

# Extraction of Oligo Therapeutics from Biological Samples

## For LC-MS Bioanalysis



- Ultra-high recoveries for low nanogram extractions
- Simple 4-step protocol in 15 minutes
- Mixed-mode SPE effectively removes biological interferences

# A Dependable Partner for Oligo Separations

Phenomenex is dedicated to the development of innovative separation science products and providing exceptional support to organizations extracting, purifying, and characterizing DNA and RNA. By collaborating with scientists doing LC-MS bioanalysis of oligonucleotide therapeutic candidates, Phenomenex developed the Clarity OTX™ buffer and SPE extraction solution. This Clarity BioSolutions portfolio is designed specifically to support high-throughput extractions of oligonucleotide therapeutics to deliver clean biological samples with excellent recoveries for LC-MS quantitation.

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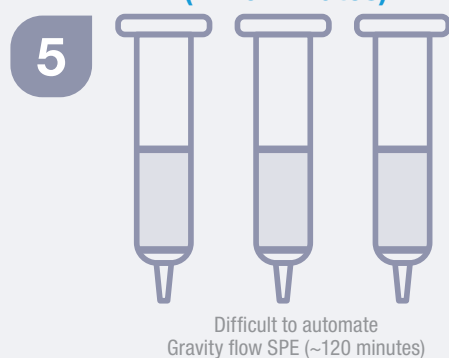
# High-Throughput Analysis

Clarity OTX™ was designed with the fast-paced requirements of the DMPK/ADME environment in mind. It is a simple, rapid, and reproducible solution that efficiently extracts oligos from biological matrices and can be easily automated to eliminate sample backlogs. By eliminating the need for liquid-liquid extractions (LLE), providing a 96-well plate format for liquid handler compatibility, and specifically targeting oligo therapeutic chemistries, Clarity OTX delivers a streamlined 15-minute extraction procedure.

## Common Extraction Procedure (~60 minutes)



## Solid Phase Extraction (SPE) (~120 minutes)



### Liquid-Liquid Extraction

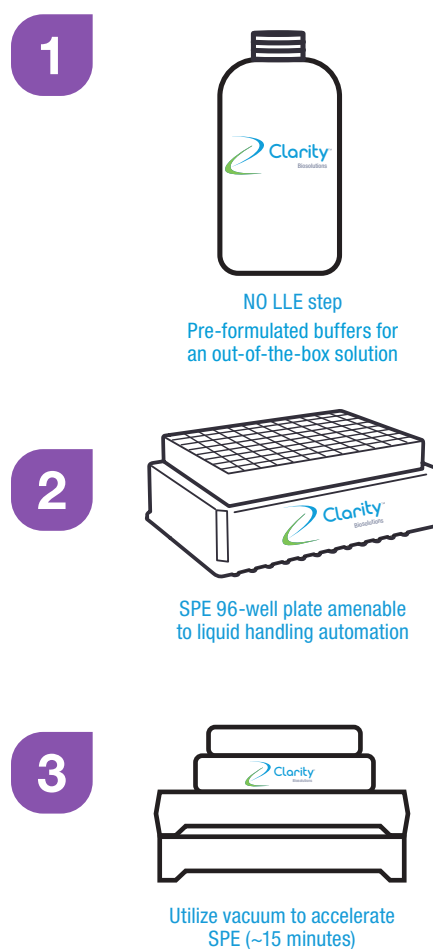
- Labor intensive
- Not automation friendly
- Toxic solvent mixture
- Time consuming (~60 minutes)

### Solid Phase Extraction

- Difficult to automate
- Gravity flow SPE (~120 minutes)

**TOTAL TIME = ~180 minutes**

## Clarity OTX Extraction Procedure (~15 minutes)



### Clarity OTX Protocol

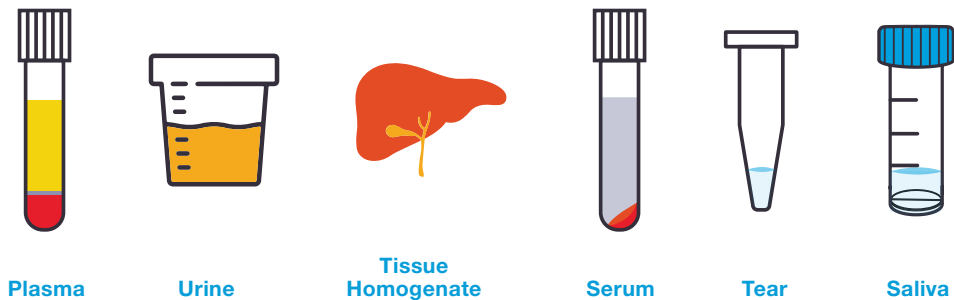
- No LLE step
- Pre-formulated buffers for out-of-box solution
- Utilize vacuum manifold to accelerate SPE (~15 minutes)
- SPE 96-well plate amenable to liquid handling automation

