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Coupling Miniaturized SPE with bioZen™ XB-C18 Nano Core-Shell Technology for the Detection of SARS-CoV-2 Nucleocapsid Protein Viral Peptides in Nasopharyngeal Samples

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Application Scientist

Roxana likes to spend time with her dog and family. She has a German Shepperd mix named Cobi who was named after the official mascot of the 1992 Summer Olympics and not the basketball player. Besides the lab, you might find Roxana in old episodes of the Ghost Whisperer, MAD TV, The L word and others as she used to be a TV extra during her school years.

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Introduction

Amplification of genetic material by PCR (Polymerase Chain Reaction) has been the gold standard for viral detection in complex sample matrices. Although PCR methods for viral detection are well established, scientists still face many challenges such as dealing with sub-optimal sensitivity due to rapid viral mutation rate, low throughput due to long experimental steps and the accessibility of specialized reagents.[1,2] LC-MS methods have emerged as either complementary or as replacements for viral detection in complex matrices. LC-MS viral detection relies on the presence of peptides that are specific to the virus and not the virus host. For an LC-MS method to achieve the sensitivity needed for the detection of viral peptides, the sample cleanup method and the choice of LC column employed are key aspects of the task. Sample cleanup enhances the signal to noise ratio, which enables trace level detection of the viral peptides. SPE (Solid Phase Extraction) is the most effective sample extraction technique to help eliminate the interferences associated with sample matrix components, while selectively retaining the peptides of interest. Similarly, achieving the desired sensitivity is also dependent on the LC column format utilized. Nano LC has been long known to enhance sensitivity and allows for the user to scale down the chromatographic separation by decreasing the column inner diameter, resulting in a decrease in flow rate, an increase in analyte concentration and thus an increase in sensitivity.[3-5] While nano LC can increase sensitivity, the combination of narrow column inner diameters and Phenomenex bioZen core-shell technology results in minimal band broadening, a reduced diffusion path, an increase in peak height and thus an increase in sensitivity compared to fully porous column technology.[6] Microelution SPE can successfully be coupled to nano LC-MS workflows. Microelution is a miniaturized SPE format which is ideal when working with small sample volumes, low analyte concentration, and the need for an increase in analyte signal.[7] Thus, this technical note provides a miniaturized LC-MS and SPE method for the reliable and accurate detection of viral peptides at 1 fmol/µL using SARS-CoV-2 nucleocapsid protein (N-protein) as a model system, using Strata-X microelution SPE for sample cleanup and a bioZen 2.6 µm Peptide XB-C18 nano column coupled to a Sciex QTRAP 6500+ mass spectrometer for maximum sensitivity.

Sample Preparation

Solid Phase Extraction

Cartridge:	Strata®-X 96-Well Plate, 2 mg/well
Part No.:	8M-S100-4GA
Condition:	200 µL Methanol
Equilibrate:	200 µL Water
Load:	400 µL diluted nasal matrix (200 µL sample with 200 µL 4% Phosphoric acid in water spiked with heavy peptide)
Wash 1:	200 µL 2% Formic acid in water
Wash 2:	200 µL Water
Elute:	2x 40 µL 1% TFA in ACN:Water (75:25)
Dry:	Under N_2 at ≤ 40° C
Reconstitute:	Resuspend dried samples in 100 µL 0.1% Formic acid in water

LC Conditions

Column:	bioZen 2.6 µm Peptide XB-C18
Dimensions:	150 x 0.075 mm
Part Number:	00F-4768-AW-21
Pressure (bar):	200
Mobile Phase:	A: 0.1% Formic Acid in Water B: 0.1% Formic Acid in Acetonitrile

Gradient:	**Time (min)**	**% B**
	0	3
	30	40
	35	70
	40	80
	41	3
	50	3

Flow Rate:	250 nL/min
Temperature:	25 °C
LC System:	NanoLC™ 425 (SCIEX)
Detection:	MS/MS
Detector:	6500 QTRAP® (SCIEX)
Injection Volume:	1 µL

MS Parameters

CUR:	30
IS:	2500
GS1:	35
GS2:	A: 0
IHT:	150
CAD:	Low
EP:	10

Materials and Methods

Nasopharyngeal swab samples were collected in PBS buffer followed by tryptic digest. Digested samples were spiked with synthetic peptides (**Table 1**) followed by serial dilution. The synthetic isotopically labeled peptides were labeled at the C-terminal of arginine (R) or lysine (K) (**Table 1**) and were spiked for quantification purposes. Microelution SPE purification was performed as shown.

