive for MS, NMR, CE, and HPLC. Simple in practice and theory, the product offers speed and efficacy in formats that can be easily automated for high-throughput parallel purification and is suitable for both combinatorial-scale and large-scale purifications. Quick, simple, and pure perfectly and accurately summarizes the Clarity QSP product line.

- **Formats easily amenable to high-throughput parallel processing**
- **Simple three-step process delivers highly purified DNA/RNA in minutes**
- **90 % average purities and yields for both DNA & RNA**
- **Minimizes depurination**

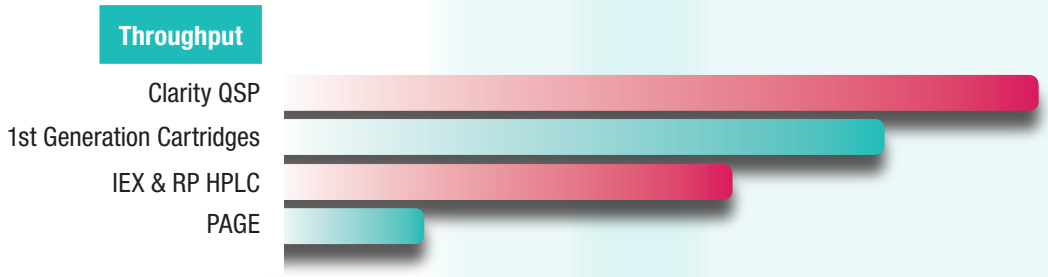
## QSP Purification – a guarantee, not a promise

Clarity QSP is a next generation purification platform that was specifically designed to complement contemporary synthetic processes. We guarantee complete discrimination between full-length trityl-on sequences from DNA and RNA synthetic impurities, yielding highly purified and concentrated target oligonucleotides. In addition, as your partner in purification, we also guarantee full application and technical support when it is time to investigate new purification platforms.

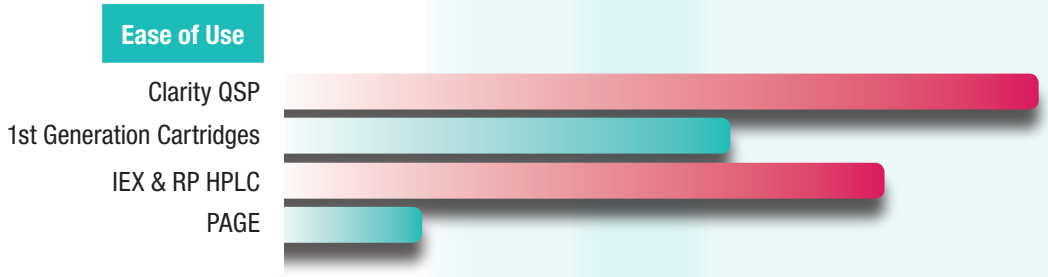
| | |
|---|---|
| **FORMATS** | 96-Well Plate and Cartridges |
| **OLIGO CHEMISTRY** | DNA & RNA |
| **SYNTHESIS SCALE LOAD** | 1 nmole – 50 µmole |
| **OLIGO LENGTH** | ≤ 100 nt |
| **TYPICAL PURITY[1]** | ≥ 90 % |
| **TYPICAL RECOVERY YIELD[2]** | ≥ 80 % |
| **PURIFICATION TIME** | ~ 8 minutes/ cartridge<br>~ 45 minutes/ well plate (96 samples) |
| **EQUIPMENT REQUIRED** | Vacuum manifold or<br>Automated liquid handling system |

*1 Purity value based on ion exchange chromatography and capillary electrophoresis*
*2 $OD_{260}$ used for quantitation*

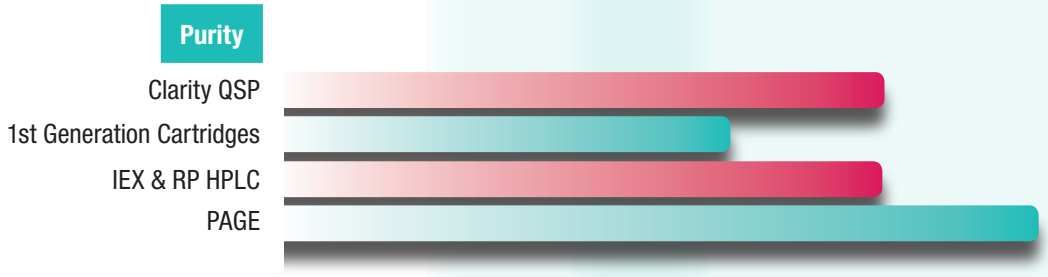
# Introducing Clarity QSP - Quick, Simple, Pure *(cont'd)*




Clarity QSP takes advantage of the hydrophobic trityl group for purification. By doing so, we are able to provide a sorbent, buffer, and method that alleviates the throughput shortfalls of conventional HPLC and PAGE purification. The unique product chemistry is packed into cartridge-based and 96-well plate-based formats that function as cost effective alternatives that are fast, efficacious, and tailored for parallel purification platforms.




