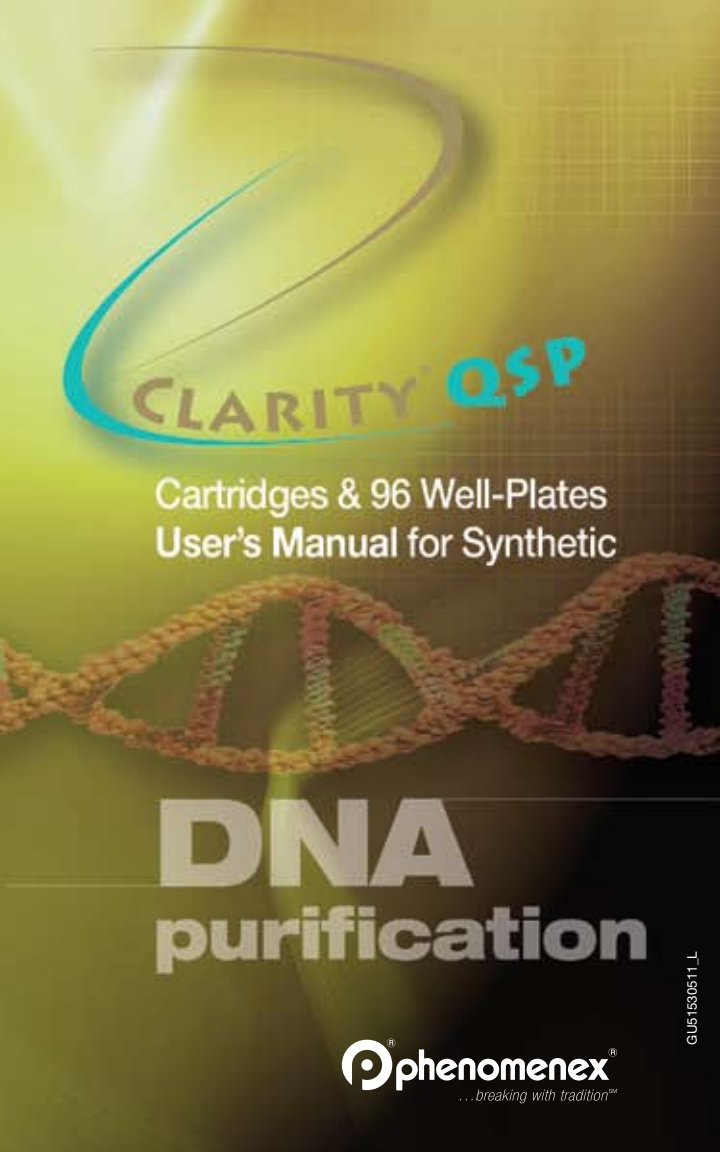
CLARITY QSP
Cartridges & 96 Well-Plates
User's Manual for Synthetic
DNA
purification
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phenomenex
. . . breaking with tradition℠

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# 1.0 Introduction

DNA

Advances in functional genomics have caused a dramatic surge for both synthetic ribo- and deoxyribonucleotides. The increase in nucleotide demand and their recent therapeutic applications have fostered a pressing need for more efficient and efficacious purification platforms. To better match the need of oligo manufacturers and their customers, Phenomenex introduces Clarity QSP; a newly developed cartridge-based format that offers a quick and simple alternative for obtaining highly purified and concentrated synthetic DNA and RNA. Utilizing trityl-on chemistry, this innovative approach provides in one-step complete discrimination of the full-length sequence from unwanted synthetic contaminants delivered in a physiological pH buffered solution. Clarity QSP is a next generation purification product that was designed specifically to complement contemporary synthetic processes.

## 1.1 DNA Synthesis

State-of-the-art synthesis employs solid-phase phosphoramidite chemistry to construct deoxyribonucleotides through a succession of phosphodiester linkages. Although designed over 40 years ago, the majority of modern oligo synthesis platforms continue to adhere to dimethoxytrityl chemistry (DMT). The synthetic approach is rather straightforward as assembly begins with the initial protected nucleotide tethered to a solid porous support. Sequence elongation then proceeds as subsequent phosphoramidites are assembled from 3' to 5' through sequential automated cycles of detritylation, coupling, capping, and oxidation. Upon sequence completion, an alkaline solution is added to release the crude oligonucleotide from the solid support then heated to remove base-labile protecting groups from the nucleobases and phosphate backbone. Purification follows and depending on the practice used, the final 5' DMT group can be retained (trityl-on) or removed (trityl-off) prior to the final cleavage and deprotection steps.

Modern synthetic chemistry has merged with advanced fabrication technology to significantly improve synthetic efficiencies and thus the quality of crude synthetic oligonucleotides. Today, instruments failure rates rarely exceed 1.0 % per coupling event. Meaning, for a 21 nt sequence, instruments providing 99 % efficiency will typically yield 80 % of full-length product. Notwithstanding of such low cumulative population failures, during the assembly process, sequence fragments and other synthetic contaminants are introduced that lower the quality of the crude product. Moreover, as the chain length grows, coupling efficiencies diminish thereby increasing the cumulative

population failures in the full-length sequence. Consequently, these remnant impurities most notably truncated sequences and depurinated fragments require robust purification techniques to increase the purity and recovery yields of the final oligonucleotide product.

