Peptide clean-up

After isolation of peptides, salts and buffers can be removed using either C18 reversed-phase (RP) or graphite resin.

The peptides bind to reversed-phase columns in high-aqueous mobile phase; the salts and buffers are then washed off, and the peptides are eluted using a high-organic mobile phase. In RP, HPLC compounds are separated based on their hydrophobic character. As very hydophilic peptides, including phosphopeptides, may bind to C18 resins poorly, graphite spin columns may provide better peptide recovery.

However, C18 and graphite resins do not efficiently remove contaminants such as detergents. Detergent removal from peptide samples is a challenge, especially for MS analysis in which even low detergent concentrations contaminate instruments and interfere with column binding, elution, and ionization.

Detergent removal can be attempted in a number of ways. Acetone or acid precipitation can be used for proteins (not peptides) and generally has poor recovery. Ion exchange chromatography can be used to bind and remove selective detergents, but only if they are anionic or cationic detergents, such as SDS. These methods can be somewhat labor- and/or time-intensive or detergent-specific.

The Thermo Scientific™ Pierce™ detergent removal products contain a proprietary resin that specifically bind a wide variety of detergents. The spin-column format provides a convenient and rapid method for removing detergents before downstream analysis.

Table 42. Peptide clean-up selection guide.

	C18 Spin Tips	C18 Tips	C18 Spin Columns	Graphite Spin Columns	Detergent Removal Products
					
Primary application	Concentrate, desalt peptides	Concentrate, desalt peptides	Concentrate, desalt peptides	Concentrate, desalt phosphopeptides	Remove detergents
Format(s)	Spin tip	Tip	Spin column	Spin column	Spin columns, spin plates, loose resin
Resin bed volume	20 µL	100 µL	8 mg	10 mg (in 0.5 mL of slurry)	125 µL–4 mL slurry
Binding capacity or loading volume	10 µg	80 µg	30 µg	100 µg	0.1–1,000 mL
Processing time	5–10 min	5 min	25 min	10 min	15 min

Pierce C18 Spin Tips

Easy-to-use C18 spin tips for fast and efficient clean-up of peptides for MS analysis

Thermo Scientific™ Pierce™ C18 Spin Tips enable fast and efficient capture, concentration, desalting, and elution of up to 10 µg peptides per 20 µL sample for MS analysis.

Pierce C18 Spin Tips are 20 µL-capacity pipette tips with accompanying adaptors for microcentrifuge sample processing. The tips contain a C18 reversed-phase sorbent that minimizes flow resistance and provides excellent binding and recovery characteristics at a wide range of peptide concentrations, upstream of MALDI or nanoelectrospray ionization techniques. Sample clean-up with C18 resin significantly improves protein analysis results by removing urea, salts, and other contaminants before MS. Each Pierce C18 Spin Tip has a 20 µL volume capacity with a 10 µg peptide-binding capacity.

Highlights:

- **Rapid**—C18 fast-flow tips have low resistance and improved flow characteristics compared to other commercially available tips
- **High capacity**—up to 10 µg peptide per 20 µL solution
- **Convenient**—spin tips come with tip adaptors for easy centrifugation
- **Cleaner sample**—device design filters out particulates that can cause autosampler and column clogging

Pierce C18 Spin Tips offer excellent flow properties with a high-efficiency C18 sorbent for fast wetting, loading, washing, and eluting. Sample is simply loaded in prepared tip and washed and eluted using multiple centrifugation steps. The procedure is simple and typically requires less than 5 min to process protein digests, strong cation-exchange fractions, and other protein and peptide samples for mass spectrometric analyses. The unique tip design also removes any particulates in the sample, eliminating clogging of the auto sampler or column upstream of the MS.

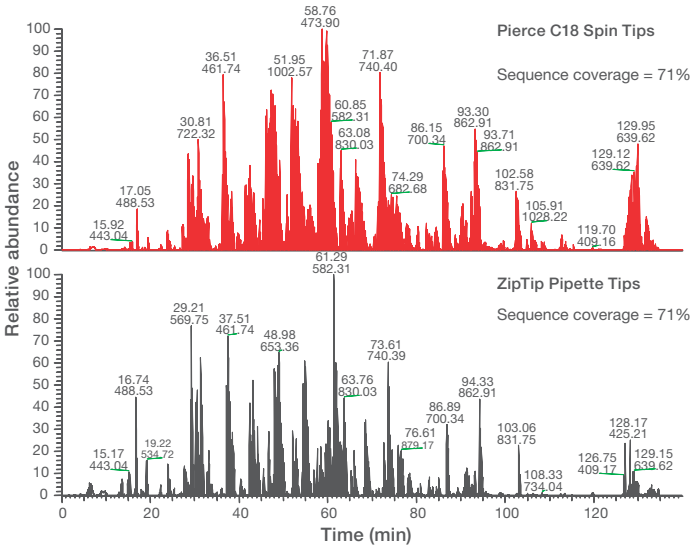


Figure 59. Pierce C18 Spin Tips outperform other popular C18 tips. BSA tryptic digests were analyzed on a Thermo Scientific™ LTQ Orbitrap™ XL ETD Mass Spectrometer after processing 10 µg aliquots of the same digest with Pierce C18 Spin Tips or ZipTip™ Pipette Tips (10 µL tips with 0.6 µL C18 resin; EMD Millipore). Base peak chromatograms of the peptide elution were extracted from the data sets to evaluate sample complexity and chromatographic resolution. MS results were analyzed using the SEQUEST™ search engine and the SwissProt™ database to determine protein sequence coverage.

