Pierce™ C18 Tips

Catalog Numbers 87782, 87784

Doc. Part No. 2162237 Pub. No. MAN0011713 Rev. B.0

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

The Thermo Scientific™ Pierce™ C18 Pipette Tips enable fast and efficient capture, concentration, desalting, and elution of peptides. Each tip contains a monolithic C18 reversed-phase sorbent that minimizes flow resistance and provides excellent binding and recovery characteristics at a wide range of peptide concentrations. The 100-µL tip allows processing of peptide samples for desalting after digestion and before mass spectrometric analysis. The 10-µL tip is ideal for off-line desalting of smaller samples.

Matrix-assisted laser desorption ionization (MALDI-) and electrospray ionization (ESI-) mass spectrometry (MS) are vital tools for studying biological compounds because of the 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 Tips remove interfering contaminants and release peptides in MS-compatible solutions, resulting in increased sensitivity and high-quality spectra. Although Pierce™ C18 Pipette Tips are designed primarily for MS applications, they may be used for applications such as peptide concentration and clean-up for peptide sequencing.

Contents and storage

Item	Cat. No.	Amount[1]	Storage
Pierce™ C18 Tips	87782	96 x 10 µL	Room temperature
	87784	96 x 100 µL	

[1] Each tip can bind up to 8 µg (10 µL) or 80 µg (100 µL) of total peptide.

Additional information

- The Pierce™ C18 Pipette Tips can bind up to 8 µg or 80 µg of total peptide in the 10-µL or 100-µL tip, respectively. For best results, use these tips with peptides derived from at least 20 ng of protein containing at least 0.5 ng of each singular peptide product. Minimum sample load requirements depend on the sensitivity limits of the downstream analysis system. Sample recovery for typical peptides is > 85%, but could be as low as 35% for hydrophilic peptides.
- To avoid inaccurate volumetric dispensing, do not use Pierce™ C18 Pipette Tips for measuring volume.
- 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 centrifugal vacuum evaporator but avoid complete dryness, which might result in sample loss. Carefully dilute or resuspend the sample in water with 0.1–1.0% trifluoroacetic acid (TFA) before processing with the Pierce™ C18 Pipette Tips.
- For optimal results, prepare all solutions and collection tubes in advance and proceed with the entire procedure in a timely manner. Do not introduce air through the membrane during any portion of the procedure for optimum flow and peptide recovery.
- Plastics used during handling of peptide samples can introduce contaminants that interfere with MS analysis and result in sample loss from nonspecific adsorption. Use high-quality receiver tubes. If necessary, receiver tubes used for the final collection may be rinsed with 70% ACN/0.1% formic acid before use. Minimizing sample transfers and freeze/thaw cycles before analysis will help minimize plastic contamination and sample loss.

Required materials not supplied

- 10-µL or 100-µL pipettor
- Ultrapure water
- Acetonitrile (ACN)
- Trifluoroacetic acid (TFA)
- Autosampler vials or 0.5-mL, 1.5-mL microcentrifuge tubes
- Formic acid (FA) or acetic acid
- *(Optional)* MALDI matrix
- *(Optional)* Methanol

Prepare materials

- Sample treatment solution: 2.5% TFA
- Wetting solution: 50:50 ACN:water; 20 µL or 200 µL per sample
- Equilibration solution: 0.1% TFA in water; 20 µL or 200 µL per sample
- Rinse solution: 0.1% TFA in 5% ACN:water; 20 µL or 200 µL per sample
- Elution solution: 0.1% TFA in 50–95% ACN:water for MALDI-MS or 0.1% FA in 50–75% ACN:water for ESI-MS, up to 100 µL per sample

Process sample with the 10-µL C18 tips

Note: For optimal flow and peptide recovery, do not introduce air through the membrane at any time during the procedure.

1. Set the pipettor to 10 µL and secure the pipette tip tightly to the end of the pipettor for optimum tip-to-pipettor seal and sample aspiration.
2. Adjust the sample to 0.1–1.0% TFA using 2.5% TFA.
3. Wet the tip by aspirating 10 µL of 50% ACN in water and then discarding the solvent. Repeat once.
4. Equilibrate the tip by aspirating 10 µL of 0.1% TFA and discarding the solvent. Repeat once.
5. Aspirate up to 10 µL of sample (prepared in Step 2 on page 2) into the C18 tip. For maximum efficiency, dispense and aspirate the sample for 3–10 cycles.
6. Rinse the tip by aspirating 10 µL of 0.1% TFA/5% ACN and discarding solvent. Repeat once.
7. Elute the sample as follows:

   MALDI-TOF analysis: Slowly aspirate 2–10 µL of 0.1% TFA in 50–95% ACN elution solution with or without matrix and dispense directly onto a MALDI plate.

   LC/MS or LC/MS/MS analysis: Slowly aspirate 2–10 µL of 0.1% formic acid or 0.1% acetic acid in 50–95% ACN or methanol and dispense into an autosampler vial or well plate.

Process sample with the 100-µL C18 tips

Note: For optimal flow and peptide recovery, do not introduce air through the membrane at any time during the procedure.

1. Set the pipettor to 100 µL and secure the pipette tip tightly to the end of the pipettor for optimum tip-to-pipettor seal and sample aspiration.
2. Adjust the sample to 0.1–1.0% TFA using 2.5% TFA.
3. Wet the tip by aspirating 100 µL of 50% ACN in water and then discarding the solvent. Repeat once.
4. Equilibrate the tip by aspirating 100 µL of 0.1% TFA and discarding the solvent. Repeat once.
5. Aspirate up to 100 µL of sample (prepared in Step 2 on page 3) into the C18 tip. For maximum efficiency, dispense and aspirate the sample for 3–10 cycles.
6. Rinse the tip by aspirating 100 µL of 0.1% TFA/5% ACN and discarding solvent. Repeat once.
7. Elute the sample as follows:

   MALDI-TOF analysis: Slowly aspirate 5–100 µL of 0.1% TFA in 50–95% ACN elution solution with or without matrix and dispense directly onto a MALDI plate.

   LC/MS or LC/MS/MS analysis: Slowly aspirate 5–100 µL of 0.1% formic acid or 0.1% acetic acid in 50–95% ACN or methanol and dispense into an autosampler vial or well plate.

Troubleshooting

Observation	Possible cause	Recommended action
Poor or incomplete sample binding	High pH, lack of ion-pairing agents.	Ensure TFA was added to the sample.
	Tip not sufficiently wetted.	Check buffers and prepare new tip.
	Sample contains organic solvent.	Dry sample and resuspend in 10 µL or 100 µL of 0.1–1.0% TFA.
	Sorbent became dry before adding sample.	Ensure that air is not drawn into the tip and that sorbent does not dry during sample processing.
	Sample not sufficiently hydrophobic to bind C18 sorbent.	None.
Poor or incomplete sample recovery	Highly hydrophobic sample.	Use 70% ACN for elution.
	Peptides binding to plastics can cause significant loss at low peptide concentrations.	Minimize contact with plastics, excessive drying, and storage at low concentrations (i.e., < 300 fmol).
	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.

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. MAN0011713

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