**TOTAL TIME = ~15 minutes**

# Simplified Extraction Workflow

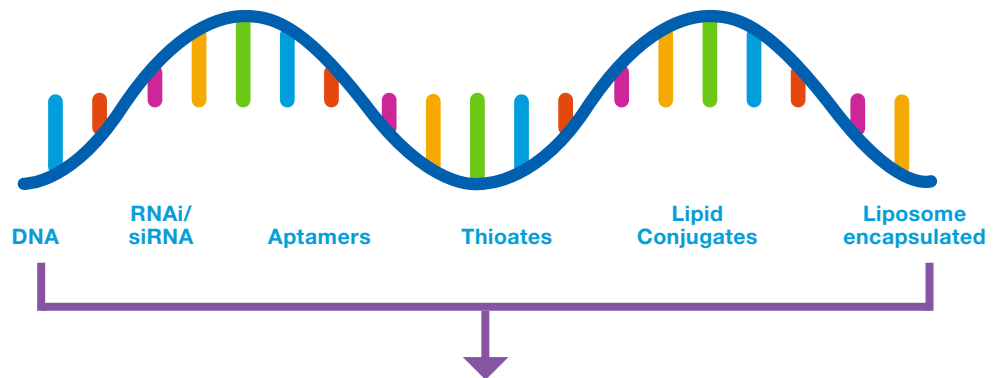
## Biological Samples

- Extract from a wide variety of oligonucleotide matrices



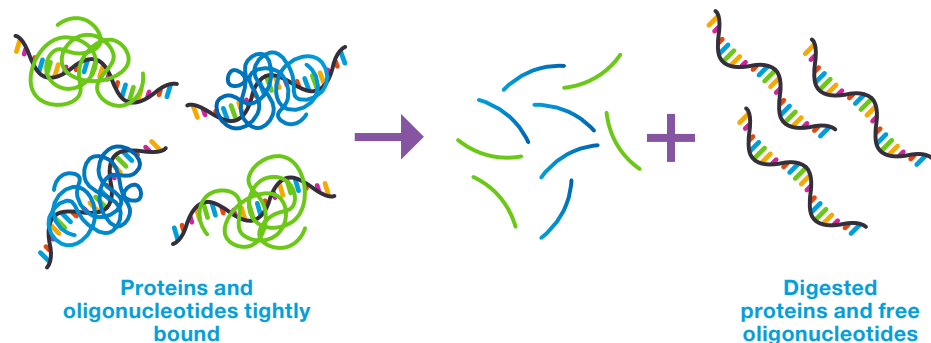
## Oligo Type

- Extract a wide variety of oligonucleotide classes



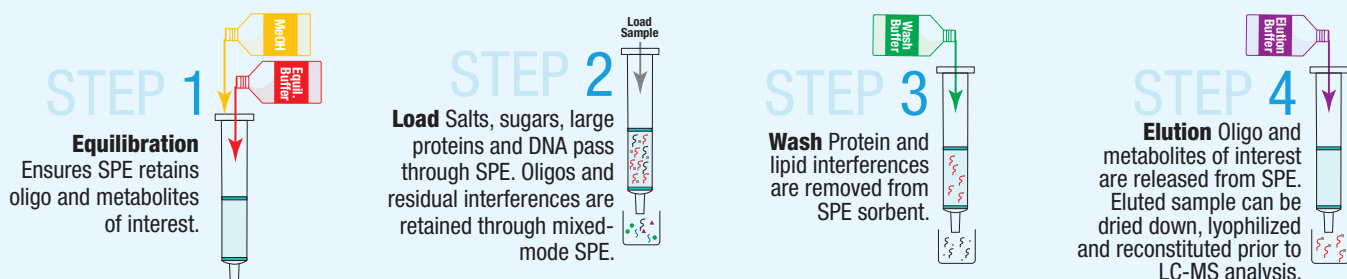
## Sample Pre-Treatment

- Lysis buffer inhibits nuclease activity
- Proteinase K digestion disrupts protein-nucleic acid reactions



