

Proteomics

Chromatography columns and consumables

# Proteomics workflow solutions

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# Complete proteomics workflow solutions

For researchers pursuing the next scientific breakthrough the correct workflow selection - from when the sample enters the lab until the sample is analyzed - can be imperative for their results and thesis.

We strive to create a better understanding of how to compose an optimal workflow allowing scientists to go beyond by resolving and understanding more resulting in breaking new grounds in proteomics. The workflows in this brochure offer a sampling of available solutions from Thermo Fisher Scientific.

## Bottom-up: Peptide mapping

- Thermo Scientific™ SMART Digest™ kits
- Thermo Scientific™ EASY-Spray™ columns
- Thermo Scientific™ PepMap™ Neo columns
- Thermo Scientific™ Acclaim™ PepMap™ Neo columns
- Thermo Scientific™ μPAC™ HPLC columns
- Thermo Scientific™ SureSTART™ vials and caps

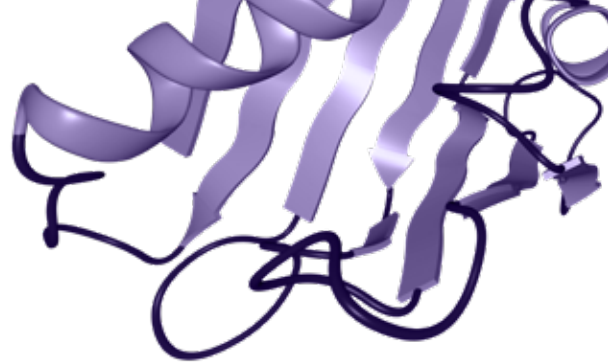
## Middle-down

- Thermo Scientific™ MAbPac™ (U)HPLC columns
- Thermo Scientific™ Double nanoViper™ PepMap™ Neo columns
- SureSTART vials and caps
- Thermo Scientific™ WebSeal™ well plates and mats

## Top-down: Intact mass and aggregates

- MAbPac (U)HPLC columns
- SureSTART vials and caps





# Contents

## Proteomics workflow solutions

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●	Bottom-up workflow: EASY-Spray columns	4
●	Bottom-up workflow: $\mu$ PAC columns	5
●	Middle-down workflow: MAbPac RP columns	6
●	Top-down workflow: MAbPac RP columns	7

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## Featured products

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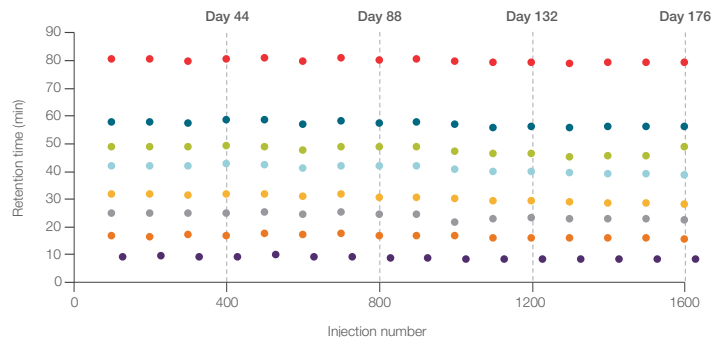
●	PepMap Neo columns	8
●	$\mu$ PAC HPLC columns	9
●	Capillary columns	10
●	Trapping columns	11
●	Emitters	12
●	Digest solutions	13
●	Vials and caps	14
●	Well plates and sealing tapes	15

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# Bottom-up workflow: EASY-Spray columns

## Proteomics research high resolution peptide mapping

Bottom-up proteomics liquid chromatography mass spectrometry (LC-MS) analyses using long separation columns and nano-flow rates have long been established as the mainstay of proteomics research. However, general concerns regarding robustness and reproducibility of the methodology combined with the technical challenges considered inherent to nanoLC have prevented its widespread adoption into “routine” applications, despite the potential benefits including increased sensitivity and reduced solvent consumption. Thermo Scientific™ Vanquish™ Neo UHPLC system and the Thermo Scientific™ EASY-Spray™ PepMap™ Neo UHPLC columns deliver levels of chromatographic robustness and reproducibility required for longterm trouble-free nanoLC operation under maximum performance and pressure conditions. A total of 1,600 injection cycles of BSA protein digest were performed over 176 days of continuous operation using a single EASY-Spray column.