Pierce C18 Tips

Monolithic C18 sorbent in a pipette tip for fast sample desalting and concentrating

Thermo Scientific™ Pierce™ C18 Tips enable efficient purification of peptides and small proteins before MS. They provide a reproducible method for capturing, concentrating, desalting, and eluting femtomole to nanomole quantities of peptides for improved data generation and analysis. The Pierce C18 Tips have unique monolithic C18 sorbent technology and offer superior flow and exceptional binding capacity, delivering uniform flow and strong analyte-to-surface interactions. They are designed to consistently achieve better sequence coverage, higher peak intensities, and improved peptide capture for accurate protein identification. With the quick and easy-to-use protocol, peptides and small proteins bind to the C18 resin while contaminants are washed away. The target peptides are then recovered in their concentrated and purified form with an aqueous, organic solvent blend.

Highlights:

- **Better sequence coverage**—obtain high sequence coverage for more reliable protein identification
- **Higher peak intensities**—assure correct protein identification with significant signal improvements
- **Increased recoveries**—isolate more peptides using superior binding capacity of Pierce C18 Tips
- **Flexible tip formats**—available in 10 and 100 µL bed volumes for processing up to 8 or 80 µg of samples, respectively
- **Expandable**—our design conveniently adapts to a variety of automated liquid-handling systems with pipetting stations for maximum performance, speed, and hands-off convenience

Improve protein analysis results with Pierce C18 Tips by removing urea, salts, and other contaminants before MS analysis (Figures 60 and 61). The tips are ideal for matrix-assisted laser desorption ionization (MALDI) or nanoelectrospray ionization techniques.

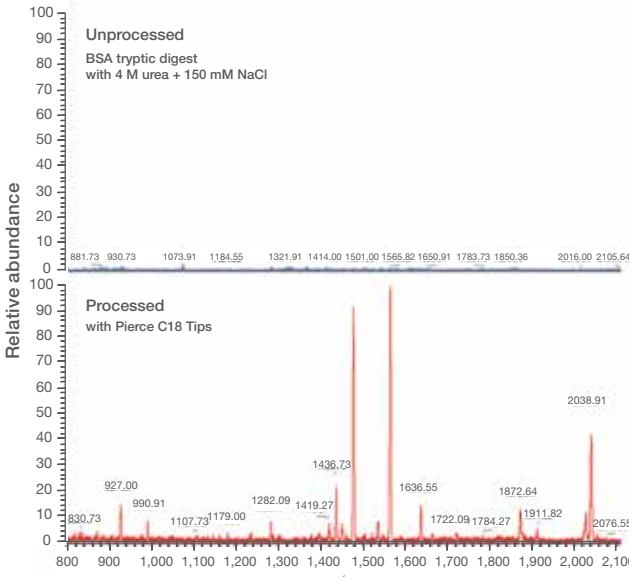


Figure 60. Removing urea and NaCl eliminates interference in MS chromatograms. A bovine serum albumin (BSA) tryptic digest was analyzed on a Thermo Scientific™ MALDI-Orbitrap™ XL Hybrid Mass Spectrometer. Digests and samples containing 150 mM NaCl and 4 M urea were analyzed with or without processing with Pierce C18 Tips (10 µL).