## 1.2 Purification Platforms

Over the decades, various purification techniques have been used to clean up crude synthetic oligonucleotides. Similar to synthetic advances, improvements have been made in the purification process. Solvent precipitation, once customary, has been replaced by improved chromatographic resins and automated systems. Gel filtration sorbents, one of the first chromatographic modalities, are effective for desalting applications but fail to remove more tenacious contaminants. Today, synthetic oligonucleotides are predominately purified with HPLC techniques using either trityl-on or trityl-off methodologies. Those employing trityl-on procedures rely primarily on reversed-phase chromatography, while IEC (ion-exchange chromatography) is the mode of choice for the trityl-off contingent. The two chromatographic modalities come with their particular advantages and disadvantages.

## 1.3 Reversed-Phase Trityl-On Cartridge Purification

Trityl-on RP cartridge purification was introduced soon after solid phase automation became the synthetic mainstay. Originally designed to alleviate the shortfalls of sequential HPLC purification, cartridge-based formats were to function as cost effective alternatives that were fast, efficacious, and tailored for parallel purification platforms. Mired with conventional reversed-phase wisdom, trityl-on RP cartridge systems have not delivered as advertised. The standard RP-cartridge design requires multiple solvents, sequential wash steps, introduces toxic and analytically problematic ion-pairing agents, but more importantly has failed to produce generally acceptable purity and recovery yields. Further diminishing the appeal, 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. Consequently, many in the field are disappointed with the performance of cartridge-based products that once promised unmatched ease and efficacy.

DNA

## 1.4 Clarity QSP Trityl-On Purification

Addressing the global aim of a purification process, Clarity QSP delivers near impurity-free, concentrated full-length DNA sequences in a stable media suitable for in-vivo applications and downstream analysis conducive for MS, NMR, CE, and HPLC. Simple in practice and in theory, the product offers speed and efficacy in formats that can be readily automated for high-throughput parallel purification and is suitable for both combinatorial-scale and large-scale purifications. Clarity QSP consists of two components, a loading buffer and a polymeric sorbent. Housed in three cartridge formats and a 96-Well plate, the QSP resin is pH-stable and purifies DNA 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 accompanying loading buffer is composed of biological compatible agents and is free of toxic and meddlesome ion-pairing agents. Together, the sorbent and buffer create a simple three-step process that in minutes delivers highly purified synthetic DNA with exceptional recovery yields.

## 1.5 The QSP process

QSP purification of trityl-on DNA begins after an equal volume of loading buffer is mixed with the cleavage and deprotection solution. After brief conditioning of the sorbent with methanol and water, the solublized crude oligo is passed through the sorbent. The unique buffer formula synergistically works with ammonia-based deprotecting solutions to selectively retain the full-length trityl-on DNA sequence, while eliminating tenaciously bound unlabeled truncated sequences and damaged fragments. The improved cleaning proficiency of the buffer when mixed directly with an alkaline solution eliminates the need for subsequent wash steps leaving only detritylation and elution to follow. The result is a final product of synthetic DNA sequences with purities typically ranging from 90 % to 95 %[1] and reliable recovery values of 90 % or higher[2].

1. Ion-exchange chromatography and capillary electrophoresis tested.

2. $OD_{260}$ used for quantitation.

## 2.1 Clarity QSP Components

Clarity QSP DNA Loading Buffer (ion pairing free)
- 100 mL
- 1 L

Clarity QSP Cartridges
- 60 mg/ 3 mL
- 150 mg/ 3 mL
- 5 g/ 60 mL

Clarity QSP 96-Well Plate
- 50 mg/ well

## 2.2 Equipment and Materials Required

Vacuum manifold
- 12- or 24-position
- 96-Well plate

Vacuum pump

Reagents
- Methanol
- Water (DI or Nuclease free)
- DCA (Dichloroacetic acid)
- Acetonitrile

Recommended eluting buffer solutions and their intended applications.

| Buffer | pH | Primary Application | Dry Down | Recon-stituting 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 (24 hr shelf-life) | 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 (24 hr shelf-life) | 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 |

***Note:*** *Buffer can contain up to 40 % Acetonitrile*

DNA

**IMPORTANT:** For trityl-on DNA purification it is imperative that the final 5' DMT group is retained following synthesis completion.

## 3.1 Cleavage and Deprotection

- If using concentrated $NH_4OH$, add appropriate volume to CPG column according to synthesis scale. Typically, the volume used is 150 µL per 100 nmole.
- Allow 24 hours for room temperature incubation or at 6 hours at 55 °C.

- For AMA (50:50 Aqueous ammonium hydroxide /Aqueous methylamine), add appropriate volume to CPG column according to synthesis scale. Typically, the volume used is 150 µL per 100 nmole.
- Incubate at 60 °C for 15 minutes.

## 3.2 Clarity QSP Sample Preparation

- Following deprotection, sample preparation for Clarity QSP purification involves simply adding an equal volume (see section 11.2 for example) of DNA loading buffer to either of the cleavage solutions mentioned above. Buffer addition can be performed immediately after deprotection and stored at -20 °C indefinitely or added just prior to administering to cartridges or 96-Well plates.

- A minimum total load volume of 200 µL is required for optimal QSP resin performance. Accordingly, for synthesis scales less than 100 nmole, it is recommended that at least 100 µL of deprotection solution be used.

- Proceed to appropriate purification format (cartridge or 96-Well plate) and follow protocol accordingly.