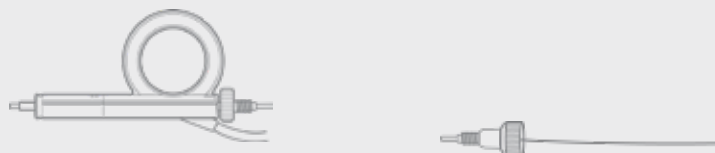


**Figure 1. The retention time for 8 selected peptides from 1,600 injections of BSA protein digest over 176 days (approximately 6 months)**



### Workflow solution

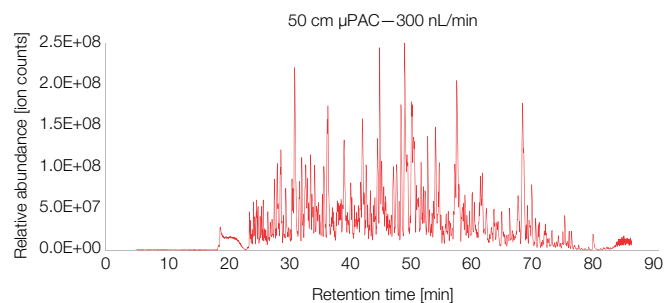
Thermo Scientific instruments	Cat. no.
Vanquish Neo UHPLC system	NC2207296
Thermo Scientific™ Orbitrap™ Exploris 480 mass spectrometer	—
Thermo Scientific™ EASY-Spray™ ion source	NC2061618
Thermo Scientific columns and trap columns	Cat. no.
EASY-Spray PepMap Neo columns	03255023
Thermo Scientific™ Acclaim™ PepMap™ 100 C18 trap column	164946
Thermo Scientific vials and caps	Cat. no.
Thermo Scientific™ SureSTART™ 0.2 mL TPX screw vials with glass insert	03452575
Thermo Scientific™ SureSTART™ 9 mm screw caps	03452313
This workflow includes the newest recommended products	



# Bottom-up workflow: μPAC columns

## Proteomics core routine high resolution, high throughput peptide mapping

When aiming for comprehensive proteome analysis with deep coverage, relatively long columns such as the Thermo Scientific™ μPAC™ HPLC columns (200 cm) or Thermo Scientific EASY-Spray columns (75 cm), are typically operated with long and shallow solvent gradients, delivering the highest chromatographic performance. This is indeed a good strategy if very complex samples need to be analyzed and as much information as possible needs to be retrieved from these samples. However, daily routine proteome analysis often deals with less complex samples or could demand increased sample throughput, making total analysis times above 120 min undesirable or even impossible. Using μPAC HPLC column (50 cm), proteomics analysis can be performed with runtimes of 20-90 minutes and outstanding peak capacities and resolution. The μPAC HPLC columns offer the highest resolution for peptide separations with increased column lifetimes and column-to-column reproducibility.

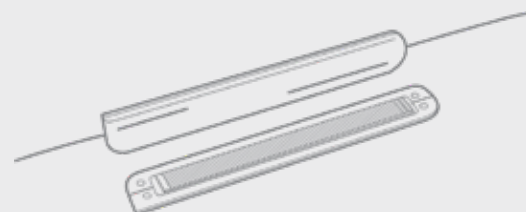


**Figure 2. Basepeak MS chromatograms of 500 ng HeLa cell digest for 60 min gradients on 50 cm μPAC HPLC column operated at 300 nL/min**