Using Clarity QSP is as easy as 1-2-3. In three simple steps, Clarity QSP delivers highly purified synthetic DNA or RNA with exceptional recovery yields. The unique buffer formula works synergistically with post-synthesis cocktails to eliminate truncated and damaged fragments. The improved cleaning proficiency of the buffer mixed with the specially engineered sorbent eliminate the need for extra steps, which often plague other commercial purification platforms.




Clarity QSP is an ideal combination of throughput, simplicity, and purity. The QSP resin is pH-stable and purifies oligo sequences of lengths ranging from 10 nt to 100 nt. In addition, the QSP media has enhanced flow characteristics to ensure consistent flow rates for increased analyte contact time resulting in unfailing performance. The result is a final product of synthetic DNA sequences with purities typically ranging from 90 % to 95 % and reliable recovery values of 80 % or higher. RNA purification, being much more difficult, still delivers excellent purities ranging from 89 % to 93 % and reproducible recovery values of 75 % or higher.

# Reinventing Trityl-on Purification

Trityl-on reversed phase purification was introduced soon after solid phase automation became the synthetic mainstay. It was originally thought to be a superior purification method due to the fact that the lipophilic tag could be used as a discrimination factor, the majority of impurities are trityl-off, and formats that are amenable to parallel processing can be used. Until Clarity QSP, however, reversed phase trityl-on purification systems have not delivered as expected. Clarity QSP has reinvented trityl-on purification by overcoming the shortfalls of the first generation products.

## No toxic ion-paring reagents

The standard reversed phase (RP) cartridge design requires the use of toxic and problematic ion-pairing reagents (ie – TEAA) to retain trityl-on DNA/RNA. Ion-pairing reagents are often very difficult to remove from purified oligonucleotides and thus these oligos are unsuitable for downstream applications, especially *in-vivo* research. The QSP DNA & RNA buffers are composed of biological compatible agents and are free of toxic and meddlesome ion-pairing reagents.

## Load from synthesis/deprotection cocktails

Nearly every commercial RP-cartridge format lacks the convenience of direct or undiluted loading in alkaline cocktails, thereby limiting their utility for serial high-throughput purification. A major goal in the development of Clarity QSP was for individuals to be able to load synthesized oligos from concentrated ammonium hydroxide or ammonium hydroxide/AMA (1:1) for DNA and from TEA 2' deprotection solutions for RNA onto the sorbent. With QSP, direct loading is a reality and saves manufacturers precious time by eliminating lengthy dry down steps.

# Reinventing Trityl-on Purification *(cont'd)*

## Compatible with downstream applications

First generation trityl-on purification cartridges typically require the use of a proprietary elution buffer, which is not always compatible with downstream applications. Clarity QSP, on the other hand, allows you to select your own elution buffer based on your goals for the synthesized oligo. We provide recommended eluting conditions, but you are also welcome to create tailored elution conditions.

| BUFFER | pH | PRIMARY APPLICATION | DRY DOWN | RECONSTITUTING SOLVENT |
|---|---|---|---|---|
| 15 mM $Na_2CO_3$ / 20 % Acetonitrile | 7.5 - 8 | *In-vivo* & tissue based investigations | YES | Water |
| 20 mM $Na_2HCO_3$ / 20 % Acetonitrile | 7.5 | *In-vivo* & tissue based investigations | YES | Water |
| 10 mM Tris pH 8 / 20 % Acetonitrile | 8 | *In-vivo* & tissue based investigations | YES | Water |
| 20 mM $NH_4HCO_3$ / 20 % Acetonitrile | 7.5 | MS, LC/ MS- ESI, MALDI / NMR | Customer Preference | Water |
| 15 mM $NH_4CO_3$ / 20 % Acetonitrile | 7.5 - 8 | MS, LC/ MS- ESI, MALDI / NMR | Customer Preference | Water |
| 20 mM $NH_4CH_3CO_2$ pH 8 / 20 % Acetonitrile | 7 | MS, LC/ MS- ESI, MALDI / NMR | Customer Preference | Water |