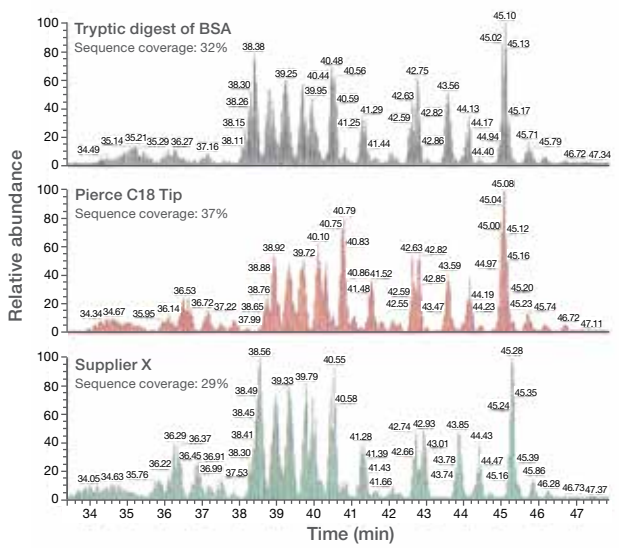


Figure 61. Pierce C18 Tips outperform other suppliers' tips. BSA tryptic digests were analyzed either directly on a Thermo Scientific™ LTQ XL™ Ion Trap Mass Spectrometer or after processing with Pierce C18 Tips (100 µL) or supplier X tips. Base peak chromatograms of the peptide elution were extracted from the data sets to evaluate sample complexity and chromatographic resolution. MS results were analyzed with Matrix Science Mascot software and the SwissProt Release 52 database to determine protein sequence coverage.

Pierce C18 Spin Columns

Purify and/or concentrate multiple peptide samples in less than 30 min

Peptide samples can be purified and concentrated for a variety of applications using Pierce C18 Spin Columns. Each spin column contains a porous C18 reversed-phase resin with excellent binding and recovery characteristics for a wide range of peptide concentrations. The spin column format allows simultaneous processing of multiple samples (10–150 µL) in approximately 30 min without laborious repeat pipetting or specialized equipment. Pierce C18 Spin Columns can be used effectively for processing peptides derived from 10 ng to 30 µg of protein. Sensitivity and detection limits are dependent on the downstream application.

Highlights:

- **Removes MS-interfering contaminants**—helps to reduce signal suppression and improves signal-to-noise ratios and sequence coverage; works on a variety of reversed-phase–compatible contaminants
- **Robust**—works with a wide variety of load volumes (10–150 µL) and concentrations; no need to reduce sample volume before application
- **Convenient**—easy to handle and requires no special equipment for processing multiple samples simultaneously (unlike tip-driven systems that require samples to be processed one at a time)
- **Sensitive**—special C18 resin allows for excellent recovery percentages, even at low (sub-picomole) sample loads

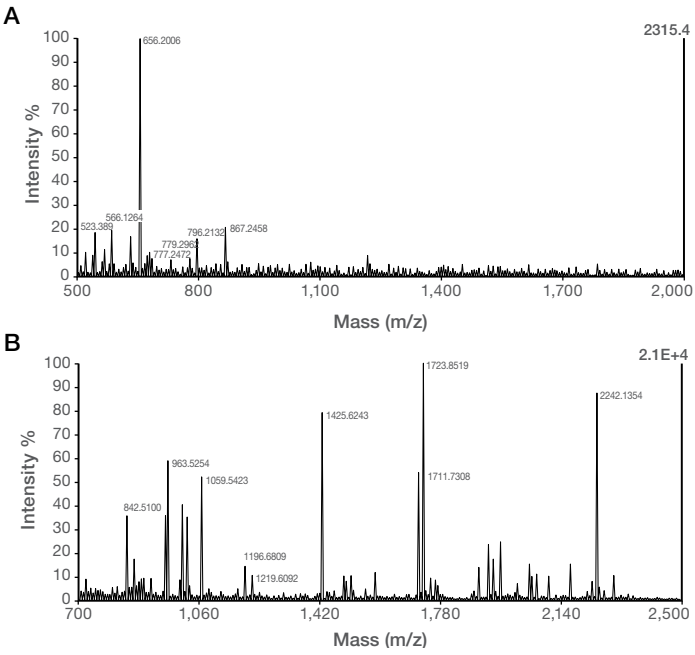


Figure 63. Effective clean-up of MS sample with Pierce C18 Spin Columns. (A) MALDI-TOF MS analysis of an unknown protein isolated from a mitochondrial extract separated by 2D electrophoresis and subjected to in-gel tryptic digestion followed by processing with Pierce C18 Spin Columns. **(B)** MALDI-TOF MS analysis of an identical digest that has not been C18 processed.

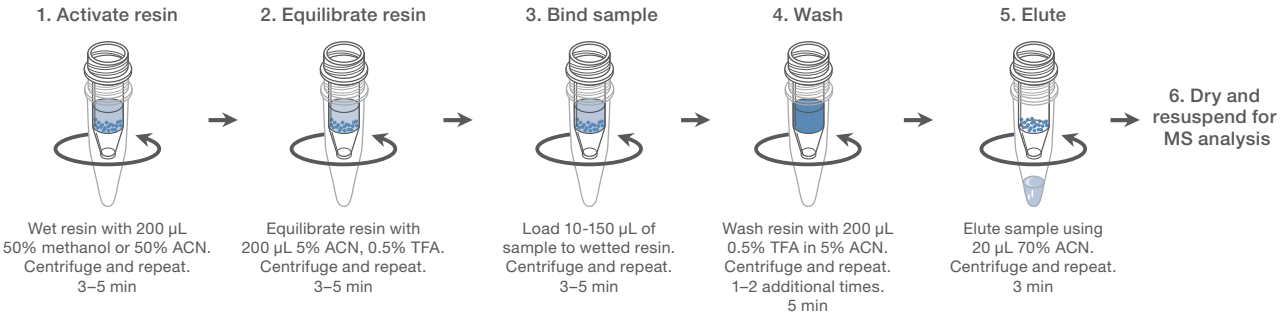


Figure 62. Thermo Scientific Pierce C18 Spin Column protocol summary.