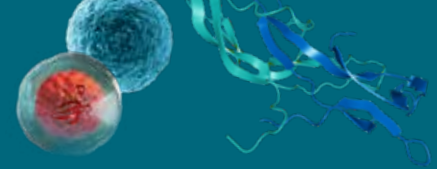
### Workflow solution

Thermo Scientific instruments	Cat. no.
Vanquish Neo UHPLC system	NC2207296
Orbitrap Exploris 480 mass spectrometer	—
EASY-Spray ion source	NC2061618
Thermo Scientific emitter	Cat. no.
Thermo Scientific™ μPAC™ compatible EASY-Spray emitter	—
Thermo Scientific columns and trap columns	Cat. no.
μPAC HPLC column (50 cm)	NC2162914
Thermo Scientific vials and caps	Cat. no.
Thermo Scientific™ SureSTART™ 0.3 mL screw vial	03452247
SureSTART 9 mm screw cap	03452313
This workflow includes the newest recommended products	





# Middle-down workflow: MAbPac RP columns



## Proteomics research of post-translational modifications

The Thermo Scientific™ MAbPac™ RP capillary column is a useful tool for analyzing tryptic peptides from protein therapeutics or monoclonal antibodies. The column's moderately hydrophobic resin is demonstrated to be particularly useful when uncovering an important protein attribute such as the glycosylation site occupancy.

The short and reproducible separations, as well as the high sensitivity attributable to capillary flow highlight the applicability of this column in the clone selection and early optimization phases of biopharmaceutical development.

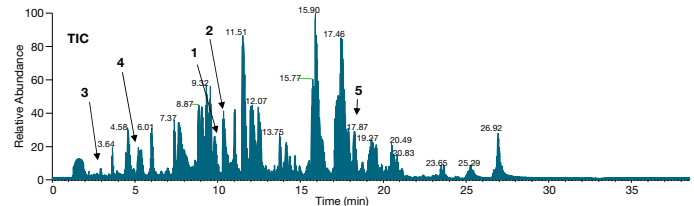


Figure 3. Labeling with  $H_2^{18}O$  discriminates masses and helps identify glycosylation and deamidation sites



### Workflow solution

Thermo Scientific instruments	Cat. no.
Vanquish Neo UHPLC system	NC2207296
Orbitrap Exploris 480 mass spectrometer	—
EASY-Spray ion source	NC2061618
Thermo Scientific™ AccelerOme™ automated sample preparation platform	—
Thermo Scientific columns and trap column	Cat. no.
MAbPac RP capillary column	03254130
Thermo Scientific emitter	Cat. no.
Thermo Scientific™ EASY-Spray™ capillary emitter, without transfer line	03254133
Thermo Scientific digest solutions	Cat. no.
Thermo Scientific™ SMART Digest™ Proteinase K kit	03252216
Thermo Scientific plates and sealing tapes	Cat. no.
Thermo Scientific™ WebSeal™ Plate+ well plate	03253924
Thermo Scientific™ WebSeal™ sealing tape	03252444
This workflow includes the newest recommended products	

# Top-down workflow: MAbPac RP columns

## Intact protein determination

The MAbPac RP capillary column and its unique polymer construction – very wide pores to allow the separation of large proteins – are a great alternative column to silica C4 resin technology. The silica C4 resin is often prone to carry-over contamination that can reach over 20% for a monoclonal antibody of 150,00Da.

With the minor carry-over contamination and a very sharp peak shape, the MAbPac RP capillary column's polymer construction allows washing with harsh conditions such as solvents, acids, and base making this column very robust and long-lasting.

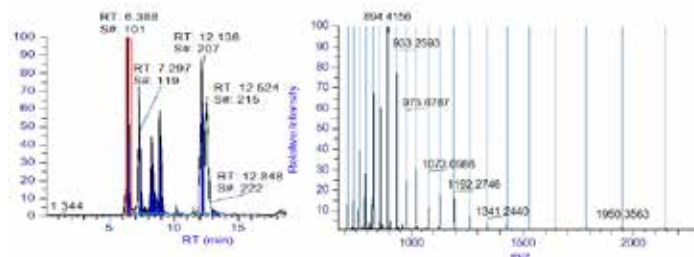


Figure 4. Deconvoluted experimental: 21429.787, theoretical monoisotopic: 21429.759