## 4.1 Clarity QSP for DNA Purification

**Format:** 96-well plate (50 mg/ well)
**Synthesis Scale:** ≤ 0.2 µmole

The 96-well plate format can be performed using automated liquid handling systems or may also be used with manual 96-well plate manifolds. The protocol can perform using either vacuum or positive pressure systems. The optimal vacuum or pressure settings differ between the various available automated systems; therefore the following serve only as suggested and modifications may be required for each system.

**Remember:** Follow DNA sample preparation protocol in section 3.0 to ensure highest purity and recovery.

**IMPORTANT:** For each of the following steps, allow the entire volume to pass through the sorbent.

**1. Condition Cartridge:** 1 mL Methanol (0.5 mL x 2)
Initiate vacuum to 2-3" Hg. Flow at 2 drops / second

**2. Equilibrate Cartridge:** 2 mL Water (1.0 mL x 2)
Flow at 2 drops / second, vacuum setting 3-4" Hg

**3. Load DNA:** 200 µL-600 µL
Monitor vacuum to ensure flow rate of 1 drop / 2-3 second (typically 3" Hg).
***Important:*** *After the volume has passed through the sorbent, increase vacuum to ~5" Hg for approximately 30 seconds to expel remaining liquid.*

**Wash (optional):** 0.5 mL. Dilute loading buffer (50 % water / 50 % DNA Buffer) flow at 1 drop / second, vacuum setting 3" Hg
***Important:*** *After the volume has passed through the sorbent, increase vacuum to ~5" Hg for approximately 30 seconds to expel remaining liquid.*

DNA

**4. Detritylate:** 1 mL Aqueous 0.5 % or 1.0 % DCA
Flow at 2 drops / second, vacuum setting 4-5" Hg.
***Important:*** *After the volume has passed through the sorbent, increase vacuum to ~5" Hg for approximately 30 seconds to expel remaining liquid.*

***Note:*** *A faint orange band will appear at the top half of the sorbent indicating DMT retention.*

***Note:*** *Automated parallel systems will experience a longer exposure to acid. Consequently it is suggested to lower the concentration of DCA to the lowest level possible that still allows complete DMT removal.*

**5. Rinse:** 1 mL Water (0.5 mL x 2)
Flow at 2 drops / second, vacuum setting 4-5" Hg

**6. Dry sorbent:**
Increase vacuum to 10" Hg for 1 minute

**7. Elution:** 1 mL 20 mM $NH_4HCO_3$ / 20 % Acetonitrile (see section 2.2)
Flow at 1 drop / second, vacuum setting 3-4" Hg.
***Note:*** *The initial eluting volumes may appear cloudy, but as the pH increases the solution will become water clear.*

***Caution:*** *During and after detritylation the unprotected DNA analyte is exposed to low pH, which over time can cause depurination (please see section 5.0 for more information). It is therefore strongly recommended to elute in an appropriate buffer to ensure a final pH between 7 & 8. The various buffer salts mentioned in section 2.0 can be used at the discretion of the end user.*

**TIP:** It is not recommended to load more than 70 $ODs_{260}$ of crude synthetic DNA on 50 mg of resin.

**TIP:** Please contact Phenomenex for any assistance in optimizing your vacuum or pressure settings.

## 4.2 Clarity QSP for DNA Purification

**Format:** 60 mg/ 3 mL Cartridge
**Synthesis Scale:** ≤ 0.2 µmole

**Remember:** Follow DNA sample preparation protocol in section 3.0 to ensure highest purity and recovery.

**IMPORTANT:** For each of the following steps, allow the entire volume to pass through the sorbent.

**1. Condition Cartridge:** 1 mL Methanol (0.5 mL x 2)
Initiate vacuum to 2-3" Hg. Flow at 2 drops / second

**2. Equilibrate Cartridge:** 1 mL Water (0.5 mL x 2)
Flow at 2 drops / second, vacuum setting 3-4" Hg

**3. Load DNA:** 200 µL-600 µL
Monitor vacuum to ensure flow rate of 1 drop / 2-3 second (typically 3" Hg).
***Important:*** *After the volume has passed through the sorbent, increase vacuum to ~5" Hg for approximately 30 seconds to expel remaining liquid.*

**Wash (optional):** 1 mL. Dilute loading buffer (50 % water / 50 % DNA Buffer) flow at 1 drop / second, vacuum setting 3" Hg
***Important:*** *After the volume has passed through the sorbent, increase vacuum to ~5" Hg for approximately 30 seconds to expel remaining liquid.*

**4. Detritylate:** 1 mL Aqueous 1 % DCA
Flow at 2 drops / second, vacuum setting 4-5" Hg.
***Important:*** *After the volume has passed through the sorbent, increase vacuum to ~5" Hg for approximately 30 seconds to expel remaining liquid.*

***Note:*** *A faint orange band will appear at the top half of the sorbent indicating DMT retention.*

**5. Rinse:** 1 mL Water (0.5 mL x 2)
Flow at 2 drops / second, vacuum setting 4-5" Hg

**6. Dry sorbent:**
Increase vacuum to 10" Hg for 1 minute

**7. Elution:** 1 mL 20 mM $NH_4HCO_3$ / 20 % Acetonitrile (see section 2.2)
Flow at 1 drop / second, vacuum setting 3-4" Hg.
***Note:*** *The initial eluting volumes may appear cloudy, but as the pH increases the solution will become water clear.*