Pierce Graphite Spin Columns

Graphite spin columns efficiently purify and concentrate hydrophilic phosphopeptides

The Thermo Scientific™ Pierce™ Graphite Spin Columns enable fast and efficient capture, concentration, desalting, and elution of hydrophilic peptides. The five-step procedure is simple and requires less than 10 min to process. These columns are ideal for improving mass spectrometric analyses of samples from protein digests (Figure 64), strong-cation exchange fractions, and enriched phosphopeptides eluted from TiO_2 and immobilized metal affinity chromatography (IMAC) columns and tips.

Highlights:

- **Convenient**—spin format enables parallel processing of multiple samples
- **High-binding capacity**—excellent recovery of up to 100 µg of hydrophilic peptides per column
- **Efficient**—porous graphite resin enables efficient clean-up of phosphopeptide samples before MS analysis

C18 resins and tips, commonly used to desalt peptides, do not efficiently capture hydrophilic peptides, like phosphopeptides. The Pierce Graphite Spin Columns improve phosphopeptide analysis by efficiently binding hydrophilic peptides and efficiently removing urea, salts, and other contaminants before MS analysis (Figure 65). The spin columns are ideal for MALDI or nanoelectrospray ionization techniques.

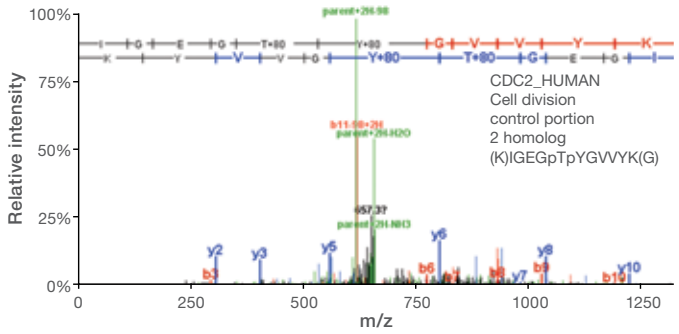


Figure 64. Graphite clean-up enables phosphopeptide identification. U2OS human osteosarcoma cells synchronized at the G_2/M boundary with nocodazole (200 ng/mL, 36 hr) were lysed with 6 M guanidine·HCl. After enzymatic protein digestion (100 µg), phosphopeptides were enriched with IMAC and desalted with Pierce Graphite Spin Columns or C18 tips before LC-MS/MS analysis on an LTQ Orbitrap XL Mass Spectrometer. Representative spectra is shown for a phosphopeptide not observed after C18 clean-up. A novel doubly phosphorylated peptide was identified within the putative ATP-binding site of cyclin dependent kinase cdc2 (CDK1). This phosphopeptide is not present in Phospho.ELM version 8.2 database.

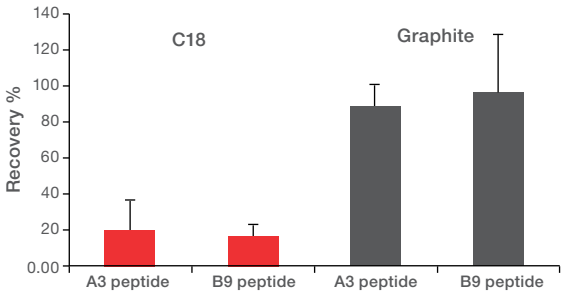


Figure 65. Improved recovery of representative hydrophilic phosphopeptides using graphite spin columns. Stable isotope–labeled A3 and B9 peptides (10 pmol) were acidified with 1% trifluoroacetic acid, processed according to instructions for C18 tips or the Pierce Graphite Spin Columns, and eluted with 50% acetonitrile/0.1% formic acid. The corresponding heavy isotope–labeled peptides (5 pmol) were spiked in the eluate, dried, and resuspended in 0.1% formic acid. Samples were analyzed by targeted LC-MS/MS with the Orbitrap XL Mass Spectrometer to quantitate percent recovery. Peptides: A3= RPRAApTFPFR*, B9 = RTPKDpSPGIPPFR*.

*Position of heavy isotope–labeled amino acid used for absolute MS quantitation.