## Solid Phase Extraction

- >80% extraction recoveries



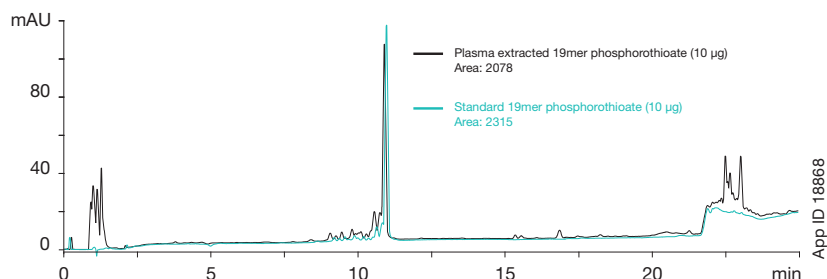
LC-MS/MS Analysis

# Dependable Recovery

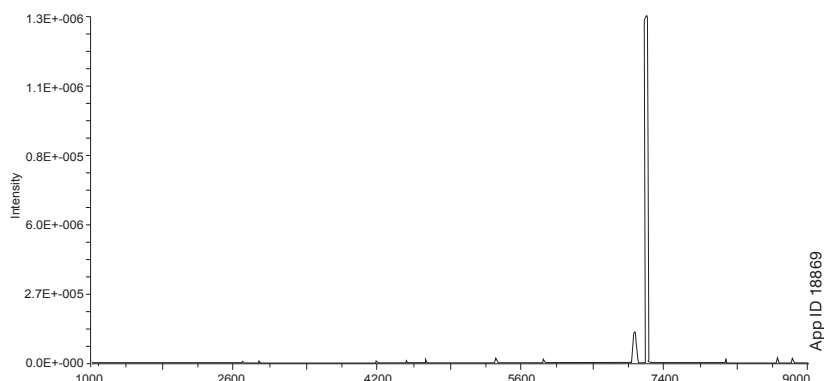
The Clarity OTX™ extraction solution is designed to effectively isolate a wide range of therapeutic oligonucleotides from fluids and tissues. It utilizes a mixed mode solid phase extraction sorbent in conjunction with carefully formulated buffers to consistently deliver greater than 80 % recoveries. In addition, the 96-well plates used for Clarity OTX have been specifically engineered to deliver consistent flow contributing to consistent recovery.

## Clarity OTX Well Plate - 100 mg/well

### UV Recovery Data of 19mer Thioate From 200 µL of Human Plasma



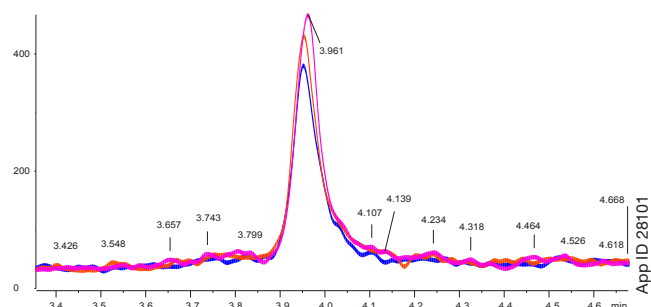
### MS Recovery Data of 19mer Thioate From 200 µL of Human Plasma



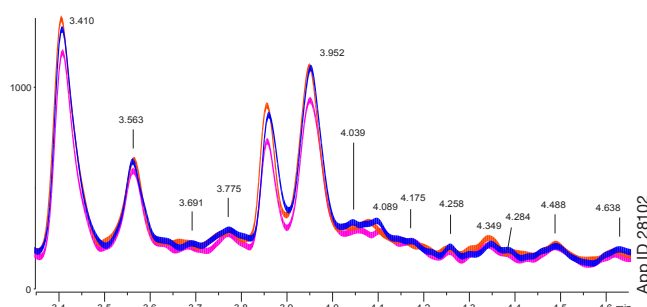
## Clarity OTX Microelution Plate - 2 mg/well

