



CLARITY[®]
OTX[™]

Extraction of **Oligo Therapeutics**
from **Biological Samples**

For LC/MS Bioanalysis

phenomenex[®]
...breaking with tradition[™]



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 synthetic 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 latest offering in the Clarity BioSolutions portfolio is designed specifically for the high-throughput extraction of oligonucleotide therapeutics to deliver clean biological samples with good recoveries for LC/MS quantitation.



OLIGO THERAPEUTIC EXTRACTION

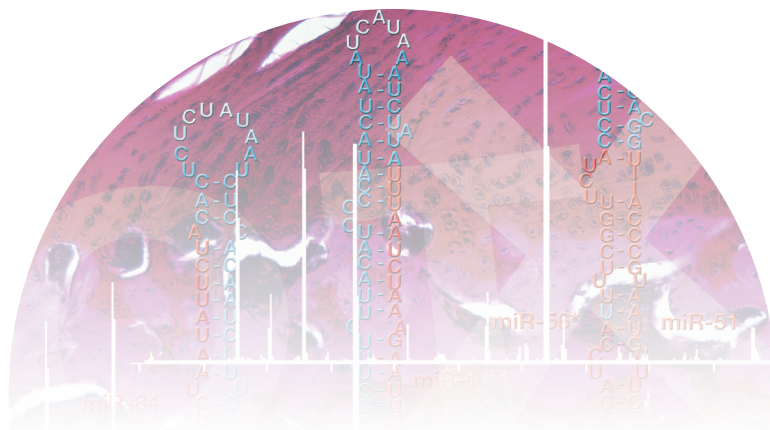


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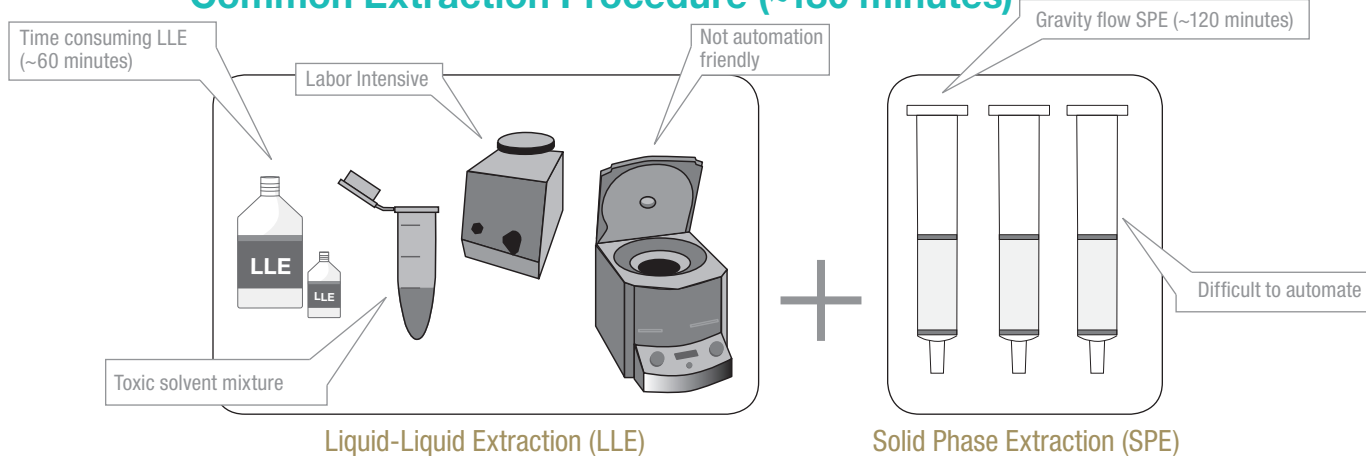
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High-Throughput

