Clarity® BioSolutions for Synthetic DNA/RNA

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

Clarity OTX™ Extraction Kits

Rapid Cleanup 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**

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.

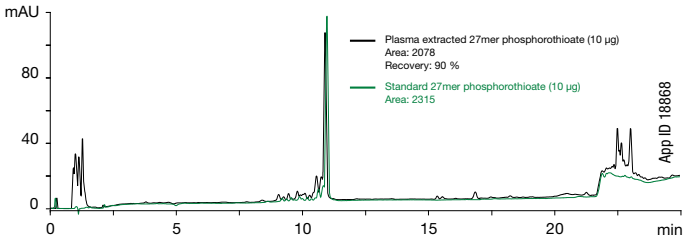
Sample Preparation:

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

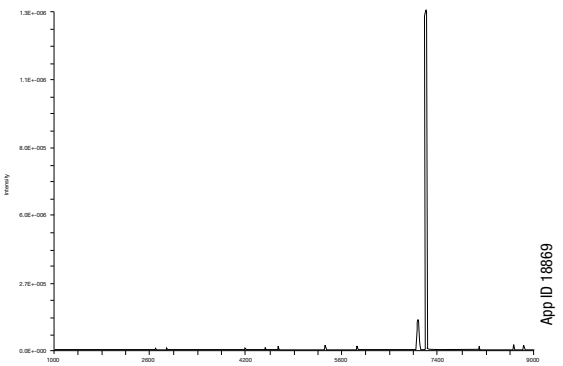
Extraction Protocol

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.

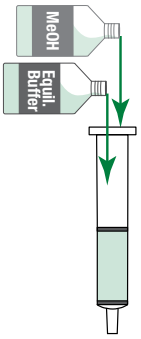
Designed for Throughput

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

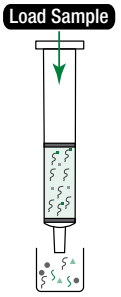
STEP 1

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



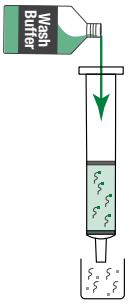
STEP 2

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.



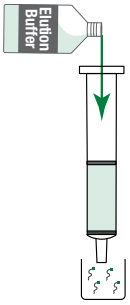
STEP 3

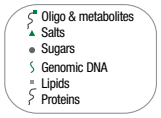
The Wash Buffer is formulated to strip off lipids and remaining proteins from the sorbent, while not disturbing the oligo therapeutics and its metabolites.



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.

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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 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.

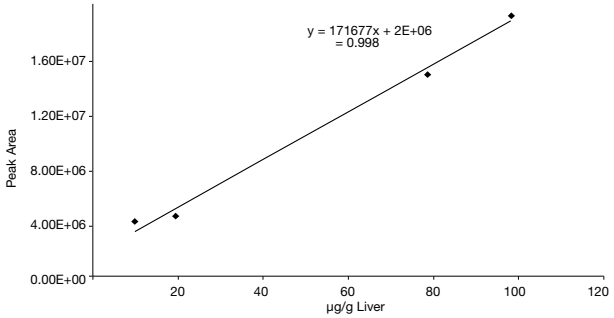
Eliminate MS Interfering Contaminants

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.

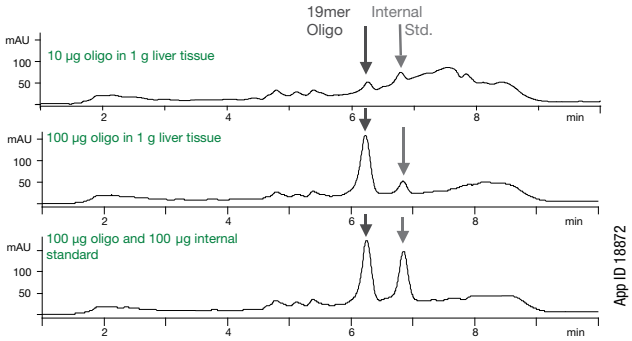
Liver Tissue Linearity Curve



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 100 µg to 10 µg. 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 10 µg 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	Price
KS0-8494	Clarity OTX Starter Kit	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	
8E-S103-EGA	Clarity OTX Well Plate	100 mg/ well	1/box	
8B-S103-EBJ	Clarity OTX Cartridge	100 mg/3 mL	50/box	
AL0-8498	Clarity OTX Lysis-Loading Buffer	1 L	ea	
AL0-8579	Clarity OTX Lysis-Loading Buffer V2.0	1 L	ea	