### OTX Extracts Eliminate More Biological Interferences, Ensuring High-Quality Data With Enhanced Confidence

**OTX: High recovery below 100 ng/mL analyte without noise**



**Competitor: Analyte at similar concentration below detection limit with noise**

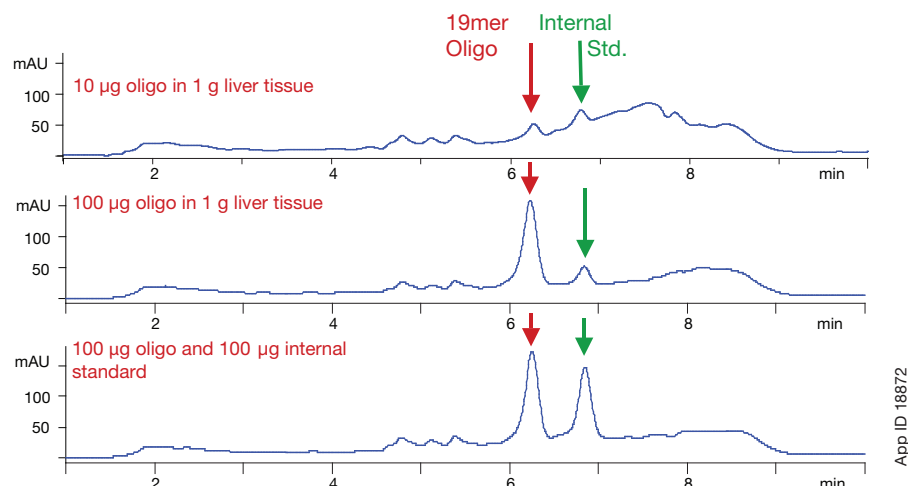




# Improved LC-MS Sensitivity

The unique extraction methodology of Clarity OTX<sup>™</sup> overcomes the challenges of low analyte recovery and MS ion suppression from interfering compounds which are associated with a lack of sensitivity. In addition, the extracted oligo nucleotide and metabolites are easily dried down (or lyophilized) so they can be reconstituted in solutions that produce sharp peaks and suppress sodium formation for high quality LC-MS data.

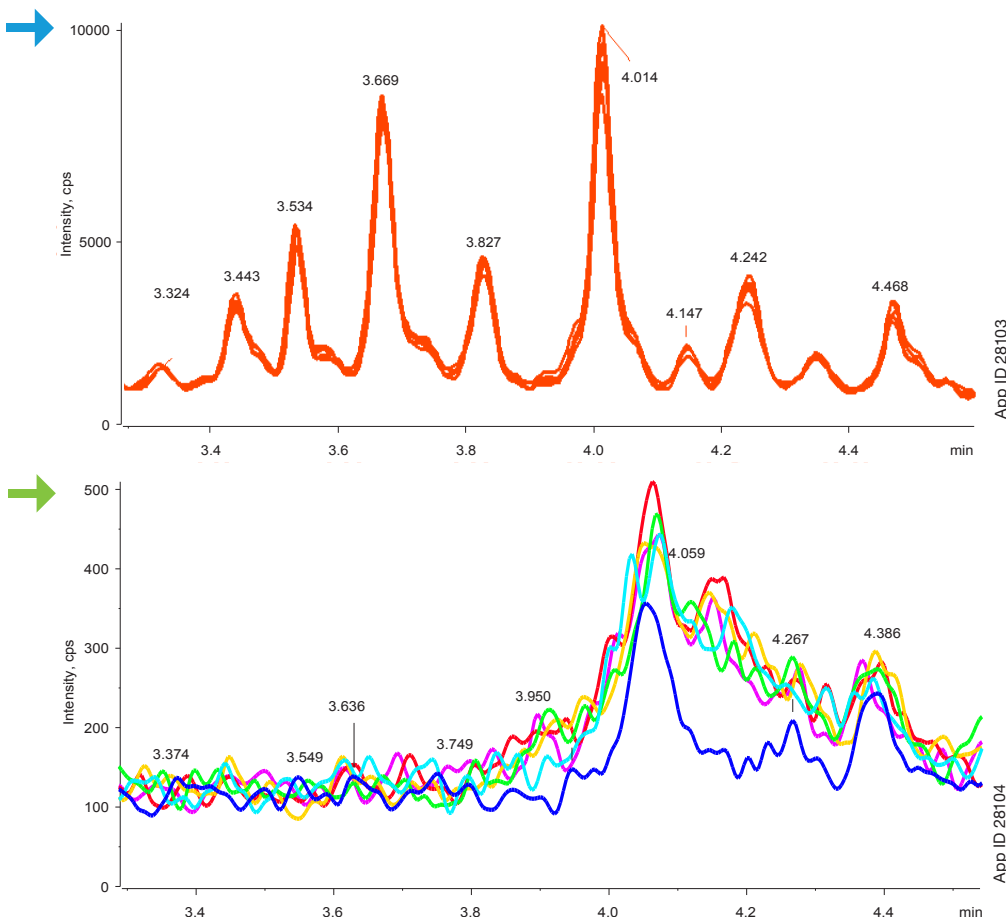
## Sensitivity Study in Liver Tissue



Clarity OTX achieves high recoveries of low-level phosphorothioate oligonucleotide with minimal co-extraction of plasma contaminants