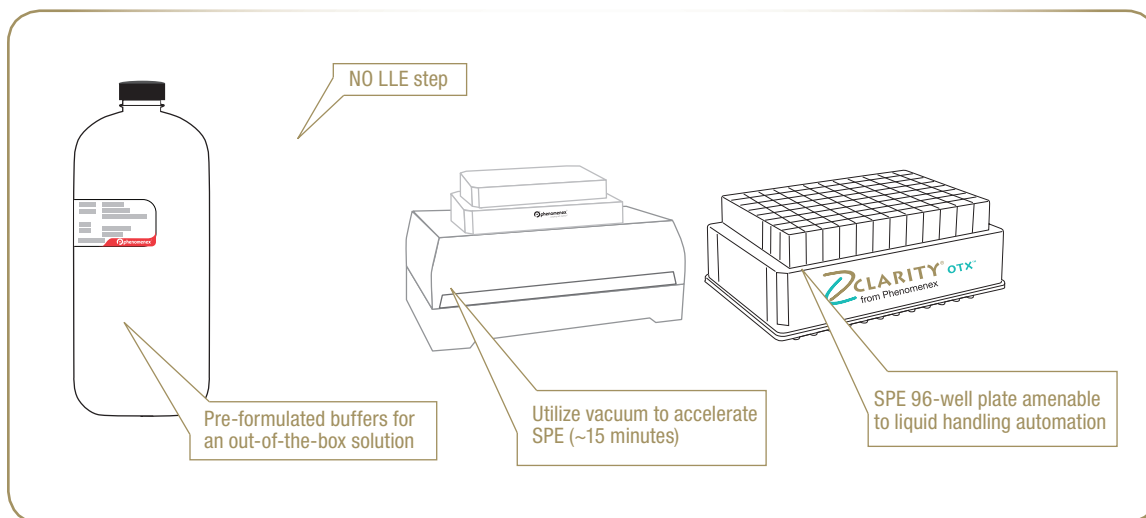
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Clarity® OTX™ was designed with the fast-paced DMPK/ADME environment in mind. It is a simple, rapid, and reproducible solution that efficiently extracts oligos from biological matrices and can be easily automated to eliminate sample backlogs and meet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

Common Extraction Procedure (~180 minutes)



Clarity OTX Extraction Procedure (~15 minutes)



Sample Compatibility

Since not all PK studies of oligonucleotide therapeutics are the same, an extraction solution needs to be compatible with different samples as well as the downstream LC/MS analysis. Clarity® OTX™ is designed to be extremely compatible in terms of formats, oligo type, sample matrix, & LC/MS preparation.

Suitable for Most Oligo Therapeutics & Samples


Oligo Types:	Sample Types:
DNA	Plasma
Aptamers	Serum
RNAi/siRNA	Urine
Thioates	Tears
Lipid-conjugates	Saliva
Liposome encapsulated	Tissue

Flexible Formats for Small & Large Sample Amounts

Starter Kit

Includes all materials needed to extract 50 samples

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




Contains 100 mg/3 mL SPE cartridges and pre-formulated buffers (load/lysis, equilibration, wash, & elution)

Individual Components

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

CONTACT US! - If you do not see the oligo or matrix type that you are working with, please contact Phenomenex. We will work with you to optimize the Clarity OTX extraction protocol for your specific sample.

Efficient Cleanup

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In just 4 steps, scientists can efficiently cleanup therapeutic oligos and their metabolites from biological samples. 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 resulting in easier quantitative bioanalysis.