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Table 1.
Multiple Reaction Monitoring (MRM) optimized transitions parameters.

ID	Q1 Mass (Da)	Q3 Mass (Da)	RT (min)	sMRM Dwell (ms)	DP	CE	CXP
GQGVPINTNSSPDDQIGYYR+3y16+2.heavy	731.015	925.433	17.1	208.871	70	31	50
GQGVPINTNSSPDDQIGYYR+3y16+2.light	727.678	920.429	17.1	208.871	70	31	50
GQGVPINTNSSPDDQIGYYR+3y9+2.light	727.678	563.762	17.1	208.871	70	31	50
NPANNAAIVLQLPQGTTLPK+3y8.heavy	690.06	849.492	20.9	224.491	80	40	40
NPANNAAIVLQLPQGTTLPK+3y8.light	687.388	841.478	20.9	224.491	80	40	40
NPANNAAIVLQLPQGTTLPK+3b9.light	687.388	865.453	20.9	224.491	80	40	40
DGIIWVATEGALNTPK+3y7.heavy	564.973	708.413	22.3	155.65	80	36	10
DGIIWVATEGALNTPK+3y7.light	687.388	865.453	20.9	224.491	80	40	40
DGIIWVATEGALNTPK. +3y5.light	562.302	572.34	22.3	155.65	80	35	18
AYNVTQAFGR +2y6. heavy	569	689.4	16.3	209.429	50	24	12
AYNVTQAFGR +2y6. light	564	679.4	16	250	50	24	12
AYNVTQAFGR +2y6. light	564	892.6	16.3	209.429	50	26	15
QQTVTLLPAADLDDFSK+2y10.heavy	935.6	1086.8	22	129.08	80	40	20
QQTVTLLPAADLDDFSK+2y10.light	931.6	1078.5	22	129.08	80	40	20
QQTVTLLPAADLDDFSK+2y11.light	931.6	1191.6	22	129.08	70	37	20

Data Processing

Sciex OS version (2.0.0) and Skyline (version 20.1.0.31) software were used for quantitative and qualitative purposes, respectively.

Results and Discussion

Trypsin digested Nasopharyngeal swab samples (containing five spiked synthetic SARS-CoV-2 Nucleocapsid Protein specific peptides as in **Table 1**) were spiked with their peptide counterpart labeled at the C-terminal of arginine (R) or lysine (K) (**Table 1**) to allow for quantification using the peak area ratio of the heavy labeled peptide to the unlabeled (light) peptide. The chemistry of the bioZen™ 2.6 µm Peptide XB-C18 allowed for adequate retention of the viral peptides, providing great resolution among the five analytes. Furthermore, the nano dimensionality of the column together with miniaturized SPE resulted in an ultrapure clean extract with minimum background noise to yield a significantly lower level of quantitation such as 1 fmol/µL.

In order to determine the dynamic range of the assay, a calibration curve constructed for the extracted samples over a concentration range of 1 to 200 fmol/µL was analyzed. All five peptides showed linearity across the entire concentration range (**Figure 3**). This demonstrates that each peptide can be reliably quantitated down to a concentration of at least 1 fmol/µL. The signal to noise (S/N) ratios were calculated at the lowest concentration tested at 1 fmol/µL and were based upon the relative response against a blank matrix extract. S/N ratios ranging 112 to 1293 (**Table 2**) were observed. QC samples were tested for replicate extraction at 3 different levels of concentration (**Table 3**). Accuracy and CV were calculated and ranged between 93-118% and <7%, respectively, which are within acceptable industry standards.

Figure 1.
Chromatographic separation of the 5 SARS-CoV-2 synthetic peptides.