***Caution:*** *During and after detritylation the unprotected DNA analyte is exposed to low pH, which over time can cause depurination (please see section 5.0 for more information). It is therefore strongly recommended to elute in an appropriate buffer to ensure a final pH between 7 & 8. The various buffer salts mentioned in section 2.0 can be used at the discretion of the end user.*

**TIP:** It is not recommended to load more than 70 $ODs_{260}$ of crude synthetic DNA on 60 mg of resin.

## 4.3 Clarity QSP for DNA Purification

**Format:** 150 mg/ 3 mL Cartridge
**Synthesis Scale:** ≤ 1.0 µmole

**Remember:** Follow DNA sample preparation protocol in section 3.0 to ensure highest purity and recovery.

**IMPORTANT:** For each of the following steps, allow the entire volume to pass through the sorbent.

**1. Condition Cartridge:** 3 mL MeOH (1.5 mL x 2)
Initiate vacuum to 2-3" Hg. Flow at 2 drops / second

**2. Equilibrate Cartridge:** 3 mL Water (1.5 mL x 2)
Flow at 2 drops / second, vacuum setting 3-4" Hg

**3. Load DNA:** ~3 mL
Monitor vacuum to ensure flow rate of 1 drop / 2-3 second (typically 3-4" Hg).
***Important:*** *After the volume has passed through the sorbent, increase vacuum to ~5" Hg for approximately 30 seconds to expel remaining liquid.*

**Wash (optional):** 1.5 mL. Dilute loading buffer (50 % water / 50 % DNA Buffer) flow at 1 drop / second, vacuum setting 3" Hg
***Important:*** *After the volume has passed through the sorbent, increase vacuum to ~5" Hg for approximately 30 seconds to expel remaining liquid.*

**4. Detritylate:** 1.5 mL Aqueous 2 % or 3 % DCA
Flow at 2 drops / second, vacuum setting 4-5" Hg.
***Important:*** *After the volume has passed through the sorbent, increase vacuum to ~5" Hg for approximately 30 seconds to expel remaining liquid.*

***Note:*** *A faint orange band will appear at the top half of the sorbent indicating DMT retention.*

**5. Rinse:** 2 mL Water (1.0 mL x 2)
Flow at 2 drops / second, vacuum setting 4-5" Hg

**6. Dry sorbent:**
Increase vacuum to 10" Hg for 2 minutes

**7. Elution:** 2 mL 20 mM $NH_4HCO_3$ / 20 % Acetonitrile (see section 2.2)
Flow at 1 drop / second, vacuum setting 3-4" Hg.
***Note:*** *The initial eluting volumes may appear cloudy, but as the pH increases the solution will become water clear.*

***Caution:*** *During and after detritylation the unprotected DNA analyte is exposed to low pH, which over time can cause depurination (please see section 5.0 for more information). It is therefore strongly recommended to elute in an appropriate buffer to ensure a final pH between 7 & 8. The various buffer salts mentioned in section 2.0 can be used at the discretion of the end user.*

**TIP:** It is not recommended to load more than 200 $ODs_{260}$ of crude synthetic DNA on 150 mg of resin.

## 4.4 Clarity QSP for DNA Purification

**Format:** 5 g/ 60 mL Cartridge
**Synthesis Scale:** 10-50 µmole

**Remember:** Follow DNA sample preparation protocol in section 3.0 to ensure highest purity and recovery.

**IMPORTANT:** For each of the following steps, allow the entire volume to pass through the sorbent.

**1. Condition Cartridge:** 30 mL Methanol (15 mL x 2)
Initiate vacuum to 2-3" Hg. Flow at 2 drops / second

**2. Equilibrate Cartridge:** 30 mL Water (15 mL x 2)
Flow at 2 drops / second, vacuum setting 3-4" Hg

**3. Load DNA:** 30 mL
Monitor vacuum to ensure flow rate of 1 drop / 2-3 second (typically 3-4" Hg).
***Important:*** *After the volume has passed through the sorbent, increase vacuum to ~5" Hg for approximately 30 seconds to expel remaining liquid.*

**Wash (optional):** 15 mL. Dilute loading buffer (50 % water / 50 % DNA Buffer) flow at 1 drop / second, vacuum setting 3" Hg
***Important:*** *After the volume has passed through the sorbent, increase vacuum to ~5" Hg for approximately 30 seconds to expel remaining liquid.*

**4. Detritylate:** 15 mL Aqueous 2 % DCA
Flow at 2 drops / second, vacuum setting 4-5" Hg.
***Important:*** *After the volume has passed through the sorbent, increase vacuum to ~5" Hg for approximately 30 seconds to expel remaining liquid.*

***Note:*** *A faint orange band will appear at the top half of the sorbent indicating DMT retention.*

**5. Rinse:** 20 mL Water (10 mL x 2)
Flow at 2 drops / second, vacuum setting 4-5" Hg

**6. Dry sorbent:**
Increase vacuum to 10" Hg for ~2 minute

**7. Elution:** 10 mL 20 mM $NH_4HCO_3$ / 20 % Acetonitrile (see section 2.2)
Flow at 1 drop / second, vacuum setting 3-4" Hg.
***Note:*** *The initial eluting volumes may appear cloudy, but as the pH increases the solution will become water clear.*

***Caution:*** *During and after detritylation the unprotected DNA analyte is exposed to low pH, over time can cause depurination (please see section 5.0 for more information). It is therefore strongly recommended to elute in an appropriate buffer to ensure a final pH between 7 & 8. The various buffer salts mentioned in section 2.0 can be used at the discretion of the end user.*

## 4.5 Clarity QSP for DNA with Modified Base-Labile Conjugates or Tags

**Synthesis Scale:** nmole – 50 µmole

**Sample Preparation:** Particular tags or sequence modifications that are not stable in ammonia-based solutions will require adjustments to the Clarity QSP protocol. When working in solutions other than ammonia-based cocktails, evaporate to dryness prior to cartridge purification.