## Minimal depurination

Trityl-on purification regardless of the modality will induce some level of depurination. However, in developing Clarity QSP, significant effort was made to monitor the causes and minimize the degree of damage to the oligonucleotide during detritylation. The Clarity QSP detritylation protocol uses DCA (minimizing acid exposure time) as opposed to TFA and introduces pH buffered solutions in the final elution. This protocol not only drastically reduces the level of depurination (typically < 3 %) in the final product, but also improves detritylation efficiencies.

Please request a FREE copy of technical note TN-0008 *Avoiding Depurination During Trityl-on Purification* for the detailed Clarity QSP/ depurination investigation

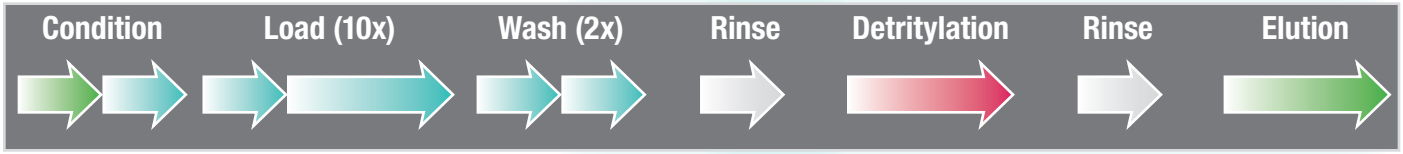
# Reinventing Trityl-on Purification *(cont'd)*

## Simplified protocol

Compared to first generation trityl-on procedures, the QSP procedure is extremely quick and simple. Mired with conventional reversed-phase wisdom, the standard trityl-on RP-cartridge design requires multiple solvents, serial dilutions, sequential wash steps, and sometimes multiple purification rounds. QSP, however, is a very simple protocol that includes brief sample preparation and 3 purification steps.

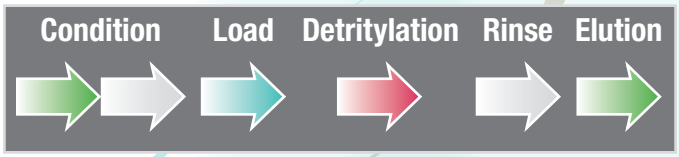
### First Generation Trityl-on Purification Procedure

(9 steps, 20 mL, 45 minutes)




### Clarity QSP Trityl-on Purification Procedure

(5 steps, 5 mL, 20 minutes)

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## Unique formats

The Clarity QSP media is packed into a variety formats to accommodate a wide variety of synthesis scales, oligo lengths, and high-throughput processing equipment. For assistance in selecting the QSP format that is right for your application, please contact Phenomenex or visit *www.phenomenex.com/Clarity* to access the Clarity QSP product calculator.

# Simple QSP Dual-Component System

Clarity QSP consists of two components, a loading buffer and a polymeric sorbent. The specially engineered buffer and sorbent synergistically work with alkaline deprotecting solutions to selectively retain the full-length trityl-on oligo sequence. A DNA buffer and an RNA buffer are available and have both been expertly formulated to match the chemistry of each type of oligo to ensure highly purified DNA/ RNA with excellent recovery. Multiple formats are available to suit a wide range of synthesis scales and oligo lengths.

## DNA & RNA loading buffers

The loading buffers can be used for any synthetic single-stranded oligonucleotide regardless of synthetic derivatization and/or adjunct. Both the DNA and the RNA buffer have been specially formulated to streamline synthetic DNA and RNA purification, respectively.

- **Complete discrimination of trityl-on full-length sequences from trityl-off impurities in presence of ammonia-based aqueous solutions and TBDMS cleavage solutions.**
- **Eliminates need for sequential wash steps**
- **One-step loading of DNA and RNA in synthetic cocktails**
- **No ion-pairing reagent included**

# Simple QSP Dual-Component System *(cont'd)*

## Formats

Clarity QSP is available in several formats to meet throughput and loading capacity requirements. The performance of the QSP media is highly reproducible in each format due to its enhanced flow characteristics that ensure consistent flow rates for increased analyte contact time. Housed in three cartridge formats and a 96-well plate, the QSP resin is pH-stable and purifies oligo sequences ranging from 10 to 100 nt.

