

Pierce™ C18 Spin Tips

84850

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Number**Description****84850****Pierce C18 Spin Tips**, 20µL, 96 tips with 24 spin adapters, each tip binds up to 10µg (20µL) of total peptide**Storage:** Upon receipt store at room temperature. Product shipped at ambient temperature

Introduction

The Thermo Scientific™ Pierce™ C18 Spin Tips enable fast and efficient capture, concentration, desalting and elution of peptides. Each tip contains a C18 reversed-phase sorbent that minimizes flow resistance and provides excellent binding and recovery characteristics at a wide range of peptide concentrations. Each 20µL tip can bind up to 10µg of total peptide and is ideal for processing peptide samples for desalting after digestion and before mass spectrometry (MS) analysis.

Matrix-assisted laser desorption ionization- (MALDI-) and electrospray ionization- (ESI-) MS are vital tools for studying biological compounds because of their high sensitivity and mass accuracy. MS methods are commonly used for examining post-translational modifications and identifying proteins by peptide mapping; however, many buffers and compounds common to biological samples (e.g., urea, guanidine, NaCl, Tris, phosphate) interfere with both MALDI-MS and ESI-MS. Pierce C18 Spin Tips remove interfering contaminants and release peptides in MS-compatible solutions, resulting in increased sensitivity and high-quality spectra. Although Pierce C18 Spin Tips are primarily designed for MS applications, they may be used for applications such as peptide concentration and clean-up for peptide sequencing.

Important Product Information

- The Pierce C18 Spin Tips can bind up to 10µg of total peptide. Minimum sample load requirements depend on the sensitivity limits of the downstream analysis system; samples must contain at least 20ng of peptide with a minimum of 0.5ng for each individual peptide. Sample recovery for typical peptides is > 85%, but may be as low as 35% for hydrophilic peptides.
- Apply solutions to the top of the spin-tip column (i.e., wide end of the tip) and use the provided spin-tip adapters for sample processing by centrifugation. Alternatively, solutions applied to the top of the spin tip may be pushed through using a pipette or syringe tip (e.g., Eppendorf Combitip™ Tips).
- For binding to C18 reversed-phase sorbents, a sample must be free of excess organic solvents such as acetonitrile (ACN) or methanol. Remove organic solvents with a speed vac (e.g., Thermo Scientific™ SpeedVac™ Vacuum Concentrator), but avoid complete dryness, which may result in sample loss.
- For optimal results, prepare all solutions and collection tubes in advance and proceed with the entire procedure in a timely manner. For optimum flow and peptide recovery after sample addition, do not completely dry the C18 resin by excessive centrifugation.
- Plastics used during the handling of peptide samples can introduce contaminants that interfere with MS analysis and result in sample loss from nonspecific adsorption. Use high-recovery collection tubes rated for low sample retention. Minimizing sample transfers and freeze-thaw cycles before analysis will help minimize plastic contamination and sample loss.

Additional Materials Required

- Microcentrifuge capable of operating at $1000 \times g$
- 1.5mL microcentrifuge tubes
- Vacuum concentrator
- LC/MS-grade or ultrapure water
- LC/MS-grade acetonitrile (ACN)
- Trifluoroacetic acid (TFA)
- pH paper (optional)
- Formic acid (FA) (optional)
- MALDI matrix (optional)

Material Preparation

| | |
|------------------------------|---|
| Sample treatment solution | 2.5% TFA |
| Wetting/Elution solution | 0.1% TFA in 80% ACN (use 60 μ L per sample) |
| Equilibration/Rinse solution | 0.1% TFA in ultrapure water (use 60 μ L per sample) |
| Re-suspension solution | 0.1% TFA in 5% ACN with ultrapure water (use 20 μ L per sample) |

Procedure for Pierce C18 Spin Tips

Note: Visually verify that each solution has completely flowed through the C18 tip after each centrifugation step. For optimal flow and peptide recovery, do not completely dry the C18 tips after equilibration by excessive centrifugation times or speed.