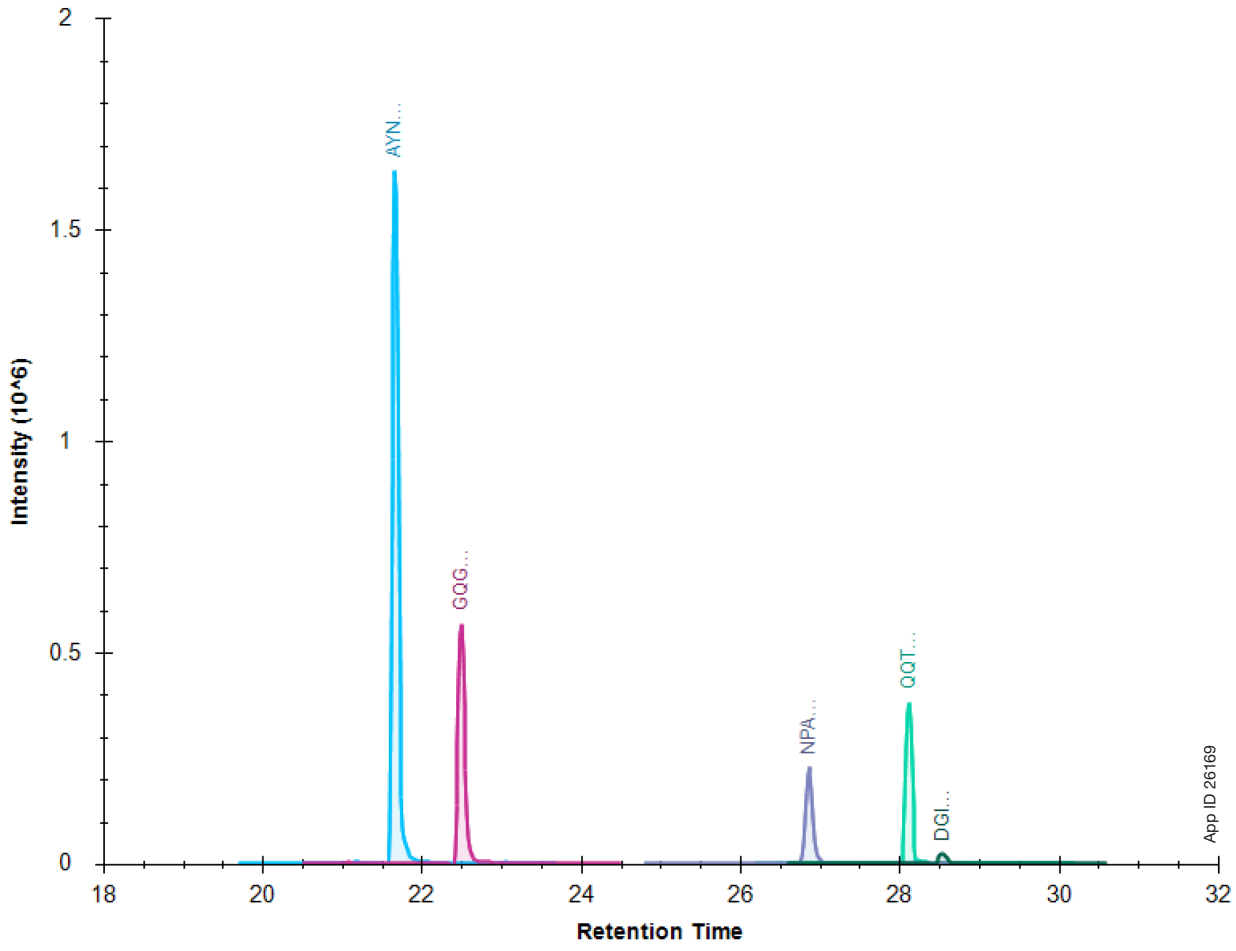

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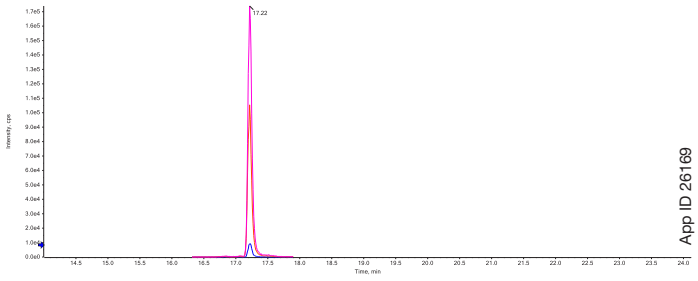
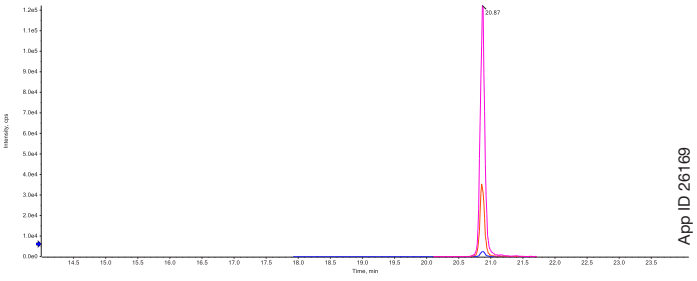
Figure 2.
Extracted ion chromatograms of the individual MRM transitions.

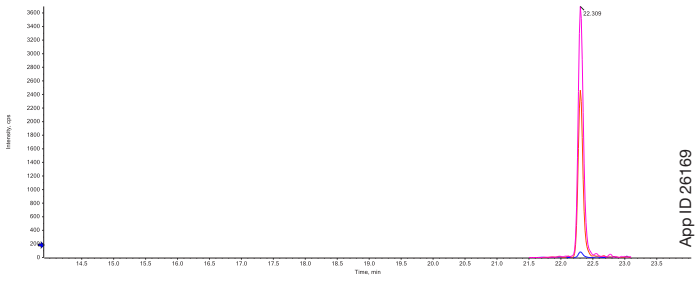