## OTX Enhances Sensitivity Through High Selectivity Extractions

### XIC for Nusinersen



Competitor Kit -  
10<sup>4</sup> background from  
plasma or diluent

OTX - No background  
from plasma or diluent

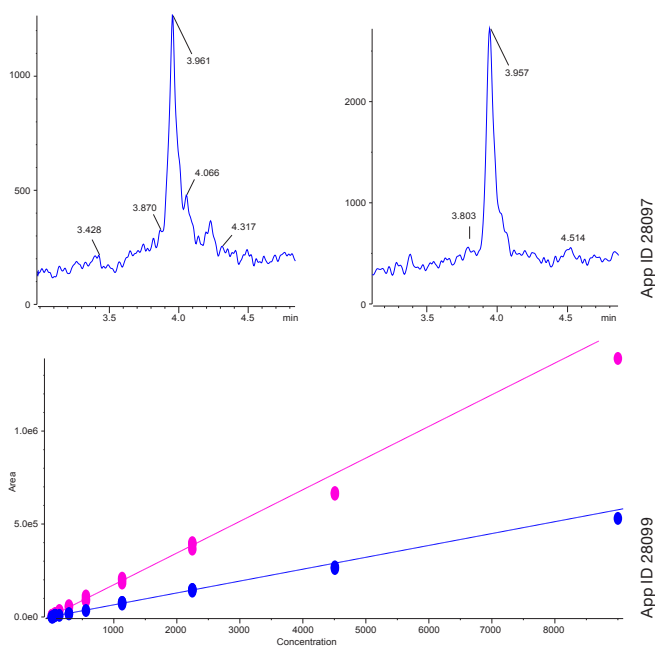
# Excellent Linearity

Significant effort was made to develop an extraction solution that would provide good linearity and reliable quantitative results. Linear dynamic ranges from 5-9000 ng/mL have been achieved when Clarity OTX™ was used to prepare biological samples combined with a signal of sensitive MS instrument to analyze them. Quantitative results like this ensure reliable bioanalytical data.

## Superior Dynamic Range & Purity - Nusinersen Extracted from Rat Plasma

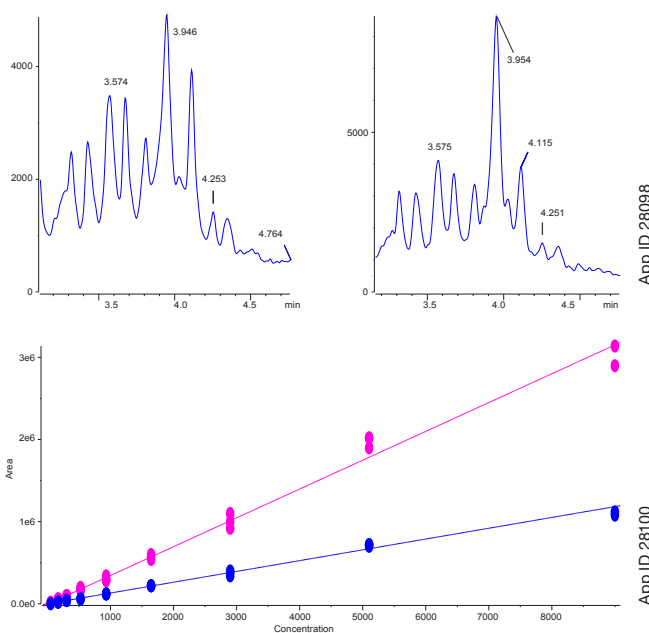
### OTX - Linearity and Purity from 35 - 9000 ng/mL