Learn more at **thermofisher.com/peptidecleanup**

Detergent removal products

The Thermo Scientific™ Pierce™ Detergent Removal Resins are provided in convenient spin-column or plate formats that quickly and efficiently remove ionic, nonionic, and/or zwitterionic detergents from protein or peptide samples to improve compatibility with downstream applications. Two formulations are available that are optimized to remove detergents from peptide samples with different concentration ranges. The Thermo Scientific™ HiPPR™ (high protein and peptide recovery) products are recommended for peptide samples </= 100 µg/mL. The standard Pierce Detergent Removal Resin products are ideal for peptide samples >100 µg/mL.

Highlights:

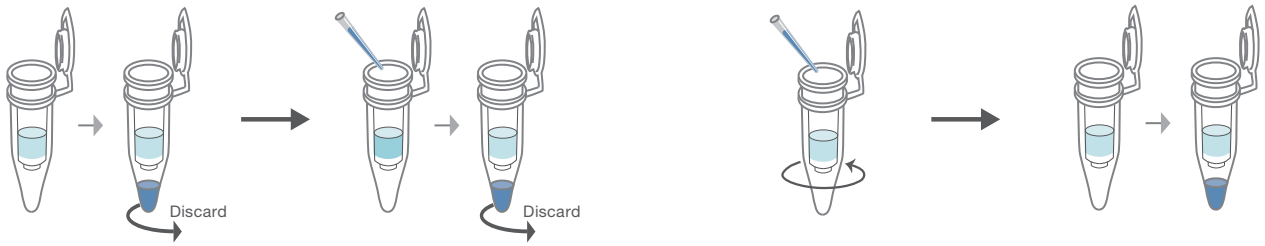
- **High performance**—removes detergent with >90% recovery and no sample dilution
- **Versatile**—effectively removes a wide variety of detergents from peptide or protein samples
- **Optimized**—separate formulations for samples with peptide concentrations ≤ or >100 µg/mL
- **Flexible**—available in various formats, including spin columns, 96-well spin plates, and loose resin
- **Convenient**—simple method that helps to improve MS peptide coverage

Table 43. Detergent removal product selection guide.

	HiPPR Detergent Removal Spin Columns	HiPPR Detergent Removal Spin Kit	HiPPR Detergent Removal Spin Plates	Pierce Detergent Removal Spin Columns	Pierce Detergent Removal Resin	Pierce Detergent Removal Spin Plates
						
Sample volume range(s)	100 µL	25–200 µL	100 µL	10–25 µL 25–100 µL 150–500 µL 500–1,000 µL	10–2,500 µL	20–100 µL
Recommended peptide sample concentration range	1–100 µg/mL	1–100 µg/mL	1–100 µg/mL	>100 µg	>100 µg	>100 µg
Format	Pre-filled spin columns	5 mL resin + 0.8 mL empty spin columns	96-well pre-filled spin plate	Pre-filled spin columns	10 mL resin	96-well pre-filled spin plate
Resin bed volume(s)	0.1 mL	0.025–0.2 mL	0.1 mL	0.125 mL 0.5 mL 2 mL 4 mL	0.125 mL–10 mL	0.55 mL

Spin columns, loose resin, and kits

The Thermo Scientific™ HiPPR™ Detergent Removal Resin is available in a predispensed 0.1 mL format or as a kit with bulk resin and empty spin columns for customizing filling and processing. The Pierce Detergent Removal Resin is available in four convenient prepacked column sizes for quick and easy sample processing; simply remove storage buffer, wash resin with equilibration buffer, add sample, incubate, and obtain detergent free sample upon final centrifugation. The resin is also available in a loose resin (10 mL pack size) for customized applications or columns.

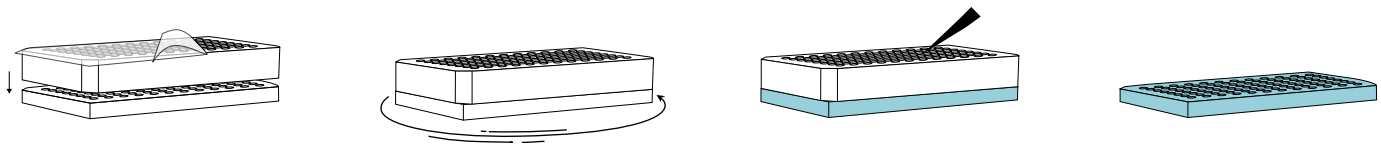


1. Centrifuge for 1 min at 1,500 x *g* to remove the storage buffer.
2. Add 0.4 mL equilibration buffer, centrifuge at 1,500 x *g* for 1 min and discard the flow-through. Repeat two additional times.
3. Add detergent-containing sample (25–100 µL) and incubate for 2 min at room temperature.
4. Centrifuge at 1,500 x *g* for 2 min to collect the detergent-free sample for downstream applications.

Figure 66. Protocol summary for Pierce Detergent Removal Spin Columns (0.5 mL).

96-well spin plates

The prepacked Thermo Scientific™ HiPPR™ and Pierce™ 96-Well Detergent Removal Spin Plates do not require resin hydration or dispensing, and offer the same high protein and peptide recovery as the spin-column format. Each plate can process up to 96 samples simultaneously, using 25–100 µL of sample per well.



