CLARITY® BIOSOLUTIONS

FAQs
for the Isolation and Purification of Synthetic Oligonucleotides

The Clarity BioSolutions portfolio is composed of unique chemistries designed to efficiently and effectively purify oligonucleotides. Learn the answers to some of the most common questions for oligonucleotide clean-up and separation.

Characterization / Analysis Sample Prep

What types of therapeutic oligonucleotides can be extracted from biological fluids with Clarity® OTX™?

DNA, RNA, miRNA, siRNA, phosphorothioates, LNA, single stranded, duplexed, and encapsulated oligonucleotides. As long as there is a phosphodiester or phosphorothioate backbone the extraction protocol should provide excellent clean-up and recovery.

What is the concentration range that can be detected with Clarity OTX?

Calibration curves are linear over the concentration range of 5-2000 ng/mL.

Can alternative lysis and/or load buffers be used in the Clarity OTX extraction protocol?

No. The Clarity OTX SPE media and buffers were developed to work in unison. Alternative solutions will not provide effective isolation or extraction of oligonucleotides.

What sequence lengths can be used with Clarity OTX?

Clarity OTX is designed for isolating and extracting therapeutic sequences ranging from 4nt to 50nt.

Is a vacuum source (or positive pressure) required when using Clarity OTX extraction cartridges or plates?

Yes. The Clarity OTX media particle size is not suitable for gravity flow. A vacuum source that can provide at least 10" Hg is required, but alternatively, a Presston™ 1000 Positive Pressure Manifold is recommended for 96-well plate applications for a more precise and accurate air flow.

What equipment is necessary to run the Clarity OTX protocol?

Vacuum manifold/Presston 1000 Postive Pressure Manifold, vortex, centrifuge, N_2 dry down station and/or lyophilizer.

What type of mechanism is used to isolate the oligo therapeutics from the biological matrix?

The Clarity OTX polymeric sorbent is a mixed-mode, anion exchanger. It works by selectively retaining the oligo based on its inherent chemical properties.

Does Clarity OTX work for tissue lysates?

Yes, Clarity OTX works with tissue lysates and most biological fluids notably, plasma, serum, urine, tears, and sputum.

Can double stranded oligonucleotides be extracted using Clarity OTX?

Yes, but only those sequences with less than 50 total base pairs are viable with Clarity OTX.

How much Proteinase K is needed for the Clarity OTX digestion protocol? What buffer do I use?

Proteinase K from Qiagen® (P/N 19133) is recommended. For each digested sample, add 40 µL of Proteinase K to 0.86 mL of 0.1 M Tris/5 mm CaCl buffer. Digest sample for 3 hours at 50 °C.

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Have questions or want more details? We would love to help!
*Visit **www.phenomenex.com/Chat** to get in touch with one of our Technical Specialists*

Purification Sample Prep

What oligos will work for Clarity® QSP™?

Most synthesized oligonucleotides (DNA, RNA, etc) with a DMT (dimethoxy trityl) or other 5' protecting group will work with Clarity QSP. Key to using the product is making sure that your oligonucleotide synthesis cycle cleave your oligo off the resin before the deprotection step of the synthesis cycle.

Is a buffer exchange or dry down required from synthesis cleavage cocktail before using Clarity QSP?

No, samples can be loaded directly from oligo deprotection cocktail without buffer exchange or a dry down! Just dilute sample 1:1 with loading buffer and follow the Clarity QSP protocol. 3 steps and 15 minutes later your oligo should be pure!

Is Clarity QSP loading buffer needed to purify your oligo?

Yes, we recommend treating the oligo with 1:1 loading buffer to purify oligos using Clarity QSP.

Doesn't trityl-on purification cause depurination of DNA during purification?

Not necessarily. Studies show that depurination occur very slowly at low pH and the oligo should only spend moments at low pH during the Clarity QSP process. As neutral/slightly basic pH is used for elution, there should not be measurable depurination using Clarity QSP.

Why use trityl-on cartridge purification?

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.

Can other 5' chemistries be used with Clarity QSP?

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.

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

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.

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

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.

Does Clarity QSP remove trityl-on contaminants?

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 a 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)

Will sequence composition effect purity or recovery?

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.

What other acids can be used for detritylation?

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 DNA 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.

Oligo LC Separation

Why are hybrid silicas preferred for reversed phase analysis of oligonucleotides?

Oligonucleotides are inherently polar and therefore not amenable to standard reversed phase HPLC. Characterization of oligos typically demands reversed phase with aggressive ion-pair and modifiers. When traditional silica materials are used lifetime is poor. Hybrid silicas have a much higher pH stability and show good lifetime for oligonucleotide analysis.

What is the role of pH in the separation of oligonucleotides?

The polarity of the structure of an oligonucleotide makes them difficult to analyze. They contain a sugar unit, phosphate backbone, and polar base repeated multiple times which makes them inherently polar in nature. To counteract this standard approach to analyzing oligonucleotides by reversed phase uses an ion-pair reagent; typically an alkylamine. Elevated pH is an unfortunate by-product of the conditions needed to retain oligonucleotides; however, the addition of HFIP serves to reduce pH as well as facilitate ionization for MS detection.

What impact does changing the nature of the alkylamine have on separation?

The alkylamine used will directly affect retention time, longer chains lead to greater retention so hexylamine will show longer retention times than triethylamine. There is also a difference in solubility so generally gradients need to be adjusted to allow for this. Generally the effect of ion-pair is oligo-specific; for Gapmers, longer amines offer better resolution whereas for siRNA shorter amines offer an improvement in resolution.

How does reducing HFIP concentration affect larger oligonucleotides?

HFIP is an acidic modifier used to improve ionization for mass spec analysis of oligonucleotides. As little as 12.5 mM HFIP has shown a benefit to improve ESI efficiency while also providing sufficient chromatographic performance for oligos up to 33mer in size. Reduction in concentration of HFIP have shown retention time loss but no reduction in MS signal.

How can you improve the stability of your mobile phase?

Mixtures of perfluorinated alcohols and alkylamines are unstable. To improve their stability the use of SecurityCAP™, with their containers can help. The SecurityCAP mobile phase safety filters have an integrated one-way valve and filter membrane that captures dust, particulates, and other air borne contaminants whilst also ensuring volatile components in your mobile phase are not lost to evaporation.

To order Clarity LC or Sample Preparation products go to

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