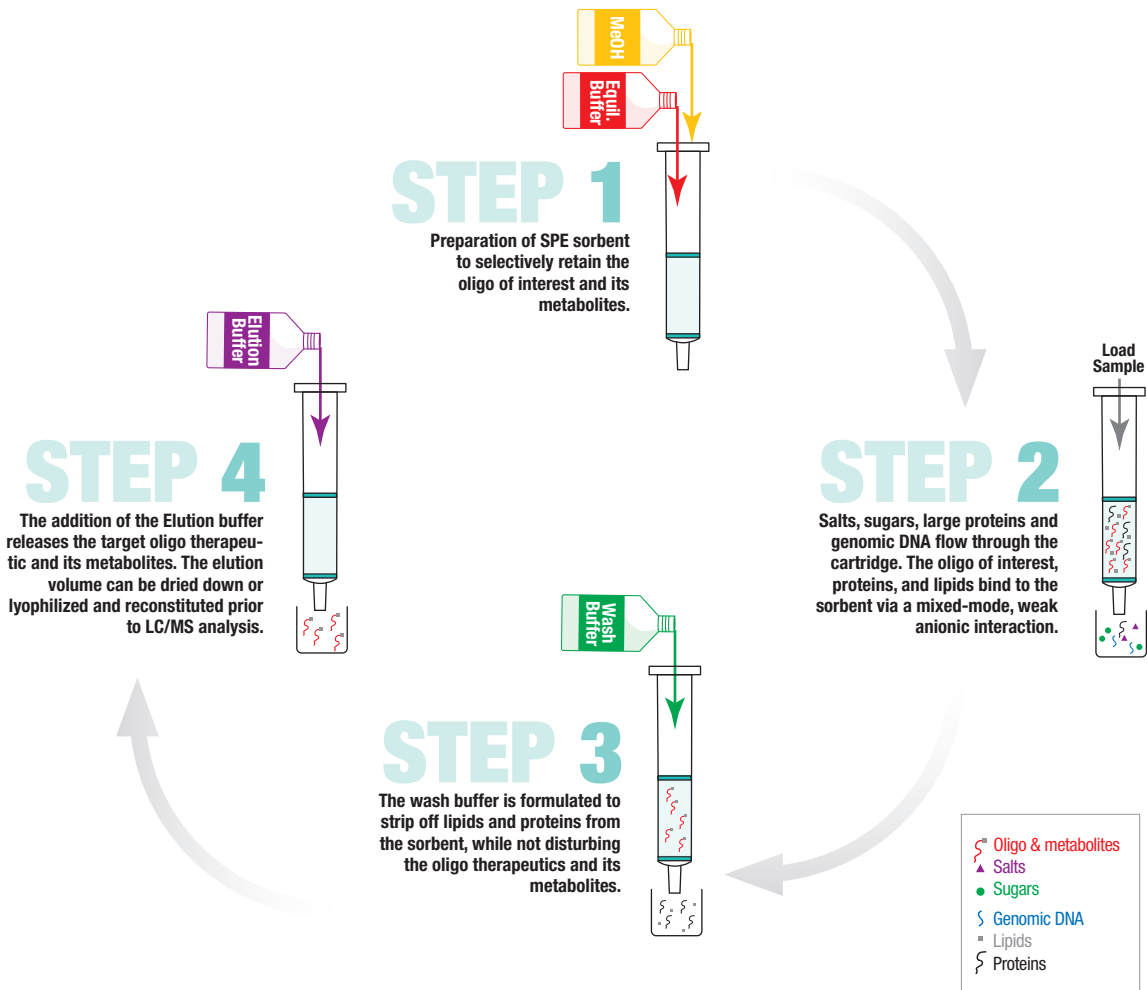
Simple 4-step Extraction Protocol

Sample Pre-Treatment

Tissue	- Proteinase K digestion or homogenize - Mix 1-to-1 with Lysis-Loading buffer
Biological Fluids	- Mix 1-to-1 with Lysis-Loading buffer



The Lysis-Loading buffer was designed with the optimal blend of chaotrope and detergent in order to facilitate the selective binding of oligo therapeutics and metabolites



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 greatly to the dependable recovery.

High Recovery from Human Plasma

Sample Preparation:

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

Extraction Protocol

Condition: 1 mL MeOH (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

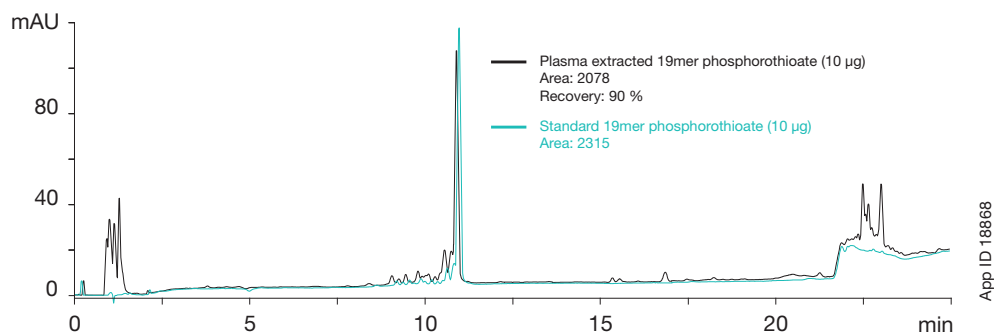
Wash: 6 mL Wash buffer (2 mL x 3) (Vacuum 3-4" Hg)

Vacuum: 10-15" Hg for ~1 minute

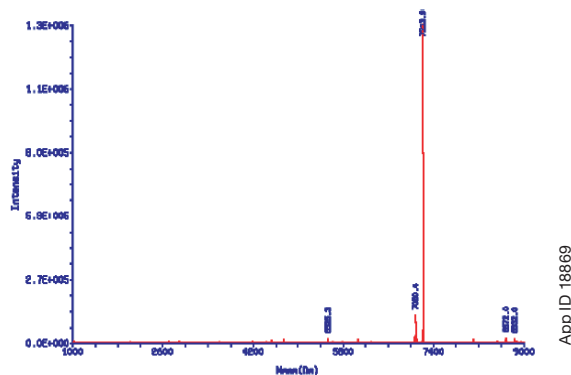
Elute: 1 mL Elution buffer (Vacuum ~3" Hg)

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 19mer 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.

