INSTRUCTIONS

Pierce® C18 Spin Columns

89870 89873

1464.3

Number	Description
89870	**Pierce C18 Spin Columns,** 25 spin columns each containing 8mg of C18 resin
89873	**Pierce C18 Spin Columns,** 50 spin columns each containing 8mg of C18 resin

Note: Each column can bind up to 30µg of total peptide from 10 to 150µL sample volumes. Depending on sensitivity limits of the mass spectrometry system used to analyze the eluted peptides, digests of as little as 20ng of protein may be processed successfully with these columns.

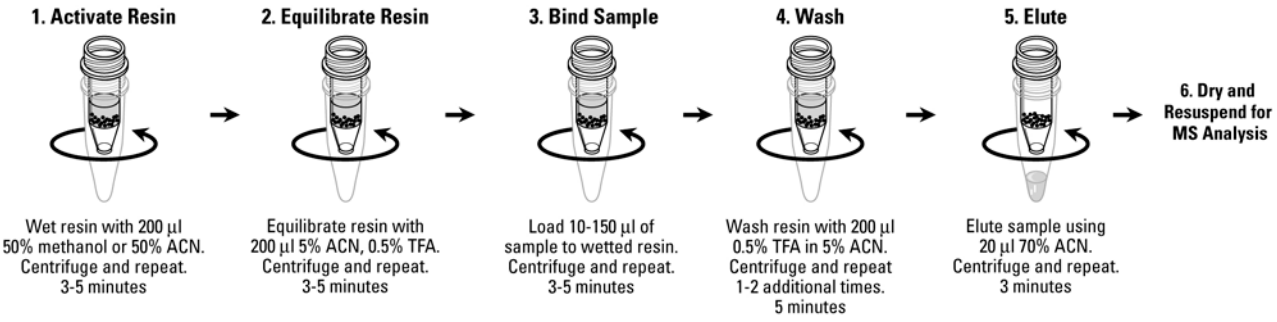
Storage: Upon receipt store at room temperature. Product shipped at ambient temperature.

Introduction

Peptide samples can be purified and concentrated for a variety of applications using the Thermo Scientific Pierce C18 Spin Columns. Each spin column contains a porous C18 reversed-phase resin with excellent binding and recovery characteristics at a wide range of peptide concentrations. The spin column format allows simultaneous processing of multiple samples without the need for laborious repeat pipetting.

Matrix-assisted laser desorption ionization (MALDI-) and electrospray ionization (ESI-) mass spectrometry (MS) are vital tools for the study of biological compounds because of the high sensitivity and mass accuracy. MS methods are commonly used for examination of post-translational modifications and identification of proteins by peptide mapping. However, many of the buffers and compounds common to biological samples interfere with both MALDI-MS and ESI-MS. Pierce C18 Spin Columns remove interfering contaminants and release peptides in MS-compatible solutions, resulting in increased sensitivity and a high-quality spectrum. Although Pierce C18 Spin Columns are designed primarily for MS applications, they may be used for other applications such as peptide concentration and clean-up for peptide sequencing.

Procedure Summary



Important Product Information

- Pierce C18 Spin Columns can bind up to 30µg of total peptide. The lower level of detection for a protein is typically 20ng (300fmol); however, at this lower level of detection, each singular peptide processed from a given protein needs to be at least 0.5ng to be detected effectively. Minimum sample load requirements depend on the sensitivity limits of the downstream analysis system.

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- For binding to C18 reversed-phase resins, a sample must be free of excess organic solvents such as acetonitrile (ACN) or methanol. If organic solvents are present, dry the sample in a vacuum evaporator. Carefully resuspend sample in 20µL of 0.5% trifluoroacetic acid (TFA) in 5% ACN before processing with Pierce C18 Spin Columns.
- For optimal results, proceed with the entire procedure in a timely manner and avoid excessive resin drying between steps.
- Plastics used during handling of peptide samples can introduce contaminants that interfere with MS analysis and result in sample loss from non-specific adsorption. Use high-quality receiver tubes. If necessary, receiver tubes used for the final collection may be rinsed with 70% ACN/0.1% TFA before use. Minimizing sample transfers and freeze-thaws before analysis will help minimize plastic contamination and sample loss.