- Dilute the DNA buffer 1:1 with water then re-dissolve oligo to a volume appropriate with scale. Proceed with cartridge purification.

**IMPORTANT:** Refer to synthesis scale for appropriate volumes

**REMEMEMBER:** Always allow the entire volume to pass through the sorbent.

### 1. Condition Cartridge:

Initiate vacuum to 2-3" Hg. Flow at 2 drops / second

### 2. Equilibrate Cartridge:

Flow at 2 drops / second, vacuum setting 3-4" Hg

### 3. Load DNA:

Monitor vacuum to ensure flow rate of 1 drop / 2-3 second (typically 3-4" Hg).

***Important:*** *After the volume has passed through the sorbent, increase vacuum to ~5" Hg for approximately 30 seconds to expel remaining liquid.*

DNA

**4. Wash:** (Dilute DNA Buffer 1:1 Water) Monitor vacuum to ensure flow rate of 1 drop / second (typically 4" Hg).
***Important:*** *After the volume has passed through the sorbent, increase vacuum to ~5" Hg for approximately 30 seconds to expel remaining liquid.*

**5. Detritylate:** Aqueous 1-3 % DCA (depending on scale)
Flow at 2 drops / second, vacuum setting 4-5" Hg.
***Important:*** *After the volume has passed through the sorbent, increase vacuum to ~5" Hg for approximately 30 seconds to expel remaining liquid.*

***Note:*** *A faint orange band will appear at the top half of the sorbent indicating DMT retention.*

**6. Rinse:** Water
Flow at 2 drops / second, vacuum setting 4-5" Hg

**7. Dry sorbent:**
Increase vacuum to 10" Hg for ~2 minutes

**8. Elution:** See section 2.2 for buffer conditions
Flow at 1 drop / second, vacuum setting 3-4" Hg.
***Note:*** *The initial eluting volumes may appear cloudy, but as the pH increases the solution will become water clear.*

***Caution:*** *During and after detritylation the unprotected DNA analyte is exposed to low pH, over time can cause depurination (please see section 5.0 for more information). It is therefore strongly recommended to elute in an appropriate buffer to ensure a final pH between 7 & 8. The various buffer salts mentioned in section 2.0 can be used at the discretion of the end user.*

**TIP:** Please contact Phenomenex for any assistance in optimizing your vacuum or pressure settings.

## 5.0 Depurination

Trityl-On purification regardless of the modality will induce some level of depurination. Clarity QSP is no different in this regard. However, altering detritylation conditions can minimize the degree of a basic prevalence in the final product. The majority of commercial trityl-on cartridge products follow conventional thinking by using 2-5 % of trifluoroacetic acid (TFA) and incubating on sorbent at ambient temperature for more than five minutes. To no surprise, under such conditions, depurination can reach as high as 30 % in the final product. This extensive amount of depurination has caused many oligo producers and end users to spurn trityl-on purification particularly, cartridge-based formats.

In developing Clarity QSP, significant effort was made to monitor the causes and minimize the degree of damage to the oligonucleotide during detritylation. While various factors influence depurination such as sequence composition, repeated studies using the **Clarity QSP protocol revealed that lower acid concentrations and limited exposure times significantly minimized depurination, yet still provided complete trityl release.** Using ESI-MS, IEC, and polyamine hydrolysis for analysis, effective DCA strengths were varied from 0.5 % to 3 % with a 1-minute (on cartridge) oligo exposure time. While maintaining complete detritylation, we observed less than 2 % depurination using 1 % DCA whereas using 3 % DCA concentration, depurination had increased to 5 %. When using 96-Well plates with automated liquid handling systems, oligo exposure time to the acid treatment will vary among the loaded samples with some seeing a five-fold increase in duration to nearly five minutes. Accordingly, an investigation was performed using 0.5 % DCA and incubated for 5 and 15 minutes at room temperature. Using the same analytical techniques described above, less than 1 % depurination was present after either 5 or 15 minutes with complete trityl detachment occurring at both time intervals. For cartridge formats, we do not recommend using this low of acid concentration, as it may not be sufficient for complete detritylation.

**Another important feature of the Clarity QSP protocol is the introduction of pH buffered solutions in the final elution.** During and after the detritylation step, the oligonucleotide is exposed to a low pH environment. Subsequent water washes do not effectively elevate the retained oligo to appropriate physiological pH. It was observed again using MS and polyamine hydrolysis, that eluants in pH of at least 7 contained no additional depurination even after 72 hours at room temperature. In sharp contrast, when eluting in a water and acetonitrile solution and left at room temperature, within hours substantial depurination had occurred.

Unavoidably, base cleavage of oligonucleotides is an inherent occurrence in not only trityl-on purification but during synthesis as well. The recommended QSP protocol will not prevent depurination from occurring however; **our investigation does present a methodology that will improve detritylation efficiencies, while also drastically reducing the level of depurination in the final purified product.** From our outcomes we hope to attract former users of cartridge-based products to once again consider the efficacy and efficiency advantages of trityl-on purification.