1. Adjust sample to 0.1-1.0% TFA using 2.5% TFA.

Note: Verify pH is < 4 using pH paper.

2. Insert C18 spin tip into spin adapter seated in microcentrifuge tube.
3. Wet tip by adding 20 μ L of 0.1% TFA in 80% ACN and centrifuging at $1000 \times g$ for 1 minute.
4. Equilibrate tip by adding 20 μ L of 0.1% TFA and centrifuging at $1000 \times g$ for 1 minute.
5. Transfer C18 spin tip and adapter to a new microcentrifuge tube.
6. Add 20-50 μ L of sample to tip and centrifuge at $1000 \times g$ for 1 minute.

Note: Larger volumes require longer centrifugation times of 2-3 minutes to completely flow through the tip. Re-applying sample to C18 spin tip is optional, but may increase sample binding.

7. Wash the tip by adding 20 μ L of 0.1% TFA and centrifuging at $1000 \times g$ for 1 minute. Repeat one additional time.
8. Transfer C18 spin tip and adapter to new microcentrifuge tube.
9. Elute the sample by adding 20 μ L of 0.1% TFA in 80% ACN and centrifuging at $1000 \times g$ for 1 minute. Repeat one additional time.

Note: For direct MALDI-MS analysis, samples can be eluted with 20 μ L of 0.1% TFA in 80% ACN with or without matrix and dispensed directly onto a MALDI plate. For direct ESI-MS analysis, samples may be eluted with 20 μ L of 0.1% FA in 50-80% ACN.

10. Speed vac sample to near dryness and re-suspend with 0.1% TFA in 5% ACN:water for LC-MS analysis.

Troubleshooting

| Problem | Possible Cause | Solution |
|------------------------------------|--|---|
| Poor or incomplete sample binding | High pH caused by lack of ion-pairing agents | Ensure TFA was added to sample and verify pH < 4 |
| | Tip was not sufficiently wetted and/or equilibrated | Check buffers and prepare new tip |
| | Sample contained organic solvent | Dry sample using speed vacuum and resuspend in 20µL of 0.1-1.0% TFA |
| | C18 resin completely dried after adding sample | Do not completely dry C18 tips after equilibration by excessive centrifugation |
| | Sample was not sufficiently hydrophobic to bind C18 sorbent | Use Thermo Scientific™ Pierce™ Graphite Spin Columns (Product No. 88302) |
| Poor or incomplete sample recovery | Peptides binding to plastic caused significant loss at low peptide concentration | Use high-recovery, low-retention microcentrifuge tubes for sample collection |
| | Detection limits of the specific application | Ensure sample is within the detection limit of the specific downstream application; limits vary considerably based on application and instrumentation |

Related Thermo Scientific Products

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| 51101 | Acetonitrile, 1L |
| 28904 | Trifluoroacetic Acid, 10 × 1mL |
| 28905 | Formic Acid, 10 × 1mL |
| 88320 | Pierce Peptide Retention Time Calibration Mixture, 50µL |
| 87781 | Pierce C18 Tips, 10µL bed, 96 tips |
| 87784 | Pierce C18 Tips, 100µL bed, 96 tips |
| 89870 | Pierce C18 Spin Columns, 25/pkg |
| 88302 | Pierce Graphite Spin Columns, 30/pkg |
| 90057 | Pierce Trypsin Protease, MS Grade, 5 × 20µg |
| 84840 | Pierce Mass Spec Sample Prep Kit for Cultured Cells |
| 89871 | In-Gel Tryptic Digestion Kit |
| 89895 | In-Solution Tryptic Digestion and Guanidination Kit |
| 90031 | CHCA MALDI Matrix, Single-Use |
| 90032 | SA MALDI Matrix |
| 90033 | DHB MALDI Matrix |
| 90034 | Iodoacetamide, Single-Use |

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