## Cartridges

- **Ideal for increased loading capacities**
- **Purify crude oligo sample in ~8 minutes**
- **Use either vacuum or positive pressure systems**


Synthesis Load: 0.2 µmole or less


Synthesis Load: 1.0 µmole or less


Synthesis Load: 50 µmole or less

## 96-Well Plates

- **Ideal for high-throughput, parallel purification**
- **Purify 96 crude oligo samples in < 45 minutes**
- **Easily amenable to automated liquid handling systems**

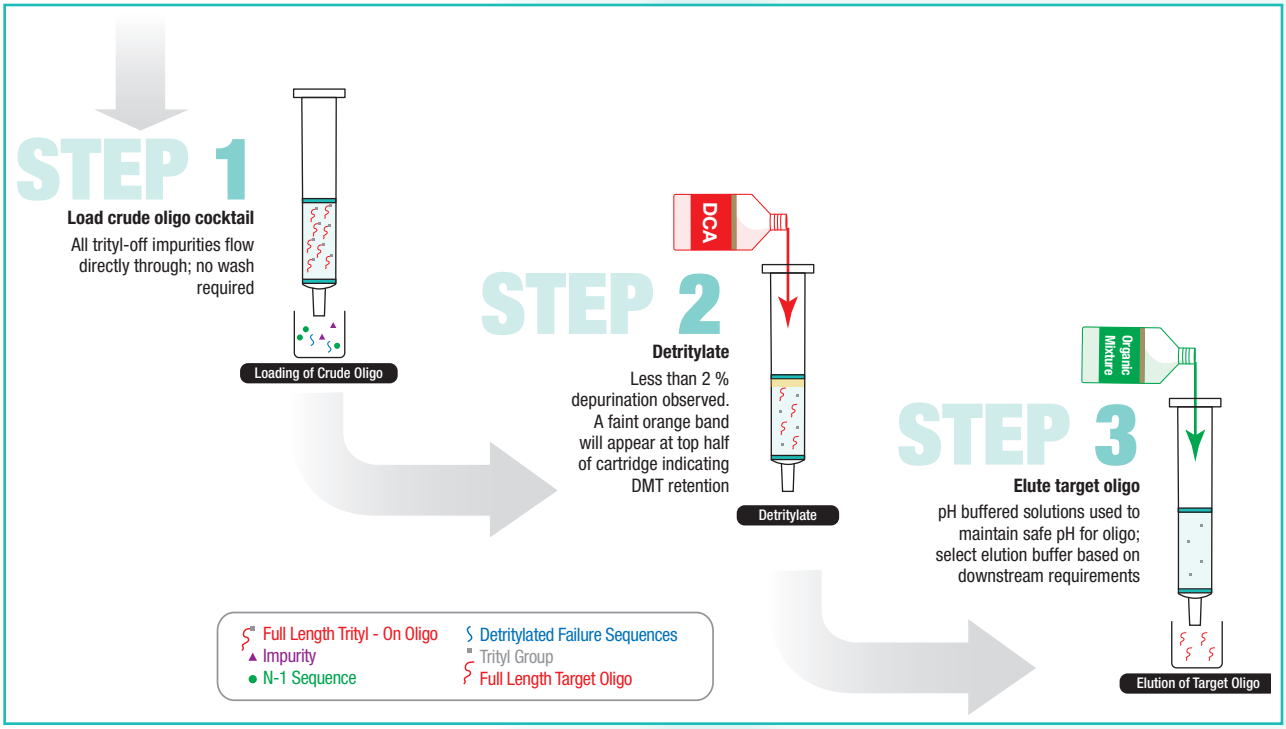

Synthesis Load: 0.2 µmole or less / well

*NOTE – No expensive capital equipment required for cartridge or well-plate purification*

*NOTE – Vacuum manifolds and 96-well collection plates available via Phenomenex for both cartridges and 96-well plate purification*

# Effortless QSP Protocol

QSP purification of trityl-on oligo sequences begins after an equal volume of loading buffer is mixed with the cleavage and deprotection solutions. After brief conditioning of the sorbent with methanol and water, the solublized crude oligo is passed through the sorbent. The buffer provides selective retention of the full-length trityl-on sequence, while impurities are not retained and flow through. Detritylation and elution follow, after which the purified full-length oligo is recovered.





Please request a **FREE** copy of the Clarity QSP User's Manual for more detailed protocol information.