GQGVPINTNSSPDDQIGYYR



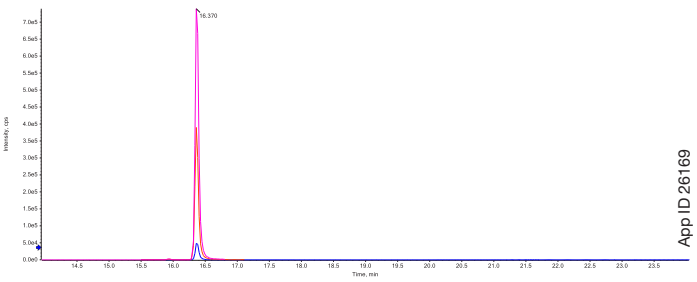
NPANNAAIVLQLPQGTTLPK



DGIIWVATEGALNTPK



AYNVTQAFGR



QQTVTLLPAADLDDFSK

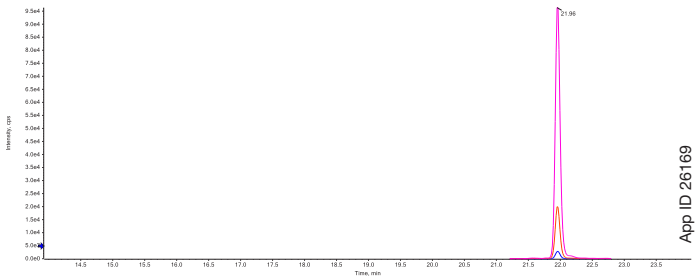
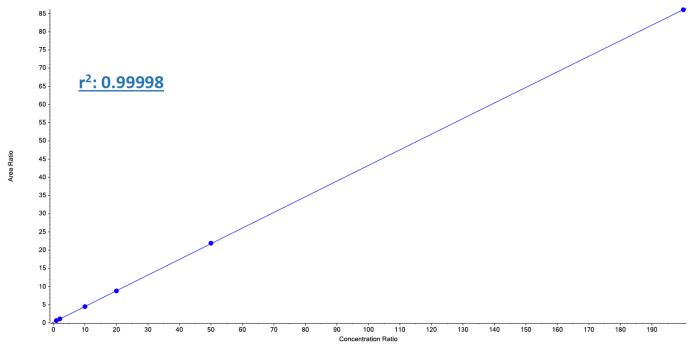


Figure 3.
Calibration curves for each peptide.

