Pierce™ C18 Spin Columns

Catalog Numbers 89870, 89873

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

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.

Contents and storage

Item	Cat. No.	Amount[1]	Storage
Pierce™ C18 Spin Columns	89870	25 spin columns each containing 8 mg of C18 resin	Room temperature
	89873	50 spin columns each containing 8 mg of C18 resin	

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

Workflow

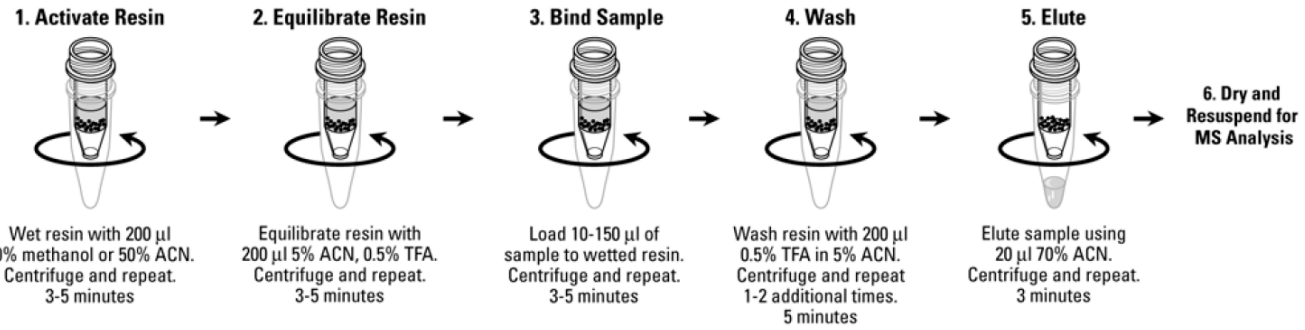

Figure 1 Pierce™ C18 Spin Columns procedure summary.

Additional information

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

Required materials not supplied

- Bench-top microcentrifuge capable of 3,000 x *g*
- Ultrapure water
- Acetonitrile (ACN)
- Trifluoroacetic acid (TFA)
- 1.5-mL microcentrifuge tubes
- *(Optional)* Methanol

Prepare materials

- Activation solution: 50% methanol; 400 µL per sample

  Note: ACN can be substituted for methanol.

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

Clean up sample

1 Prepare sample

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

Note: See “Additional information” on page 2 for additional details on sample preparation.

2 Prepare column

1. Tap the column to settle the resin. Remove the top and bottom cap. Place the column into a receiver tube.
2. Add 200 µL of activation solution to rinse the walls of the spin column and to wet the resin.
3. Centrifuge at 1,500 x *g* for 1 minute. Discard the flow-through.
4. Repeat step 2.2 to step 2.3.

2	Prepare column *(continued)*	5. Add 200 µL of equilibration solution. Centrifuge at 1,500 x *g* for 1 minute. Discard the flow-through. 6. Repeat step 2.5.
3	**Bind sample**	1. Load the sample on top of the resin bed. 2. Place the column into a receiver tube. Centrifuge at 1,500 x *g* for 1 minute. 3. To ensure complete binding, recover the flow-through and repeat step 3.1 to step 3.2. **Note:** Flow-through may be retained to confirm sample binding.
4	**Wash sample**	1. Place the column into a receiver tube. Add 200 µL of wash solution to the column and centrifuge at 1,500 x *g* for 1 minute. Discard the flow-through. 2. Repeat step 4.1. **Note:** If the sample contains high levels of contaminants (i.e., 2 M urea or ≥100 mM ammonium bicarbonate), repeat the wash step 1–2 additional times.
5	**Elute sample**	1. Place the column in a new receiver tube. Add 20 µL of elution buffer to the top of the resin bed. Centrifuge at 1,500 x *g* for 1 minute. 2. Repeat step 5.1 with the same receiver tube. 3. Gently dry the sample in a vacuum evaporator. For MALDI-MS analysis, carefully suspend the sample in 1–2 µL of matrix solution prepared just before use. For LC-ESI applications, suspend the sample in 0.1% formic acid or the appropriate buffer.

Troubleshooting

Observation	Possible cause	Recommended action
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 20 µL 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., ≤300 fmol).
		Pierce™ C18 Spin Columns are not recommended for routine use at total peptide concentrations ≤300 fmol 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.

Limited product warranty

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0011495

Revision	Date	Description
B.0	7 December 2022	The content and format were updated.
A.0	17 October 2015	New document for Pierce™ C18 Spin Columns.