# Synthetic DNA Purification Applications

## Clarity QSP 50 mg/1 mL Cartridge with DNA Buffer

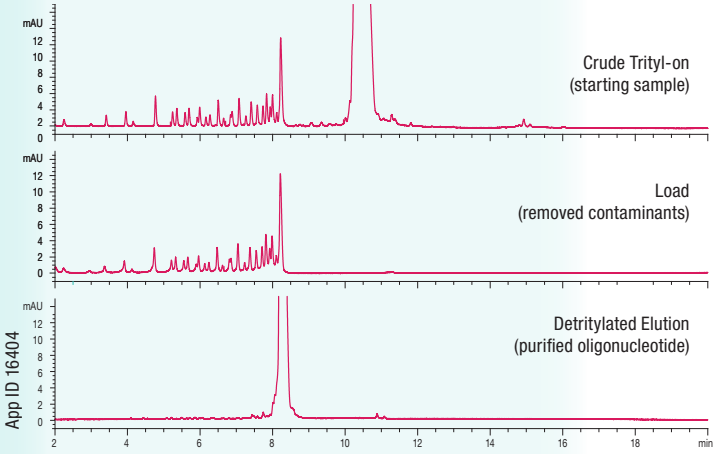
Crude 21 mer DNA- 200 nmole Sequence: CAAGTATATAAAACTTCGATC

### Sample Preparation:

- 300 µL of concentrated $NH_4OH$ was added to CPG column and heated to 55 °C for 6 hours
- Following deprotection, sample preparation for Clarity QSP purification involved simply adding an equal volume of DNA loading buffer (300 µL) to the $NH_4OH$ solution

### DNA Protocol

- Condition: 1 mL Methanol (2 x 0.5 mL)
- Equilibrate: 1 mL Water (2 x 0.5 mL)
- Load: Oligo Sample (600 µL)
- Detritylate: 1 mL 3 % DCA
- Rinse: 1 mL Water (2 x 0.5 mL)
- Dry Sorbent at 10" Hg Vacuum (~1 min)
- Elute: 1 mL 15 mM $Na_2CO_3$/ 40 % Acetonitrile




| Crude Trityl-on $OD_{260}$ | Load Fraction $OD_{260}$ | Detritylated Final Elution $OD_{260}$ | Recovery | Purity (Peak area) |
|---|---|---|---|---|
| 17.1 | 3.2 | 11.8 | 84.6 % | 97 % |

## Clarity QSP 50 mg/1 mL Cartridge with DNA Buffer

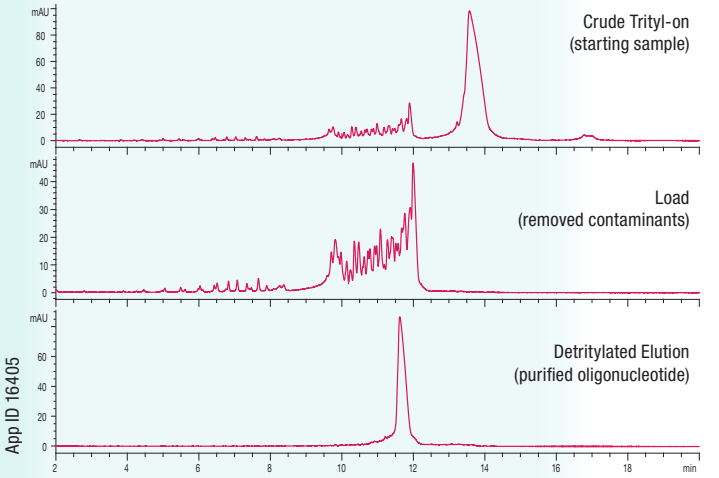
Crude 53 mer DNA- 200 nmole Sequence:
ACAGTCGTACAGTCATATATTACTATTCAGTGTCTACTGCAGTCGTTATCTAT

### Sample Preparation:

- 300 µL of concentrated $NH_4OH$ was added to CPG column and heated to 55 °C for 6 hours
- Following deprotection, sample preparation for Clarity QSP purification involved simply adding an equal volume of DNA loading buffer (300 µL) to the $NH_4OH$ solution