1. Remove the bottom seal and stack the detergent removal plate on top of a wash plate. Remove the top seal and centrifuge.*
2. Add 300 µL of buffer to each well and centrifuge. Discard the flow-through. Repeat this step two times.
3. Stack the detergent removal plate on top of a sample collection plate. Apply sample and incubate at room temperature for 2 min. Centrifuge to remove detergent.
4. Recover the detergent-free sample for downstream analysis.

*Centrifugations are performed for 2 min at 1,000 x *g*.

Figure 67. Protocol summary for Pierce Detergent Removal Spin Plates.

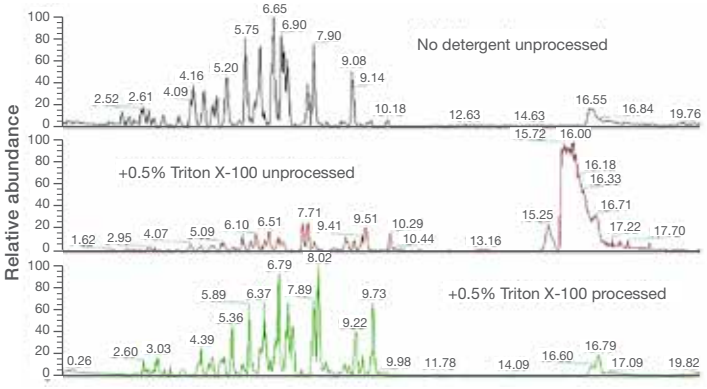
Learn more at **thermofisher.com/detergentremoval**

Table 44. Results using HiPPR Detergent Removal Resin. Each column and well plate contained ~550 µL of detergent-removal resin slurry and 0.1 mL of sample. Similar results were obtained with both process formats.

Process format†	Detergent	Detergent concentration (%)	Detergent removal (%)	BSA recovery (%)
0.5 mL spin column	Sodium deoxycholate	5	99	100
	Octyl glucoside	5	99	90
	Octyl thioglucoside	5	99	95
	Lauryl maltoside	1	98	99
	Triton X-114	2	95	100
	Brij-35	1	99	97
	Tween 20	0.25	99	87
96-well spin plate	SDS	5	99	89
	Triton X-100	4	99	100
	NP-40	1	95	100
	CHAPS	5	99	100

Table 45. Results using the standard Thermo Scientific™ Pierce™ Detergent Removal Spin Column, 0.5 mL. Detergent removal efficiency and protein recovery. BSA sample (25–200 µL) + detergent in 0.15 M NaCl, 0.05% sodium azide was mixed with an equal volume of detergent removal resin (2x volume for CHAPS removal) and processed as shown in the protocol (page 81).

Detergent	Sample volume (µL)	Protein quantity (µg)	Detergent removal (%)	Protein recovery (%)
SDS (1%)	25	0.375	>99	98
	50	0.75	>99	97
	100	1.5	>99	100
	200	3.0	>99	100
Triton X-100 (1%)	25	0.375	>95	82
	50	0.75	>95	86
	100	1.5	>95	86
	200	3.0	>95	93
NP-40 (0.75%)	25	0.375	95	90
	50	0.75	96	94
	100	1.5	97	91
	200	3.0	97	97
CHAPS (1%)	25	0.375	95	64
	50	0.75	97	70
	100	1.5	98	78
	200	3.0	98	75



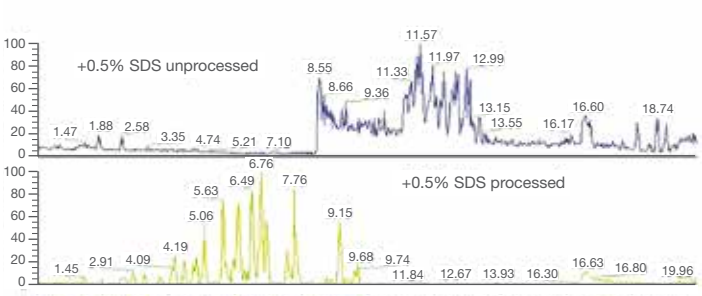


Figure 68. Results using HiPPR Detergent Removal Resin. BSA (100 µg/mL) tryptic digests were prepared without detergent, in the presence of 0.5% Triton™ X-100 or spiked with 0.5% SDS following enzymatic digestion. Samples (0.1 mL) containing detergent were processed with the HiPPR Detergent Removal Resin and compared to unprocessed or detergent-free samples by LC-MS/MS. Results demonstrate that detergent removal is effective and produces results similar to those observed for samples containing no detergent.