DNA

| Problem | Cause | Solution |
|---|---|---|
| Orange band observed throughout entire sorbent | Possible overloading of sample on the resin. | Do not exceed recommended oligo concentration as described for the given synthesis scales in section 4.0. |
| Breakthrough of DMT full-length sequence observed in load elution | Flow rate too fast. | Ensure flow rate of 1 drop / 2 seconds. Some patience is required. |
| Incomplete removal of DMT observed in final product | Detritylation step: Acid strength too weak | Gradually increase DCA concentration. Do not exceed 3 % aqueous DCA as higher acid strength will increase depurination |
| Final product is not as clean as advertised | Impurities were not eluted in the load eluant. | During each step, allow solutions to pass completely through the sorbent.<br><br>**DNA buffer should be mixed equally with an alkaline deprotecting solution.** We do not advise drying down then re-dissolving in dilute buffer. |
| Following detritylation, cloudy precipitate observed in the water wash | Exceeded recommended volume of water in rinse step. | Use the appropriate volumes as outlined for each scale |
| Orange color was not present after acid addition. No recovery of full-length sequence in final elution | No DMT group. | Ensure DMT was retained following synthesis. Do not adjust the pH in the deprotection cocktail. A pH below 5 will cleave the DMT group. Do NOT heat above 85 °C for extended period of time, or loss of trityl group may occur. |
| Flow rate too slow following conditioning and equilibration | Air is trapped in sorbent | Slowly increase vacuum to remove air pocket. A constant flow should then resume. There is no need to re-condition or re-equilibrate. |

## 7.0 Storage and Stability



### 7.1 Clarity QSP Sorbent

Store at room temperature (~25 °C) indefinitely.

### 7.2 Clarity DNA Loading Buffer

Store at room temperature (~25 °C) indefinitely.

## 8.0 Quality Assurance

The QA/QC of the sorbent include determination of the physical characteristics and a % recovery evaluation. The DNA buffer solution is tested for endo- and exo-nuclease contamination before packaging.

***NOTE:*** *see Certificate of Analysis included with cartridges and the loading buffer for more information.*

## 9.0 Safety and Handling

The resin housed in the cartridges and plates requires no special handling nor does it impose any chemical or biological hazards.

The buffer is composed of biological compatible agents and is nonflammable. Do not however store at elevated temperatures (above 40 °C) for extended periods of time. This can cause pressure to build causing a sudden and possible violent release of vapors upon opening.

***NOTE:*** *For more information, refer to the MSDS sheets.*

**Q Why use trityl-on cartridge purification?**

**A.** When properly designed, trityl-on cartridge formats offer the most efficient purification method available for synthetic oligonucleotides. Unlike HPLC or PAGE, the versatility of the cartridge format allows the end-user to tailor their purification requirements whether off-line or continuous on-line purification. Moreover, when automated, cartridge platforms can effectively purify thousands of samples per day, thereby substantially increasing the productivity for large-scale producers.

**Q. Can Clarity QSP Cartridges and 96-Well Plates be re-used?**

**A.** No. Strongly bound lipophilic and hydrophobic contaminants will remain on the sorbent thus causing cross-contamination if re-used.

**Q Can other 5' chemistries be used with Clarity QSP?**

**A.** Yes. The QSP protocol can be used with any 5' protecting group providing a lipophilic handle for hydrophobic discrimination between contaminants and full-length sequences.

**Q Can modified sequences such as amino, phosphorothioates, dyes and quenching tags be purified using QSP?**

**A.** Yes. As long as the particular sequence modification and tags are alkaline stable then the standard protocol can be used. If however modifications are base labile, please refer to the specific protocol for modified sequences.

**Q Can the DNA buffer be used for RNA (2' Silyl protection)?**

**A.** No. The DNA buffer is formulated specifically to work in ammonia-based deprotection cocktails and will not provide the same efficacy if used in RNA 2' silyl deprotecting solutions.

**Q Does Clarity QSP remove trityl-on contaminants?**

**A.** No. The QSP process discriminates only trityl-off contaminants and cannot discern trityl-on impurities that may co-elute with the full-length sequence. Please do note that the peak often seen eluting immediately to the right of full-length sequences is in fact a deprotection modification of acrylonitrile reacting with the nucleobases. Widely mistaken as an n+1 trityl-on contaminant, this by-product actually results from extended exposure to alkaline conditions at high temperatures. (Bhan, et al. US Patent 6,887,990 B1, 5/3/05)

**Q Will sequence composition effect purity or recovery?**

**A.** No. During the development of QSP we evaluated thousands of sequences with varied combinations of the nucleobases. Purine or pyrimidine concentrations along with varied sequence orders showed no difference in obtaining consistently high purity and recovery yields.

**Q What other acids can be used for detritylation?**

**A.** While we highly recommend using dilute aqueous DCA for our protocols, it is by no means a mandate. Dilute concentrations of TCA and TFA are effective for

detritylation; however, in our research we observed higher rates of depurination among these acids than with similar concentrations of DCA. Acetic acid has been mentioned with other commercial products, but we strongly discourage its usage. Acetic acid requires extreme concentrations of at least 50 % for complete trityl release. At this concentration, unwanted early elution of the full-length sequence occurs in the detritylation step resulting in substantial sample loss.