### DNA Protocol

- Condition: 1 mL Methanol (2 x 0.5 mL)
- Equilibrate: 1 mL Water (2 x 0.5 mL)
- Load: Oligo Sample (600 µL)
- Detritylate: 1 mL 3 % DCA
- Rinse: 1 mL Water (2 x 0.5 mL)
- Dry Sorbent at 10" Hg Vacuum (~1 min)
- Elute: 1 mL 15 mM $Na_2CO_3$/ 40 % Acetonitrile




| Crude Trityl-on $OD_{260}$ | Load Fraction $OD_{260}$ | Detritylated Final Elution $OD_{260}$ | Recovery | Purity (Peak area) |
|---|---|---|---|---|
| 39.7 | 6.51 | 29.6 | 89.2 % | 93 % |

## Clarity QSP 96-well plate with DNA Buffer

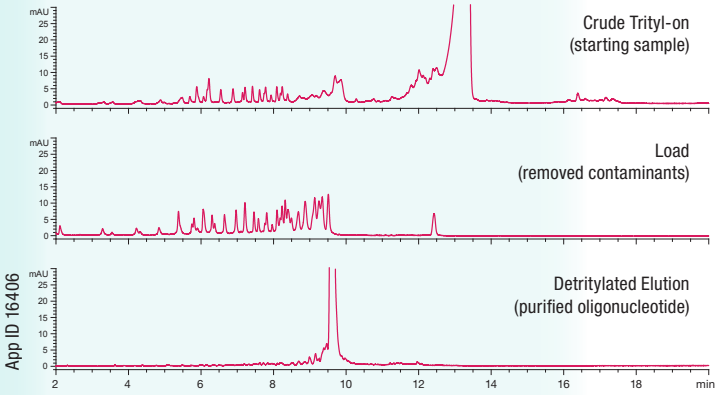
Crude 30 mer DNA- 200 nmole Sequence:
GTGGATCTGCGCACTTCAGGCTCCTGGGCG

### Sample Preparation:

- 250 µL of oligo solution in concentrated $NH_4OH$ (post deprotection) was aliquoted
- An equal volume of DNA buffer (250 µL) was added and vortexed

### DNA Protocol

- Condition: 1 mL Methanol (2 x 0.5 mL)
- Equilibrate: 1 mL Water (2 x 0.5 mL)
- Load: Oligo Sample (500 µL)
- Detritylate: 1 mL 1 % DCA
- Rinse: 1 mL Water (2 x 0.5 mL)
- Dry Sorbent at 10" Hg Vacuum (~1 min)
- Elute: 1 mL 15 mM $Na_2CO_3$/ 20 % Acetonitrile




| Crude Trityl-on $OD_{260}$ | Load Fraction $OD_{260}$ | Detritylated Final Elution $OD_{260}$ | Recovery | Purity (Peak area) |
|---|---|---|---|---|
| 28.3 | 5.3 | 20.8 | 90.3 % | 92 % |

# Synthetic RNA Purification Applications

## Clarity QSP 50 mg/1 mL Cartridge with DNA Buffer (for RNA TOM Chemistry)*

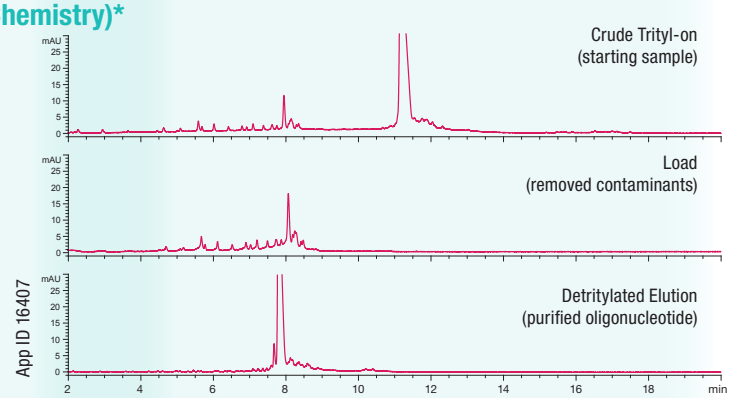
Crude 21 mer RNA- 200 nmole Sequence: GAGUGACCACCUCACUUGATT

### Sample Preparation:

- 400 µL of oligo sample was mixed with an equal volume of DNA loading buffer (400 µL) *(for RNA TOM chemistry, DNA loading buffer is used instead of RNA buffer)