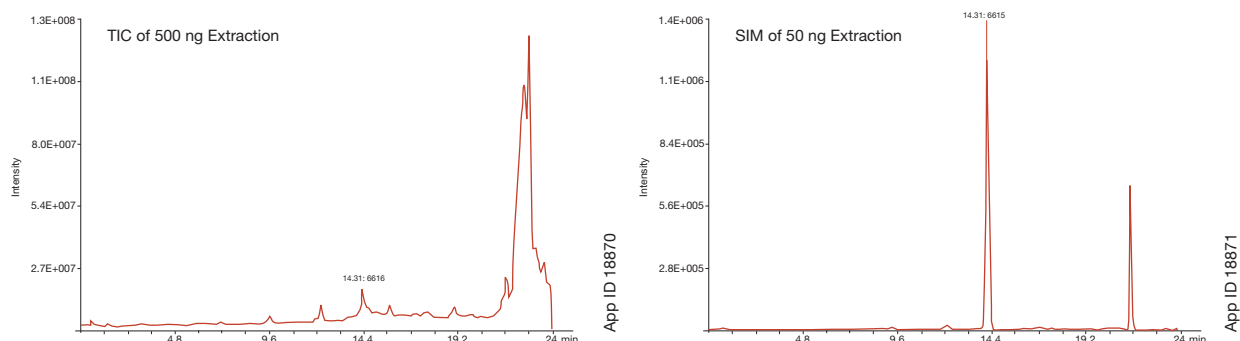
LC/MS Sensitivity

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The unique Clarity® OTX™ extraction methodology overcomes the challenges of low analyte recovery and MS interfering compounds which are associated with lack of sensitivity. In addition, the extracted oligo & metabolites are easily dried down (or lyophilized) so they can be reconstituted in solutions that produce sharp peaks & suppress sodium adduction for good LC/MS data.

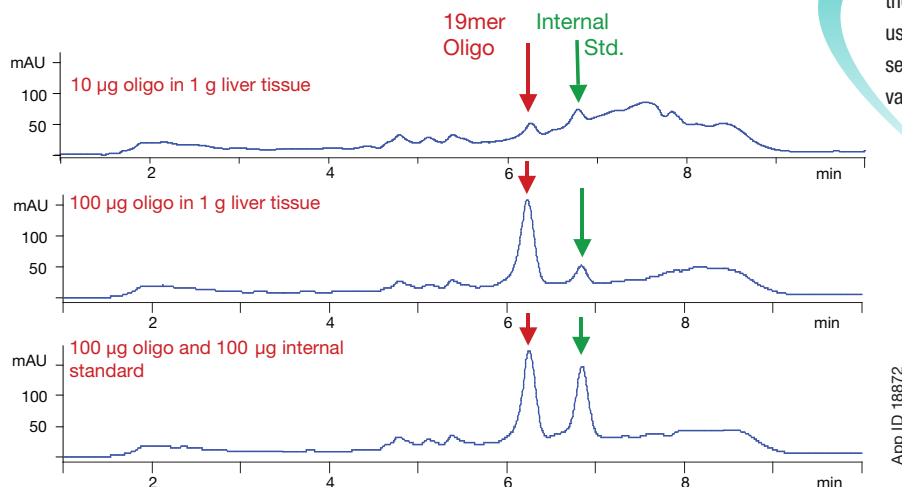
Detect Low Dosage Levels

Sensitivity Study in Plasma



Due to the typical 80% and greater recoveries of the parent oligonucleotide therapeutic and its metabolites, detection in the picomole range is possible. The TIC above illustrates detection of a dosage in this very sensitive range. Aliquots of 500 ng & 50 ng spiked into plasma were extracted using Clarity OTX to determine sensitivity limits. A TIC of the 500 ng load is shown above. The 14.3 minute peak corresponding to the 19mer P-S can be quantitated. While a peak is not observed for the TIC of the 50 ng load, using a XIC at m/z of 944 (the -7 charge state) one can still quantitate the oligonucleotide at very low levels.

Sensitivity Study in Live Tissue



The level of MS sensitivity is highly linked to the MS instrument and HPLC mobile phase used. Contact Phenomenex for questions about sensitivity levels achieved with Clarity OTX on various instruments with specific mobile phases.