- Transition 1 and 2 at 35 ng/mL, respectively, above limit of detection

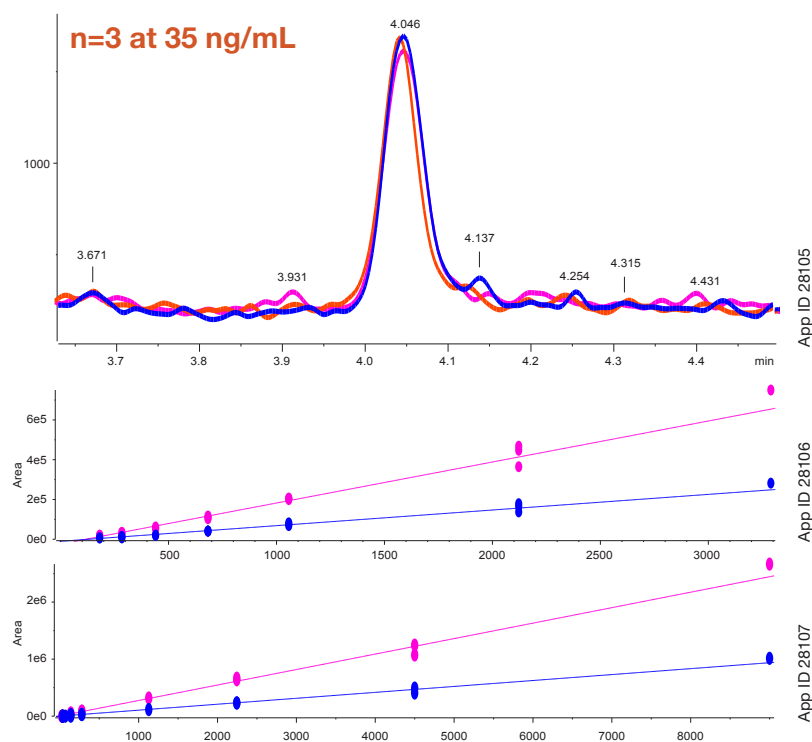


### Competitor - Linearity and Purity from 169 - 9000 ng/mL

- At 95 ng/mL, competitor kit shows signal of analyte at chemical noise level (left)
- Competitor lower limit of dynamic range >150 ng/mL (right)



## Superior Linearity & Dynamic Range - Nusinersen Extracted from Mouse Liver



OTX demonstrates linear recovery and purity from 35 - 9,000 ng/mL

- Transition 2,  $R^2=0.994$
- Transition 1,  $R^2=0.991$
- Transition 2,  $R^2=0.997$
- Transition 1,  $R^2=0.976$

# Ordering Information

The right kinds of extraction tools deliver the right LC-MS results. The chemistry and formats comprising the Clarity OTX™ product portfolio are specifically designed for the efficient and high-throughput extraction of synthetic oligonucleotide therapeutics from biological fluids for bioanalysis.

## Flexible Formats for Small & Large Sample Amounts

### Starter Kit

To test proof of concept or for low sample volumes, Clarity OTX is available as a starter kit, which includes either a 96-well plate or 50 solid phase extraction cartridges and all the buffers (lysis-loading, equilibration, wash, and elution) required for the extraction protocol.

Includes all materials needed to extract 50 samples

Designed for validating proof-of-concept before scaling up or studies with low sample volumes



### Individual Components

For labs that process large volumes of biological samples, 96-well plates, 1L quantities of Lysis-Loading buffer, and the formulations for the other three buffers are available.

Suitable for large sample studies & amenable to automation

Formulations for equilibration, wash, & elution buffers provided for liquid handling protocols

100 mg / 96-well plate & 1 L pre-formulated loading-lysis buffer available



Clarity OTX			
Part No.	Description		Unit
<a href="#">KS0-8494</a>	Clarity OTX Starter Kit- Tubes	Includes: 100 mg/3 mL cartridges (x50) Lysis-loading buffer (100 mL) Equilibration buffer (250 mL) Wash buffer (350 mL) Elution buffer (100 mL)	ea
<a href="#">KS0-9253</a>	Clarity OTX Starter Kit- 96-Well Plate	100 mg/well plate (x1) Lysis-Loading buffer (100 mL) Equilibration buffer (250 mL) Wash buffer (350 mL) Elution buffer (100 mL)	ea
<a href="#">8M-S103-4GA</a>	Clarity OTX Microelution Well Plate	2 mg/ well	1/box
<a href="#">8E-S103-CGA</a>	Clarity OTX Well Plate	25 mg/ well	1/box
<a href="#">8E-S103-EGA</a>	Clarity OTX Well Plate	100 mg/ well	1/box
<a href="#">8B-S103-EBJ</a>	Clarity OTX Cartridge	100 mg/3 mL	50/box
<a href="#">8B-S103-HCH</a>	Clarity OTX Cartridge	500 mg/6 mL	30/box
<a href="#">AL0-8579</a>	Clarity OTX Lysis-Loading Buffer V2.0	1 L	ea



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For any questions regarding Clarity OTX, chat to our technical experts:  
[www.phenomenex.com/chat](http://www.phenomenex.com/chat)



# Extraction of Oligo Therapeutics from Biological Samples

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