### RNA Protocol (TOM)

- Condition: 1 mL Methanol (2 x 0.5 mL)
- Equilibrate: 1 mL Water (2 x 0.5 mL)
- Load: Oligo Sample (800 µL)
- Detritylate: 1 mL 3 % DCA
- Rinse: 1 mL Water (2 x 0.5 mL)
- Dry Sorbent at 10" Hg Vacuum (~1 min)
- Elute: 1 mL 15 mM $Na_2CO_3$/ 50 % Acetonitrile




| Crude Trityl-on $OD_{260}$ | Load Fraction $OD_{260}$ | Detritylated Final Elution $OD_{260}$ | Recovery | Purity (Peak area) |
|---|---|---|---|---|
| 18.5 | 5.1 | 10.9 | 81.4 % | 92.6 % |

## Clarity QSP 150 mg/3 mL Cartridge with RNA Buffer (for RNA TBDMS Chemistry)

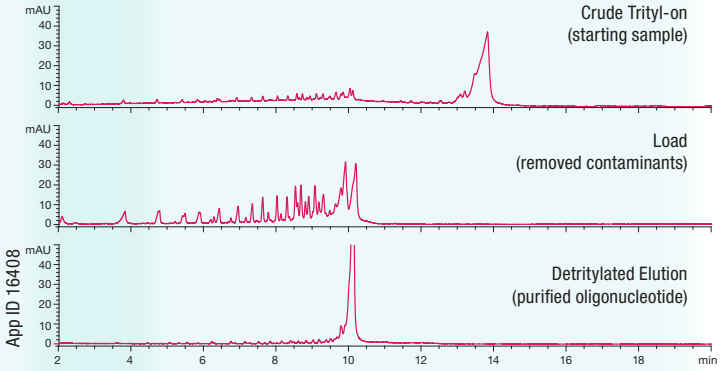
Crude 27 mer RNA- 1 µmole Sequence: Proprietary

### Sample Preparation (2' Deprotection)

- Added 250 µL of RNA deprotection (TEA.3HF) solution to dried RNA pellet
- Vortex/ mix briefly
- Heated at 65 °C for 1.5 Hour
- Cooled to room temperature
- Added 1 mL of 1.5 M ammonium bicarbonate to quench
- Added an equal volume of Clarity RNA loading buffer (1.25 mL) to quenched deprotection solution [Final volume 2.5 mL]
- Proceeded to trityl-on cartridge purification

### RNA Protocol

- Condition: 3 mL Methanol (2 x 1.5 mL)
- Equilibrate: 3 mL Water (2 x 1.5 mL)
- Load: Oligo Sample (2.5 mL)
- Detritylate: 1.5 mL 3 % DCA
- Rinse: 2 mL Water (2 x 1.0 mL)
- Dry Sorbent at 10" Hg Vacuum (~1 min)
- Elute: 2 mL 15 mM $Na_2CO_3$/ 40 % Acetonitrile




| Crude Trityl-on $OD_{260}$ | Load Fraction $OD_{260}$ | Detritylated Final Elution $OD_{260}$ | Recovery | Purity (Peak area) |
|---|---|---|---|---|
| 33.4 | 9.22 | 22.9 | 94 % | 84 % |

## Clarity QSP 96-well plate with RNA Buffer (for RNA TBDMS Chemistry)

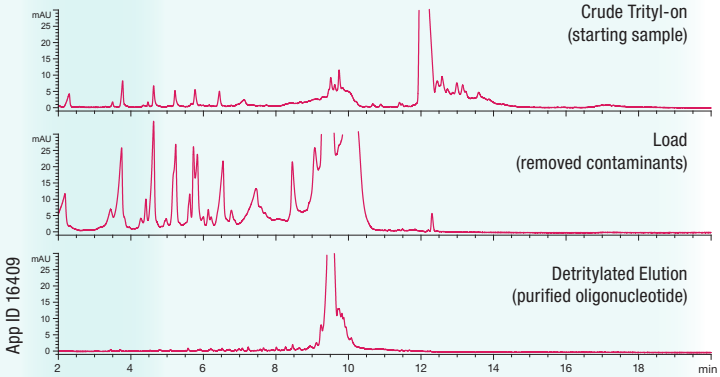
Crude 28 mer RNA- 200 nmole Sequence: GACUCACAUCAACUACGAUCGAGCACTT

### Sample Preparation (2' Deprotection)

- Added 100 µL of RNA deprotection (TEA.3HF) solution to dried RNA pellet
- Vortex/ mix briefly
- Heated at 65 °C for 1.5 Hour
- Cooled to room temperature
- Added 400 µL of 1.5 M ammonium bicarbonate to quench
- Added an equal volume of Clarity RNA loading buffer (500 µL) to quenched deprotection solution [Final volume 1 mL]
- Proceeded to trityl-on cartridge purification