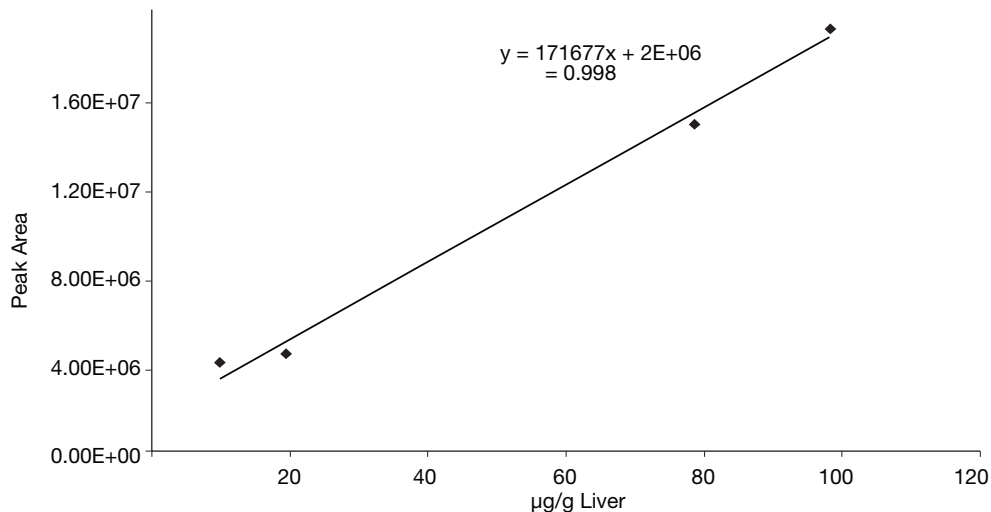
UV Chromatograms of oligonucleotide extracted from liver tissue using Clarity OTX. The 19mer extracted phosphorothioate oligonucleotide was spiked with 10 µg of an oligonucleotide internal standard before analysis. The top two chromatograms represent different levels of the incubated P-S oligo. The bottom 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.

Good Linearity

Significant effort was made to develop an extraction solution that would provide good linearity and reliable quantitative results. Dynamic ranges from 5-2000 ng/mL have been achieved when Clarity® OTX™ was used to prepare the biological samples and a sensitive MS instrument was used to analyze them. Quantitative results like this ensure reliable bioanalytical data.

Excellent Linearity at Various Concentrations

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 by the Clarity OTX methodology. Linearity for a 19 mer 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.

Development Partner

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Using expertise in separation science and synthetic RNA/DNA, Phenomenex partners with scientists investigating oligo therapeutics to develop unique solutions, give on-site technical training, provide analytical method development assistance, and offer personalized customer support.



Technical Assistance

Free method development assistance, by the experienced Clarity® BioSolutions team at Phenomenex, is available for both LC/MS methodology and extraction protocol modifications for difficult to analyze oligonucleotide candidates.

Long Lasting Relationship

Using 27 years of experience in the separation science & pharmaceutical industries, Phenomenex is committed to supporting companies and scientists whose goals are to discover, develop, & manufacture novel RNA and DNA-based therapeutics to address unmet medical needs.

Product Innovation

Together with bioanalytical scientists, Phenomenex has the capacity to research and develop new, innovative chromatography-based solutions for the advancement of oligo therapeutics down the pre-clinical & clinical path.



Educational Support

Whether by phone, internet, or in person, the Clarity BioSolutions team is available to provide training & technical assistance on the extraction, purification, & characterization of synthetic oligonucleotide therapeutics.

Dependable Customer Service

Phenomenex strives to be a reliable partner through quick responses to customer inquiries, rapid delivery of products, and complimentary technical references.



Global Availability

The Clarity® OTX™ portfolio and technical support is available globally for seamless method transfer across the globe.



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 & high-throughput extraction of synthetic oligonucleotide therapeutics from biological fluids for bioanalysis.

Part No.	Description	Unit
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 100 mg/ 96-Well Plate	1/Box
8B-S103-EBJ	Clarity OTX 100 mg/ 3 mL Tubes	50/Box
ALO-8498	Clarity OTX Lysis-Loading buffer 1 L	Ea

NOTE - Please request a Clarity OTX User's Guide for more detailed information on the protocol.

NOTE - Formulations for the equilibration, wash, and elution buffers are available to scientists using Clarity OTX 96-well plates.



Evaluate Clarity® OTX™ Biosolutions in your lab for 45 days, if you are not completely satisfied return it for a FULL REFUND.

Trademarks

Clarity is registered trademark of Phenomenex, Inc. OTX is a trademark of Phenomenex, Inc.

Disclaimer

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