QGVPINTNSSPDDQIGYYR



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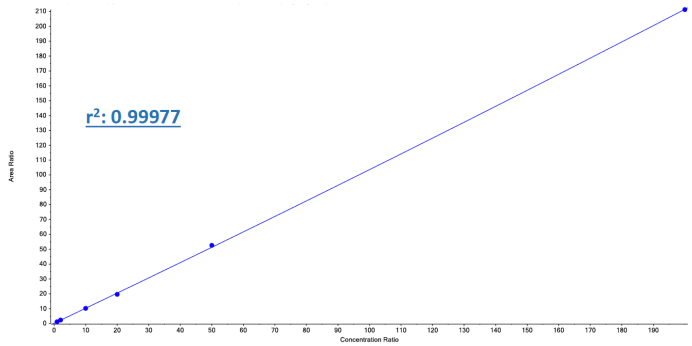

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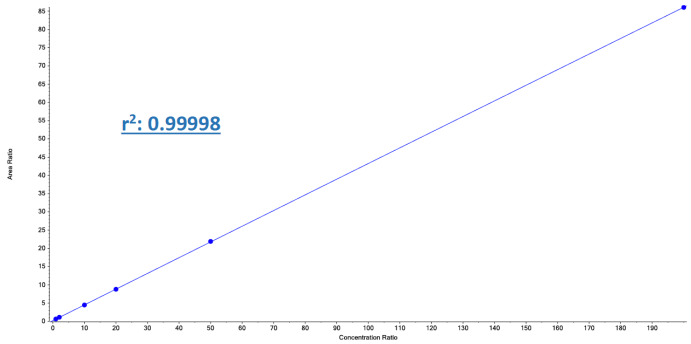

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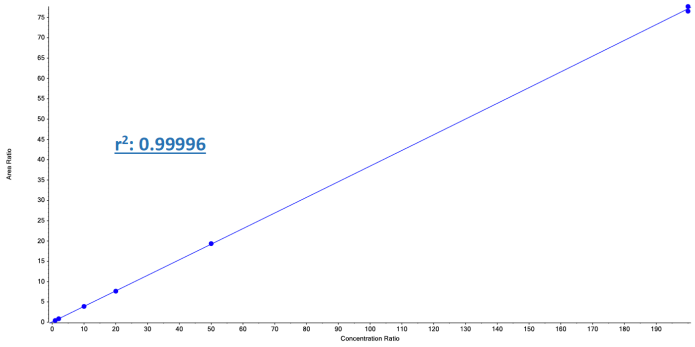

QQTVTLLPAADLDDFSK

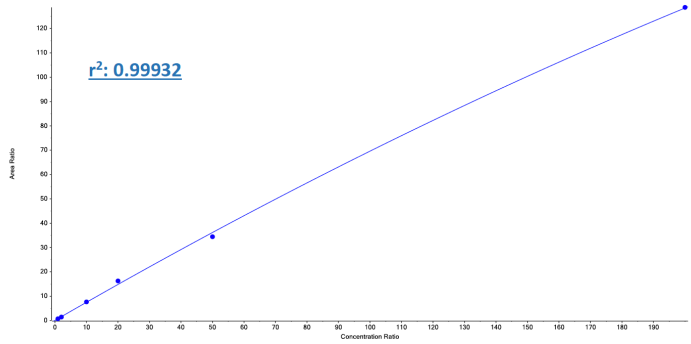

Table 2.
Signal to noise ratio for individual peptides at 1 fmol/µL.

Peptide ID	S/N
AYNVTQAFGR+2y6. light	408
DGIIWVATEGALNTPK+3y7.light	460
QQTVTLLPAADLDDFSK+2y10.light	1293
NPANNAAIVLQLPQGTTLPK+3y8.light	323
GQGVPINTNSSPDDQIGYYR+3y16+2.light	112

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Table 3.
Precision and accuracy for QC samples of each peptide.