# Ordering Information

### *Formats:*

| Part No. | Description | | Unit |
|---|---|---|---|
| 8E-S102-DGB | Clarity QSP 30 µm | 50 mg / 96-Well Plate | 1/Box |
| 8B-S102-UBJ | Clarity QSP 30 µm | 60 mg / 3 mL Cartridge | 50/Box |
| 8B-S102-SBJ | Clarity QSP 30 µm | 150 mg /3 mL Cartridge | 50/Box |
| 8B-S042-LFF | Clarity QSP 70 µm | 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 |

### *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 |

DNA

## 11.1 Conversions:

ssDNA 1 $OD_{260}$= 33 µg, 0.033 mg
Nanomoles = [milligrams / molecular weight] x $10^6$

## Molecular weights

| Purines | Pyrimidines |
|---|---|
| A: 251 | T: 245 |
| G: 267 | C: 230 |

## Molecular weight calculation of an oligonucleotide:

(nA x 251) + (nT x 245) + (nG x 267) + (nC x 230) + (61 x (n-1))
+ (54 x n) + (17 x (n-1)) + 2.

Where: nA, nT, nG, and nC equal number of given base in the sequence.
n = total number of bases
(61 x (n-1)): molecular weight of phosphate group
(54 x n): Water molecules per nucleotide
(17 x (n-1)): $NH_4$ cations associated with phosphate groups

## Cumulative Population Failure Equation:

Purity Yield = (Coupling Efficiency Rate) $^{Couplings}$

## 11.2 DNA Applications & Chromatograms

## Quantitative Analysis: $OD_{260}$

## Qualitative (Purity)

IEC Chromatography
DNAPac® 200
Mobile Phase: A: Water
C: 0.25 M Tris-HCL pH 8
D: 0.375 $NaClO_4$.
Gradient: A: 80 %, C:10 %, D:10-65 % in 20 minutes
Flow Rate: 1.2 mL/min
VWD @ 260 nm

## Enclosed Data:

DNA
IEC Chromatograms / OD quantitation
MS Spectra

## Clarity QSP 60 mg/ 3 mL cartridge with DNA loading buffer

DNA-24nt CGATATTCGCATCTAGCCAGATCC MW: 7274 [200 nmole]

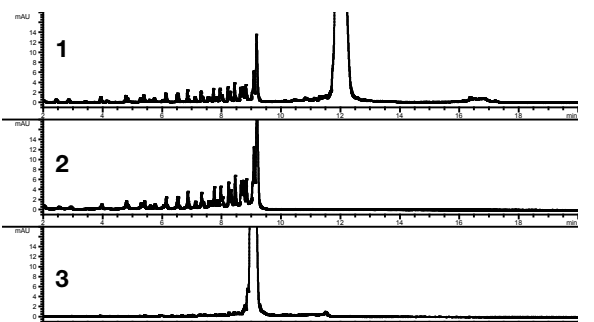
## Trityl-on DNA Sample Preparation

**Cleavage and Deprotection:**

- 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 and vortexing.

## Protocol followed

- Condition: 1 mL Methanol (0.5 mL x 2)
- Equilibrate: 1 mL Water (0.5 mL x 2)
- Load Vol of Oligo (600 µL)
- Detritylate: 1 mL 3 % DCA
- Rinse: 1 mL Water (0.5 mL x 2)
- Dried sorbent @ 10" Hg (~1 min)
- Elute: 1 mL 15 mM $Na_2CO_3$ / 20 % Acetonitrile




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) |
|---|---|---|---|---|
| 20.1 | 3.57 | 14.2 | 86 % | 96.8 % |

DNA

## Clarity QSP 60 mg/ 3 mL cartridge with DNA loading buffer

DNA-53nt ACAGTCGTACAGTCATATATTACTATTCAGTGTCTACTGCAGTCGTTATC-TAT MW: 16222 [200 nmole]

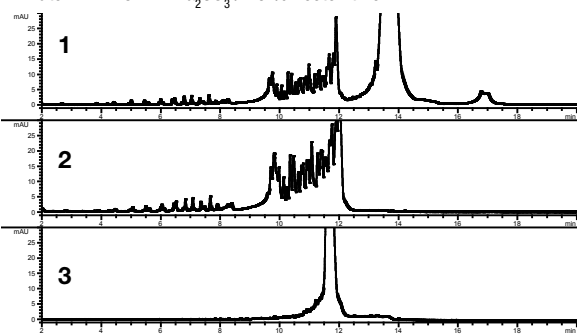
## Trityl-on DNA Sample Preparation

**Cleavage and Deprotection:**

- 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 and vortexing.

## Protocol followed

- Condition: 1 mL Methanol (0.5 mL x 2)
- Equilibrate: 1 mL Water (0.5 mL x 2)
- Load Vol of Oligo (600 µL)
- Detritylate: 1 mL 3 % DCA
- Rinse: 1 mL Water (0.5 mL x 2)
- Dried sorbent @ 10" Hg (~1 min)
- Elute: 1 mL15 mM $Na_2CO_3$ / 20 % Acetonitrile




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 % |

## Clarity QSP 150 mg/ 3 mL cartridge with DNA loading buffer

DNA-26nt GTATGGAGCAATCCTAGCTTATGTTA MW: 7998.9 [1 µmole]