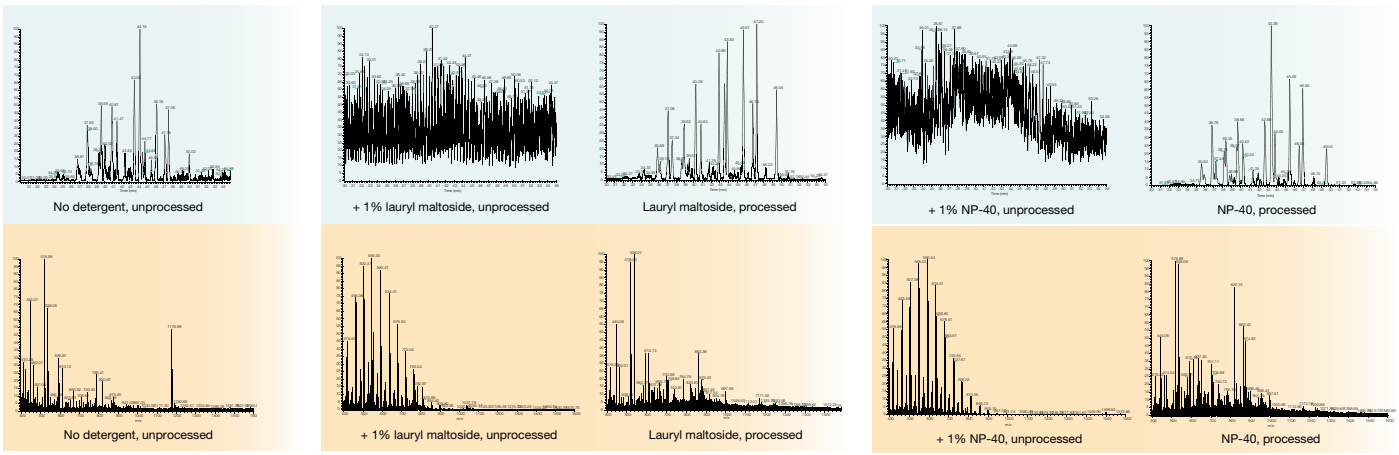


Figure 69. Results using the standard Pierce Detergent Removal Spin Column, 0.5 mL. Tryptic digests (0.1 mL, 100 µg) containing detergent were each processed through 0.5 mL of Pierce Detergent Removal Resin and subjected to LC-MS/MS analysis. Top row: Base peak LC-MS chromatograms. Bottom row: Integrated mass spectra. Similar results were produced for Thermo Scientific™ Brij™-35 detergent, octyl glucoside, octyl thioglucoside, and SDS (data not shown).

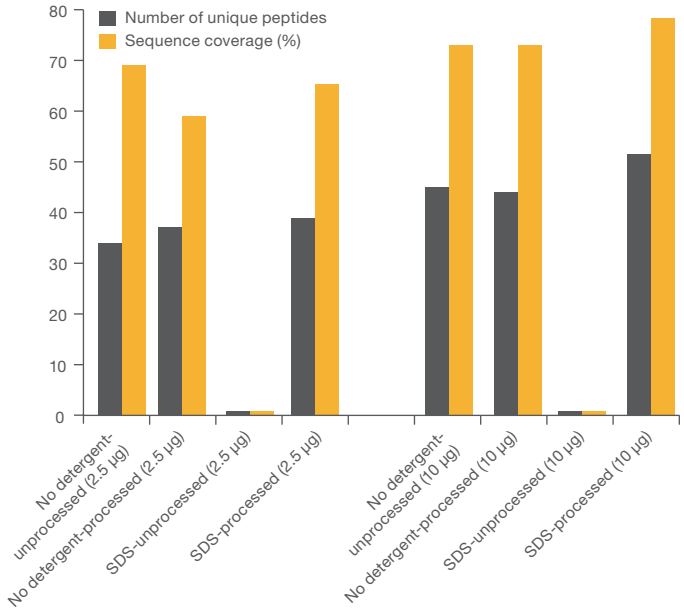


Figure 70. Results using HiPPR Detergent Removal Resin. BSA (25 and 100 µg/mL) was digested in the presence and absence of detergents, and the samples were processed for LC-MS/MS analysis. Effective detergent removal resulted in greater peptide identification and high Mascot scores.

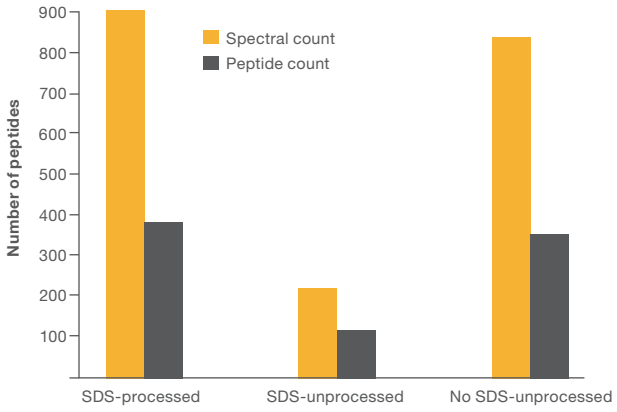


Figure 71. Results using Pierce Detergent Removal Spin Columns, 0.5 mL. A tryptic digest of HeLa cell lysate (0.1 mL, 100 µg) containing 1% SDS was processed through 0.5 mL of Pierce Detergent Removal Resin and subjected to LC-MS/MS analysis. The processed sample allowed similar numbers of identified peptides as digests containing no SDS. Peptide identification is greatly reduced in sample containing SDS. Effective detergent removal enables greater peptide identification.