AYNVTQAFGR

Expected Conc. (fmol/µL)	Sample	Replicates (N)	% CV	Accuracy
5	QC1-1	4	3.7	96
40	QC1-2	4	1.2	102
100	QC1-3	4	2.1	96

DGIIWVATEGALNTPK

Expected Conc. (fmol/µL)	Sample	Replicates (N)	% CV	Accuracy
5	QC1-1	4	4	118
40	QC1-2	4	3.9	115
100	QC1-3	4	1.2	113

GQGVPINTNSSPDDQIGYYR

Expected Conc. (fmol/µL)	Sample	Replicates (N)	% CV	Accuracy
5	QC1-1	4	0.6	110
40	QC1-2	4	6	107
100	QC1-3	4	4	93

QQTVTLLPAADLDDFSK

Expected Conc. (fmol/µL)	Sample	Replicates (N)	% CV	Accuracy
5	QC1-1	4	7	104
40	QC1-2	4	5	102
100	QC1-3	4	5	110

Conclusion

The bioZen™ Peptide XB-C18 nano core-shell column chemistry retains the SARS-CoV-2 viral peptides adequately, providing great resolution between these analytes. By employing Strata®-X microelution SPE, samples were extracted more efficiently and in a much shorter amount of time than conventional SPE. This selective extraction and sensitive chromatography yielded signal/noise ratios ≥112 at 1 fmol/µL. The selectivity at the lowest concentration tested of 1 fmol/µL, suggests that the detection range for these peptides can easily be expanded to a much lower concentration range to obtain the lowest level of quantitation. The dynamic range of microelution SPE tested demonstrates an acceptable linearity with r2 values ≥0.995 for all peptides. Also, industry standards call for an accuracy of 80-120% and a precision of <20% at LLOQ and <15% across the calibration curve, which were well met by the presented methodology. The data presented here demonstrates that using microelution SPE in conjunction with bioZen 2.6 µm Peptide XB-C18 nano core-shell columns, peptides from the COVID-19 causing SARS-CoV-2 virus can be detected with great resolution and sensitivity.

References

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Ordering Information

bioZen™ Nano LC Columns with Integrated SecurityLINK™ Fitting

Phases	150 x 0.075	250 x 0.075	500 x 0.075
bioZen 3 µm Peptide PS-C18	00F-4771-AW-21	00G-4771-AW-21	–
bioZen 2.6 µm Peptide XB-C18	00F-4768-AW-21	00G-4768-AW-21	–
bioZen 3 µm Peptide Polar-C18	00F-4782-AW-21	00G-4782-AW-21	–
bioZen 5 µm Peptide XB-C18	–	–	00J-4792-AW-11

bioZen Nano LC Columns with Open Fused-Silica Inlet/Outlet Fitting

Phases	150 x 0.075	250 x 0.075	500 x 0.075
bioZen 3 µm Peptide PS-C18	00F-4771-AW-11	00G-4771-AW-11	–
bioZen 2.6 µm Peptide XB-C18	00F-4768-AW-11	00G-4768-AW-11	–
bioZen 3 µm Peptide Polar-C18	00F-4782-AW-11	00G-4782-AW-11	–
bioZen 5 µm Peptide XB-C18	–	–	00J-4792-AW-21

Trap Columns

Trap Columns		
	10 x 0.075 mm	Unit
RP-1	05N-4252-AW	3/pk
RP-2	05N-4754-AW	3/pk

Trap Fittings

Trap Fittings		
Part No.	Description	Unit
AQ0-7602	PEEKLok™ fittings with 6-40 thread for 1/32" OD tubing (2 x fittings, 6 x ferrules and 1 x tightening tool)	ea
AQ0-7603	PEEKLok fittings with 6-32 thread for 1/32" OD tubing (2 x fittings, 6 x ferrules and 1 x tightening tool)	ea
AQ0-7600	PEEKLok fittings with 10-32 thread for 1/32" OD tubing with low profile hex head (2 x fittings, 6 x ferrules and 1 x wrench)	ea

Strata®-X Microelution 96-Well Plates

96-Well Plates (ea)	
Phase	2 mg
Strata X-AW	8M-S038-4GA
Strata X-A	8M-S123-4GA
Strata-X	8M-S100-4GA
Strata-X-C	8M-S029-4GA
Strata-X-CW	8M-S035-4GA

Strata-X Microelution Method Development 96-Well Plates

Part No.	Description	Unit
KS0-9528	**Strata-X Peptide Screening** Strata-X-CW 2 mg/well (6 rows) Strata-X-A 2 mg/well (6 rows)	ea
KS0-9529	**Strata-X Method Development** Strata-X-C 2 mg/well (3 rows) Strata-X-AW 2 mg/well (3 rows) Strata-X-CW 2 mg/well (3 rows) Strata-X-A 2 mg/well (3 rows)	ea

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