Vanquish Neo UHPLC system



Orbitrap Exploris 480 mass spectrometer



EASY-Spray ion source



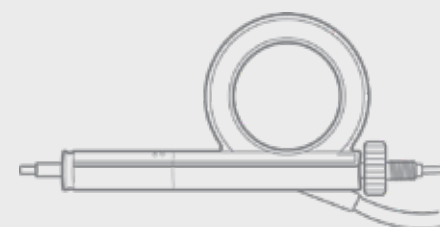
EASY-Spray column



SureSTART vial and cap

### Workflow solution

Thermo Scientific instruments	Cat. no.
Vanquish Neo UHPLC system	NC2207296
Orbitrap Exploris 480 mass spectrometer	—
EASY-Spray ion source	NC2061618
Thermo Scientific column	Cat. no.
EASY-Spray column	03254131
Thermo Scientific vials and caps	Cat. no.
Thermo Scientific™ SureSTART™ 1.5 mL screw vial	03452550
SureSTART 9 mm screw cap	03452313
This workflow includes the newest recommended products	

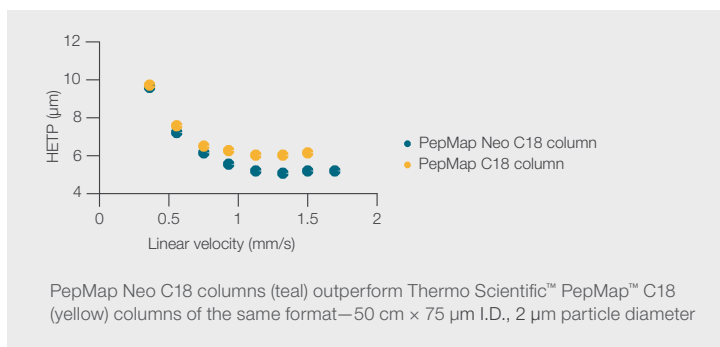


# PepMap Neo columns



## EASY-Spray and Double nanoViper PepMap Neo UHPLC columns for high resolution peptide mapping

Separate challenging peptide mapping samples using EASY-Spray PepMap Neo UHPLC columns. These columns feature easy connectivity, high reproducibility, and excellent separations. Our PepMap Neo columns provide 1,500 bar pressure capability, improved column-to-column consistency, and increased efficiency. The column media is manufactured and selected to exact standards and packed at high pressure, resulting in enhanced peak symmetry, resolution, and column-to-column reproducibility that allows you to obtain greater sample coverage and sample insights. The click-and-spray format of EASY-Spray columns enables perfect connections to our mass spectrometers every time. The same column dimensions exist in Double nanoViper PepMap Neo UHPLC columns.



Video: EASY-Spray 150 mm LC columns



### EASY-Spray PepMap Neo UHPLC columns

Diameter	Length (metric)	Particle size	Stationary phase	For use with	Cat. no.
75 µm	150 mm	2 µm	C18	Bottom-up proteomics, LC-MS	03255021
	500 mm				03255022
	750 mm				03255023

### Double nanoViper PepMap Neo UHPLC columns

Diameter	Length (metric)	Particle size	Stationary phase	For use with	Cat. no.
75 µm	150 mm	2 µm	C18	Peptide mapping, LC-MS	03255024
	500 mm				03255025
	750 mm				03255026



## μPAC HPLC columns for high throughput and high resolution peptide mapping

Learn more about your proteome, metabolome, and lipidome samples with μPAC HPLC columns. These specialized columns provide the ultra-high resolution needed to extract the maximum amount of information from highly complex samples with low-flow chromatography.

- **Sample coverage:** μPAC HPLC columns provide comprehensive coverage that is ideal for proteomics, workflows, running both data-dependent (DDA) and data-independent acquisition (DIA) MS experiments. When used for single shot proteome analysis, nearly 10,000 identifications can be achieved.
- **Column-to-column reproducibility:** Each column is manufactured using the same lithographic mask, providing consistency and performance from column-to-column.
- **Flow rate flexibility:** μPAC HPLC columns can be operated at moderate LC pump pressures up to 350 bar over a wide range of flow rates (100 – 1,000 nL/min).