Product	Quantity	Cat. No.
Stains		
Pierce Silver Stain for Mass Spectrometry	1 L kit	24600
Imperial Protein Stain	1 L	24615
To view additional pack sizes and products, go to **thermofisher.com/proteinstains**		
Protein digestion		
Pierce Trypsin Protease, MS Grade, Frozen	100 µg	90305
Pierce Trypsin Protease, MS Grade	5 x 20 µg	90057
Pierce Trypsin Protease, MS Grade	5 x 100 µg	90058
Pierce Trypsin Protease, MS Grade	1 mg	90059
Lys-C Protease, MS Grade	20 µg	90051
Pierce LysN Protease, MS Grade	20 µg	90300
Pierce LysN Protease, MS Grade	5 x 20 µg	90301
Asp-N Protease, MS Grade	2 µg	90053
Glu-C Protease, MS Grade	5 x 10 µg	90054
Chymotrypsin Protease, MS Grade	4 x 25 µg	90056
In-Gel Tryptic Digestion Kit	Kit	89871
In-Solution Tryptic Digestion and Guanidination Kit	Kit	89895
Bond-Breaker TCEP Solution, Neutral pH	5 mL	77720
Pierce DTT (Dithiothreitol), No-Weigh Format	48 x 7.7 mg	20291
Pierce Iodoacetic Acid	500 mg	35603
Pierce Iodoacetamide, Single-Use	24 x 9.3 mg	90034
Pierce MMTS	200 mg	23011
Pierce N-Ethylmalemide (NEM)	25 g	23030
To view additional pack sizes and products, go to **thermofisher.com/msdigestion**		
Peptide enrichment and fractionation		
High-Select Fe-NTA Phosphopeptide Enrichment Kit	Kit	A32992
High-Select TiO_2 Phosphopeptide Enrichment Kit	Kit	A32993
Pierce TiO_2 Phosphopeptide Enrichment Spin Tips	96 tips	88303
Pierce Magnetic TiO_2 Phosphopeptide Enrichment Kit	96-rxn kit	88811
Pierce Magnetic TiO_2 Phosphopeptide Enrichment Kit, Trial Size	24-rxn kit	88812
Pierce High pH Reversed-Phase Peptide Fractionation Kit	12 columns	84868
Low Protein Binding Microcentrifuge Tubes, 2.0 mL	250 tubes	88379
Low Protein Binding Microcentrifuge Tubes, 2.0 mL	10 x 250 tubes	88380
To view additional pack sizes and products, go to **thermofisher.com/peptidekits**		

Product	Quantity	Cat. No.
Peptide clean-up		
Pierce C18 Spin Tips	96 tips	84850
Pierce C18 Tips, 10 µL bed	8 tips	87781
Pierce C18 Tips, 10 µL bed	96 tips	87782
Pierce C18 Tips, 100 µL bed	8 tips	87783
Pierce C18 Tips, 100 µL bed	96 tips	87784
Pierce C18 Spin Columns	25 columns	89870
Pierce C18 Spin Columns	50 columns	89873
Pierce Graphite Spin Columns	30 columns	88302
Pierce 96-Well Detergent Removal Spin Plates	2 plates	88304
Pierce Detergent Removal Spin Column, 125 µL	25 columns	87776
Pierce Detergent Removal Spin Column, 0.5 mL	25 columns	87777
Pierce Detergent Removal Spin Column, 2 mL	5 columns	87778
Pierce Detergent Removal Spin Column, 4 mL	5 columns	87779
Pierce Detergent Removal Resin	10 mL	87780
HiPPR Detergent Removal Spin Column	54 columns	88305
HiPPR Detergent Removal Spin Columns, 0.1 mL	24 columns	88306
HiPPR Detergent Removal 96-Well Spin Plates, 0.1 mL	2 plates	88307
To view additional pack sizes and products, go to **thermofisher.com/peptidecleanup**		
Peptide quantitation assays		
Pierce Quantitative Colorimetric Peptide Assay	Kit	23275
Peptide Digest Assay Standard	1.5 mL	23295
Pierce Quantitative Fluorometric Peptide Assay	500 assays	23290
96-Well Black Plate	25 pack	88378
To view additional pack sizes and products, go to **thermofisher.com/peptideassays**		