## Trityl-on DNA Sample Preparation

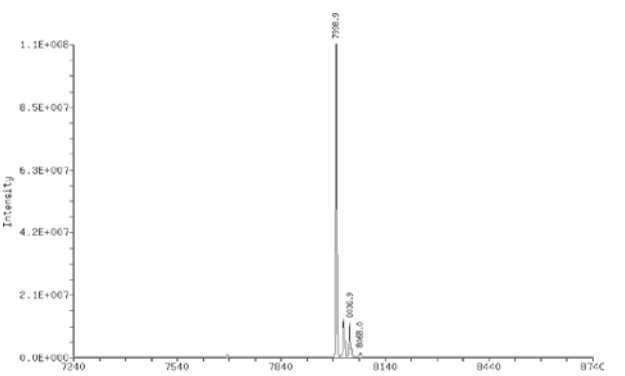
**Cleavage and Deprotection:**

- 1 mL of 40 % AMA was added to CPG column and heated to 60 °C for 15 minutes.
- Following deprotection, sample preparation for Clarity QSP purification involved simply adding an equal volume of DNA loading buffer (1 mL) to the $NH_4OH$ solution and vortexing.

## Protocol followed

- Condition: 3 mL Methanol (1.5 mL x 2)
- Equilibrate: 3 mL Water (1.5 mL x 2)
- Load Vol of Oligo (2 mL)
- Detritylate: 1.5 mL 3 % DCA
- Rinse: 2 mL Water (1 mL x 2)
- Dried sorbent @ 10" Hg (~1 min)
- Elute: 2 mL 20 mM $NH_4HCO_3$ / 40 % Acetonitrile

| Crude Trityl-on | Load Fraction | Detritylated final elution | Recovery | Peak Abundance |
|---|---|---|---|---|
| NA | NA | 270 nmole | NA | 97 % |



DNA

## Clarity QSP 96-Well Plate with DNA loading buffer

DNA-30nt GTGGATCTGCGCACTTCAGGCTCCTGGGCT MW: 9206 [200 nmole]

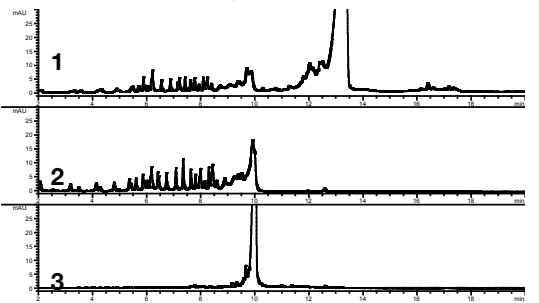
## Trityl-on DNA Sample Preparation

**Cleavage and Deprotection:**

- 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 and vortexing.

## Protocol followed

- Condition: 1 mL Methanol (0.5 mL x 2)
- Equilibrate: 2 mL Water (1.0 mL x 2)
- Load Vol of Oligo (600 µL)
- Rinse: 1 mL Water (0.5 mL x 2)
- Detritylate: 1 mL 1 % DCA
- Rinse: 1 mL Water (0.5 mL x 2)
- Dry sorbent @ 10" Hg (~1 min)
- Elute: 1 mL 20 mM $NH_4HCO_3$ / 20 % Acetonitrile



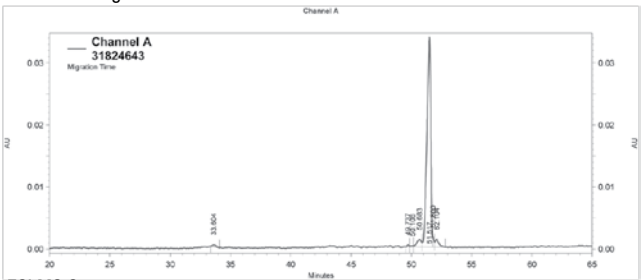

1.Crude Trityl-on
2. Load fraction
3. Detritylated final elution

$OD_{260}$

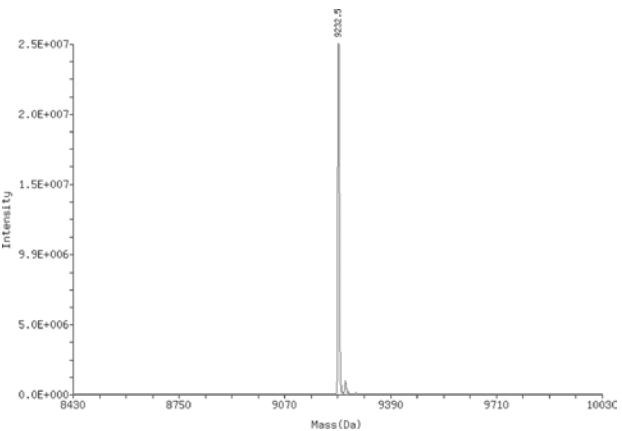
| Crude Trityl-on | Load Fraction | Detritylated final elution | Recovery | Purity (Peak area) |
|---|---|---|---|---|
| 42.4 | 4.9 | 35 | 94 % | 93 % |

## Clarity QSP 96-Well Plate with DNA loading buffer *cont.*

CE Chromatogram




ESI MS Spectra




| Mass (Da) | Std. Dev | Intensity | Identity | Delta Mass | % Relative | % Total |
|---|---|---|---|---|---|---|
| 9232.5 | 0.2 | 2.48E+07 | Target Mass | 0 | 100 | 96.4 |
| 9254.0 | 0.8 | 9.29E+05 | Na adduct | 21.5 | 3.7 | 3.6 |

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