### μPAC HPLC columns

Bed length	Bed width	Pillar length	Stationary phase	Temperature	Cat. no.
50 cm	315 μm	18 μm	C18	60° C	NC2162914

\* For capillary flow analysis

# Capillary columns

Top-down  
MAbPac RP  
workflow

## EASY-Spray HPLC column

Ensure robust nano and capillary flow into your LC-MS using EASY-Spray HPLC columns. The integrated column/emitter design eliminates dead volume and is temperature-controlled for maximum reliability and performance. Rigorously tested to ensure maximum quality, these columns deliver maximum simplicity and ease-of-use. The capillary flow HPLC columns provide sensitive protein, peptide, and monoclonal antibody (mAb) separation. They give proteomics researchers more: throughput, sensitivity, separation power, and ease-of-use.



## MAbPac reversed-phase (RP) HPLC capillary column

Use the MAbPac RP HPLC capillary column high sensitivity HPLC or LC-MS characterization of intact proteins in top-down proteomics applications where sample amount is critically limited. This versatile column can be used for peptide-mapping characterization where the capillary format balances sensitivity with throughput. Additional applications are monoclonal antibodies (mAbs), fragments, variants, antibody drug conjugates (ADCs), PEGylated proteins, and bispecific proteins. These columns have excellent resolution, long column lifetime, and low carry-over.



### EASY-Spray HPLC columns

Diameter	Length (metric)	Particle size	Phase	Temperature	Cat. no.
150 µm	150 mm	4 µm	PSDVB	60° C	03254131

### MAbPac RP HPLC capillary column

Diameter	Length (metric)	Particle size	Phase	Temperature	Cat. no.
150 µm	150 mm	4 µm	PSDVB	110° C	03254130

# Trapping columns

Bottom-up  
EASY-Spray  
workflow

Bottom-up  
 $\mu$ PAC  
workflow

## Acclaim PepMap 100 C18 HPLC column

Feature high loading capacity for exceptional suitability in the analysis of low abundant peptides in complex proteomics samples with Acclaim PepMap 100 C18 HPLC columns. Acclaim PepMap HPLC columns offer lengths targeting fast or high resolution peptide separation.



## $\mu$ PAC trapping column

Perform large volume (>5  $\mu$ L) sample injections on capillary and nano-columns with  $\mu$ PAC trapping columns with minimal impact on your total low-flow chromatography analysis time. Their C18 stationary phase support morphology is designed to perfectly match that of capillary and nano-  $\mu$ PAC HPLC columns, with carefully selected dimensions and surface chemistry.

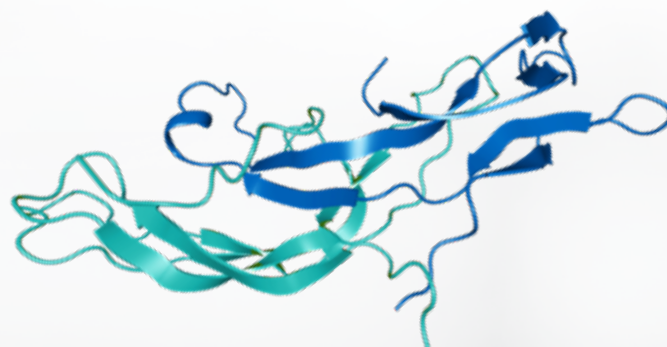


### Acclaim PepMap 100 C18 HPLC columns

Diameter	Length (metric)	Particle size	Stationary phase	Package	Cat. no.
75 $\mu$ m	20 mm	3 $\mu$ m	C18	2	164946

### PepMap Neo trap cartridge holder

Length (metric)	Package	Cat. no.
5 mm	1	03255020



## EASY-Spray nano and capillary emitters

Connect your nano or capillary column to your Thermo Fisher Scientific mass spectrometers (MS) more easily using EASY-Spray nano and capillary emitters. The nano and capillary emitters act as a column-independent sprayer, allowing the introduction of low flow from nano and capillary columns without troublesome handling of traditional spray needles. There are two versions of emitters available: with and without integrated transfer lines. These sprayers are ideal for protein and peptide separation using nano and capillary HPLC.