Additional Materials Required

- Bench-top microcentrifuge capable of 3000 × *g*
- Ultrapure water
- Acetonitrile (ACN)
- Trifluoroacetic acid (TFA)
- 1.5mL microcentrifuge tubes
- Methanol (optional)

Material Preparation

- Activation Solution: 50% Methanol; 400µL per sample

  Note: ACN can be substituted for methanol.
- Equilibration Solution: 0.5% TFA in 5% ACN; 400µL per sample
- Sample Buffer: 2% TFA in 20% ACN; 1µL for every 3µL of sample
- Wash Solution: 0.5% TFA in 5% ACN; 400-800µL per sample; wash volume will be dependent upon amount and type of contaminants present in sample
- Elution Buffer: 70% ACN; 40µL per sample

  Note: The elution buffer used can be tailored to the downstream application. Acceptable buffers include 50-70% ACN or 50-70% methanol with or without 0.1% TFA. For ESI-MS analysis, replace TFA with 0.1% formic acid for best results.

Protocol for Sample Clean-up

A. Sample Preparation

Each Pierce C18 Spin Column can process 10-150µL of sample. Mix 3 parts sample to 1 part Sample Buffer. The final sample will contain 0.5% TFA in 5% ACN.

Note: See Important Product Information section for additional details on sample preparation.

B. Column Preparation

1. Tap column to settle resin. Remove top and bottom cap. Place column into a receiver tube.
2. Add 200µL of Activation Solution to rinse walls of the spin column and to wet resin.
3. Centrifuge at 1500 × *g* for 1 minute. Discard flow-through.
4. Repeat steps B.2-B.3.
5. Add 200µL Equilibration Solution. Centrifuge at 1500 × *g* for 1 minute. Discard flow-through.
6. Repeat step B.5.

C. Sample Binding

1. Load sample on top of resin bed.
2. Place column into a receiver tube. Centrifuge at 1500 × *g* for 1 minute.
3. To ensure complete binding, recover flow-through and repeat steps C.1-C.2.

   Note: Flow-through may be retained to confirm sample binding.

D. Wash

1. Place column into a receiver tube. Add 200µL Wash Solution to column and centrifuge at 1500 × *g* for 1 minute. Discard flow-through.
2. Repeat step D.1.

   Note: If sample contains high levels of contaminants (i.e., 2M urea or ≥100mM ammonium bicarbonate), repeat the wash step one to two additional times.

E. Elution

1. Place column in a new receiver tube. Add 20µL of Elution Buffer to top of the resin bed. Centrifuge at 1500 × *g* for 1 minute.
2. Repeat step E.1 with same receiver tube.
3. Gently dry sample in a vacuum evaporator. For MALDI-MS analysis, carefully suspend sample in 1-2µL of matrix solution prepared just before use. For LC-ESI applications, suspend sample in 0.1% formic acid or the appropriate buffer.

Troubleshooting

Problem	Cause	Solution
Poor or incomplete sample binding	High pH, lack of ion-pairing agents	Ensure TFA was added to sample
	Sample contains organic solvent	Dry sample and resuspend in 20µL of 0.1-0.5% TFA
	Sample not sufficiently hydrophobic to bind C18 resin	None
	Resin became dry before sample addition	Ensure resin does not dry during activation and equilibration of the resin; keep resin in Equilibration Solution until sample addition
Poor or incomplete sample recovery	Highly hydrophobic sample	Use 70% ACN/0.1% TFA for elution conditions
	Sample loss due to nonspecific binding	Nonspecific binding of peptides to plastics can cause significant sample loss at very low peptide concentrations
		Minimize contact with plastics and storage at low concentrations (i.e., ≤ 300fmol)
		Pierce C18 Spin Columns are not recommended for routine use at total peptide concentrations ≤ 300fmol as special handling may be required
	Detection limits of application	Ensure sample is within the detection limit of the specific downstream application Note: Limits vary considerably based on application and instrumentation

Related Thermo Scientific Products

28904	**Trifluoroacetic acid,** 10 × 1mL
51101	**Acetonitrile**, 1L
51140	**Water,** LC/MS grade, 1L
87782	**Pierce C18 Tips,** 10µL bed column, 96/pkg.
87784	**Pierce C18 Tips,** 100µL bed column, 96/pkg.
88302	**Pierce Graphite Spin Columns,** 0.5mL, 30/pkg.
89871	**In-Gel Tryptic Digestion Kit**, sufficient reagents for up to 150 digests

Current product instructions are available at *www.thermoscientific.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.

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