### RNA Protocol

- Condition: 1 mL Methanol (2 x 0.5 mL)
- Equilibrate: 1 mL Water (2 x 0.5 mL)
- Load: Oligo Sample (1 mL)
- Wash: 0.5 mL 40/60 [RNA buffer/ Water]
- Detritylate: 1 mL 1 % DCA
- Rinse: 1 mL Water (2 x 0.5 mL)
- Dry Sorbent at 10" Hg Vacuum (~1 min)
- Elute: 1 mL 20 mM $NH_4CO_3$/ 20 % Acetonitrile




| Crude Trityl-on $OD_{260}$ | Load Fraction $OD_{260}$ | Detritylated Final Elution $OD_{260}$ | Recovery | Purity (Peak area) |
|---|---|---|---|---|
| 46 | 15.7 | 24 | 79.2 % | 81 % |

# Ordering Information

## Formats

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

## Buffer

| Part No. | Description | | Unit |
|---|---|---|---|
| AL0-8279 | Clarity QSP DNA Loading Buffer | 100 mL | Ea |
| AL0-8280 | Clarity QSP DNA Loading Buffer | 1 L | Ea |
| AL0-8281 | Clarity QSP RNA Loading Buffer | 100 mL | Ea |
| AL0-8282 | Clarity QSP RNA Loading Buffer | 1 L | Ea |
| AH0-7858 | Clarity Nuclease Free Water | 1 L | Ea |

*Note: for RNA TOM Chemistry, DNA loading buffer is used instead of RNA buffer. Please contact Phenomenex for questions or clarification.*

## Accessories

| Part No. | Description | | Unit |
|---|---|---|---|
| AH0-7284 | 96-Well Plate Manifold | Acrylic | Ea |
| AH0-6024 | 24-Position Vacuum Manifold | Complete Set | Ea |
| AH0-7194 | 96 Square Well Collection Plate | 2 mL/Well (Polypropylene) | 50/pk |
| AH0-7408 | Solvent Waste Reservoir Tray | For Well Plate Manifolds | 25/pk |
| AH0-7195 | 96-Well Pierceable Sealing Mat | Square Well | 50/pk |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**Australia**
PO Box 4084
Lane Cove, NSW 2066
Australia
tel.: 02-9428-6444
fax: 02-9428-6445
email: info@phenomenex.com.au

**Austria**
Zeppelinstr. 5
63741 Aschaffenburg
Germany
01-319-1301
01-319-1300
anfrage@phenomenex.com

**Canada**
411 Madrid Ave.
Torrance, CA
90501-1430
USA
(800) 543-3681
(310) 328-7768
info@phenomenex.com

**Denmark**
Gydevang 39-41
3450 Allerød
Denmark
4824 8048
4810 6265
dkinfo@phenomenex.com

**France**
Parc des Grillons, Bat.3
60 route de Sartrouville
78232 Le Pecq Cedex
France
01 30 09 21 10
01 30 09 21 11
franceinfo@phenomenex.com

**Germany**
Zeppelinstr. 5
63741 Aschaffenburg
Germany
06021-58830-0
06021-58830-11
anfrage@phenomenex.com

**Ireland**
Queens Avenue,
Hurdsfield Ind. Est.,
Macclesfield, Cheshire
SK10 2BN, UK
tel.: 01 247 5405
fax: +44 1625-501796
email: eireinfo@phenomenex.com

**Italy**
Via Emilia, 51/C
40011 Anzola Emilia (BO)
Italy
051 736176
051 735302
italiainfo@phenomenex.com

**New Zealand**
P O Box 31-601
Milford 0741
North Shore City
New Zealand
09-4780951
09-4780952
info@phenomenex.co.nz

**Puerto Rico**
273 Sierra Morena,
Suite #104
San Juan,
Puerto Rico 00926
(800) 541-HPLC
(310) 328-7768
info@phenomenex.com

**United Kingdom**
Queens Avenue,
Hurdsfield Ind. Est.,
Macclesfield, Cheshire
SK10 2BN, UK
01625-501367
01625-501796
ukinfo@phenomenex.com

**USA**
411 Madrid Ave.
Torrance, CA
90501-1430
USA
(310) 212-0555
(310) 328-7768
info@phenomenex.com

# QUICK SIMPLE PURE


## CLARITY® QSP™ CARTRIDGES AND 96-WELL PLATES

HIGH-THROUGHPUT SYNTHETIC DNA/RNA PURIFICATION

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