## µPAC compatible EASY-Spray emitter

The µPAC compatible EASY-Spray emitter is equipped with a stainless steel 50 µm through-bore union. Achieve high resolution separations in low-flow applications when using this emitter to connect a µPAC HPLC column and a transfer line with a Thermo Scientific EASY-Spray ion source.



### EASY-Spray nano and capillary emitters

Diameter	Type	Cat. no.
20 µm	Nano EASY-Spray emitter with transfer line	03251869
75 µm	Capillary EASY-Spray emitter with transfer	03251870
10 µm	Nano EASY-Spray emitter, bullet type without transfer line	03254132
15 µm	Capillary EASY-Spray emitter, bullet type without transfer line	03254133

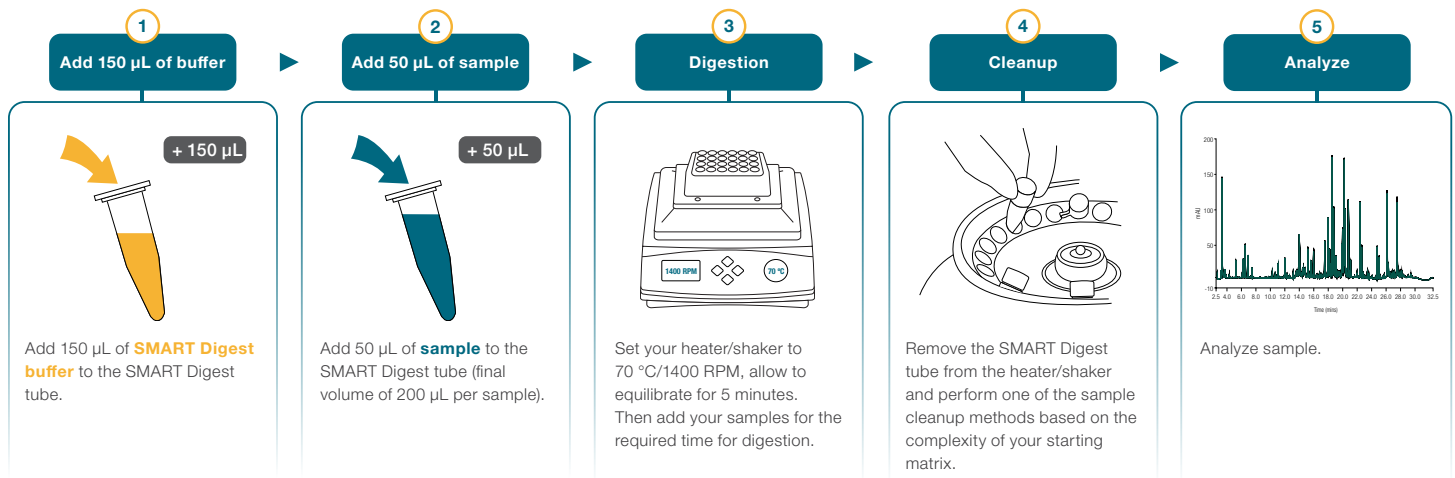
# Digest solutions

## SMART Digest kits for fast and robust partial or full protein digestion

Obtain high quality analytical results from protein digests using SMART Digest kits. A significant advance in sample preparation for biopharmaceutical protein research, the kits provide fast and simple protein digestion with high reproducibility, high sensitivity, and high levels of data quality in a format that's compatible with automation. The SMART Digest kits are a significant improvement over current in-solution protein digestion technologies, which are not reproducible, have poor sensitivity, and employ protracted methodologies not amenable to automation.

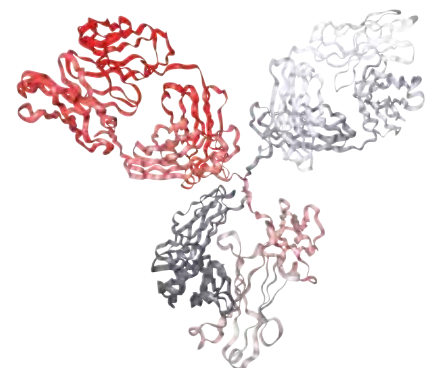


### SMART Digest Kit workflow

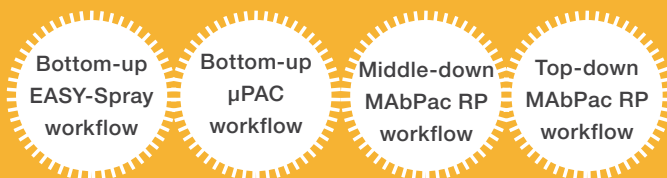


### SMART Digest Proteinase K kit with SOLAµ/collection plate

Type	For use	Cat. no.
Protein digestion	Peptide quantitation, peptide mapping	03252216



# Vials and caps



## SureSTART 0.3 mL screw glass vials

Choose SureSTART 0.3 mL glass screw top microvials, performance level 3, when you need to maximize the injection volume for <2 mL samples.



## SureSTART 1.5 mL screw glass vials

Choose SureSTART 1.5 mL total recovery glass screw top microvials, performance level 3, when you need to maximize the injection volume for <2 mL samples.



## SureSTART 0.2 mL TPX screw vial with glass insert

Use the SureSTART TPX screw top microvial with glass insert, performance level 3, when you are working with <2 mL samples and need maximum sample injection. This microvial is made of amber TPX polymethylpentene (PMP), which is lightweight and transparent and has both heat and chemical resistant properties. The fixed glass conical insert removes the need to assemble a vial and insert prior to use.



## SureSTART 9 mm screw caps

Use SureSTART 9 mm screw caps with screw vials that have a 9 mm opening, including our SureSTART 2 mL glass screw vials, 2 mL gold-grade glass screw vials, total recovery glass screw microvials for <2 mL samples, 1.7 mL high recovery glass screw vials, and glass screw microvials for <2 mL samples (Level 3).



### SureSTART vials

Material	Total volume	Usable volume	Cat. no.
Clear glass	0.3 mL	0.25 mL	03452247
Clear glass	1.5 mL	1.10 mL	03452550
Amber TPX	0.2 mL	0.15 mL	03452575

### SureSTART screw caps

Septum	Closure material	Hardness	Thickness	Closure size	Cat. no.
White silicone/red PTFE	Black polypropylene	54 shore	1.3 mm	9 mm	03452313



# Well plates and sealing tapes

## WebSeal Plate+ glass coated plastic well plates, 2nd generation

Samples that require glass vials can now benefit from high-throughput analysis using these glass-coated well plates. Our second-generation WebSeal Plate+ glass coated plastic plates are the solution to pure plastic plates that cause poor solvent stability or loss of product due to adsorption onto plastic well walls. The WebSeal Plate+ products are glass coated using state-of-the-art technology for higher density coating (thickness of 200 Å) to improve solvent stability and reproducibility, especially for biomolecules that adsorb onto plastic walls.



## WebSeal sealing tapes

Effectively seal all microplate formats with WebSeal sealing tapes. Available in a selection of thin, lightweight materials and adhesives to meet the needs of any application or assay, they minimize evaporation and protect samples from contamination and spilling.

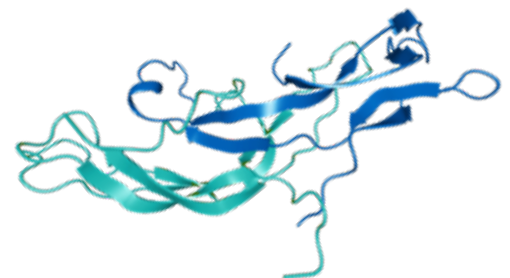


### WebSeal Plate+ well plates

Type	Material	Diameter	Volume	Unit size	Cat. no.
Microplate	Polypropylene	14.6 x 7 mm	220 µL	10	03253924

### WebSeal sealing tapes

Type	Material	Color	Compatibility	Unit size	Cat. no.
Non-sterile	PET, silicone	Clear	All microplates	100	03252444



# Chromatography columns and consumables

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