
Technical Note

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Title: **Sample Preparation of Peptides or Proteins Prior to Electrospray /
Nanospray MS Using ZipTip_{C18}, ZipTip_{μ-C18} and ZipTip_{C4} Pipette Tips**

INTRODUCTION

ZipTip™ is a 10 μL (P-10) pipette tip with a bed of chromatography media fixed at its end such that there is no dead volume. It is intended for purifying and concentrating femtomoles to picomoles of protein, peptide or oligonucleotide samples prior to analysis, providing better data quality. The sample is aspirated and dispensed through ZipTip to bind, wash, and elute. Recovered samples are contaminant-free and eluted in 0.5-4 μL for direct transfer to a mass spectrometer target or vial.

This protocol provides a guideline for using ZipTip_{C18} and ZipTip_{C4} to facilitate protein or peptide binding, salt and detergent removal, and sample elution for direct electrospray/nanospray MS analysis. C₁₈ is offered in two bed volumes; ZipTip_{C18} - a standard bed of 0.6 μL for sample elution in 1 to 4 μL, and ZipTip_{μ-C18} - a micro bed of 0.2 μL for elution in < 1 μL.

MATERIALS

- ZipTip_{C18}, ZipTip_{μ-C18} or ZipTip_{C4} pipette tips
- P-10 pipettor, multi-channel P-10 pipettor (Biohit Proline® pipettor recommended) or compatible automated liquid handling work station
- Wetting solution: 50% methanol (MeOH) in (TFA) in Milli-Q® water
- Equilibration and washing solution: 0.1%TFA in Milli-Q water
- Sample preparation solution: 2.5% TFA in Milli-Q water (5X stock solution)
NOTE: in the case of proteins a 8M guanidine and 2.5% TFA in Milli-Q water solution (5X stock solution) may substituted if sample solubility is a problem
- Elution solution: Peptides - 50% MeOH/0.1% TFA or 0.1% formic acid in Milli-Q water
Proteins - 75% MeOH/0.1% TFA or 0.1% formic acid in Milli-Q water

NOTE: Since the resin bed provides a slight backpressure, the pipettor should not be used as an accurate volumetric dispenser. To achieve optimal sample uptake and delivery, set pipettor to 10 μL and attach tip securely. Depress plunger to dead stop and slowly release or dispense plunger throughout operation.

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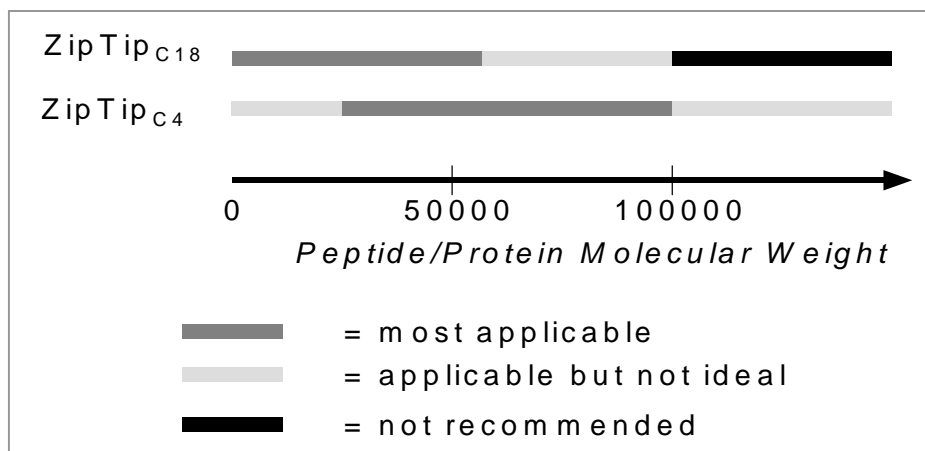
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GUIDELINES FOR SELECTING ZIPTIP_{C18} AND ZIPTIP_{C4}



ZipTip_{C18/μ-C18} is most applicable for low molecular weight proteins and peptides, while ZipTip_{C4} is most suitable for low to intermediate molecular weight proteins. In many cases, the two devices can be used interchangeably, as indicated by the overlapping bars in the above figure. Because higher molecular weight proteins tend to adsorb tenaciously to hydrophobic surfaces, ZipTip_{C4} is recommended for proteins over 100,000 MW.

PROCEDURE

Prepare the Sample:

Maximum binding to the ZipTip is achieved in the presence of TFA or other ion-pairing agents. To maximize analyte binding, use the appropriate **sample preparation solution**. The final TFA concentration should be between 0.1%–1.0% at a pH of <4. Optimal binding of protein to ZipTip may also require a chaotropic agent (e.g. guanidine-HCl at a final concentration of approximately 1–4M). If sample does not already contain a chaotropic salt, add a few minutes before binding. In the case of excess detergent, dilute sample with 0.1% TFA to achieve acceptable binding conditions, for example, SDS (<0.1%), Triton[®] (<1%), and Tween[®] (<0.5%).

Equilibrate the ZipTip for Sample Binding:

1. Prewet the tip by depressing pipettor plunger to a dead stop using the maximum volume setting of 10 μL. Aspirate **wetting solution** into tip. Dispense to waste. Repeat.
2. Equilibrate the tip for binding by washing with the **equilibration solution** 3 times.

Bind and Wash the Peptides or Proteins:

Follow these steps after equilibration.

1. Bind peptides and proteins to ZipTip by fully depressing the pipettor plunger to a dead stop. Aspirate and dispense sample 3 to 7 cycles for simple mixtures and up to 10 cycles for maximum binding of complex mixtures.
2. Wash tip and dispense to waste using at least five cycles of **wash solution**. A 5% methanol in 0.1% TFA/water wash can improve desalting efficiency.

Elute the Peptides or Proteins:

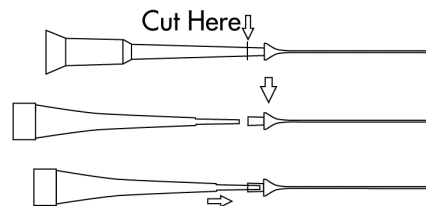
For ZipTip_{C18} and ZipTip_{C4}, dispense 1 to 4 μL of **elution solution** into a clean vial using a standard pipette tip. In the case of ZipTip _{μ -C18}, dispense 0.5 to 2 μL of elution solution into a clean vial. Carefully, aspirate and dispense eluant through ZipTip at least three times without introducing air. Sample recovery can be improved (at the expense of concentration) by increasing elution volume to 10 μL or by performing multiple elutions.

CAUTION: Methanol is volatile and evaporation can occur rapidly. If this occurs, add more eluant to recover sample.

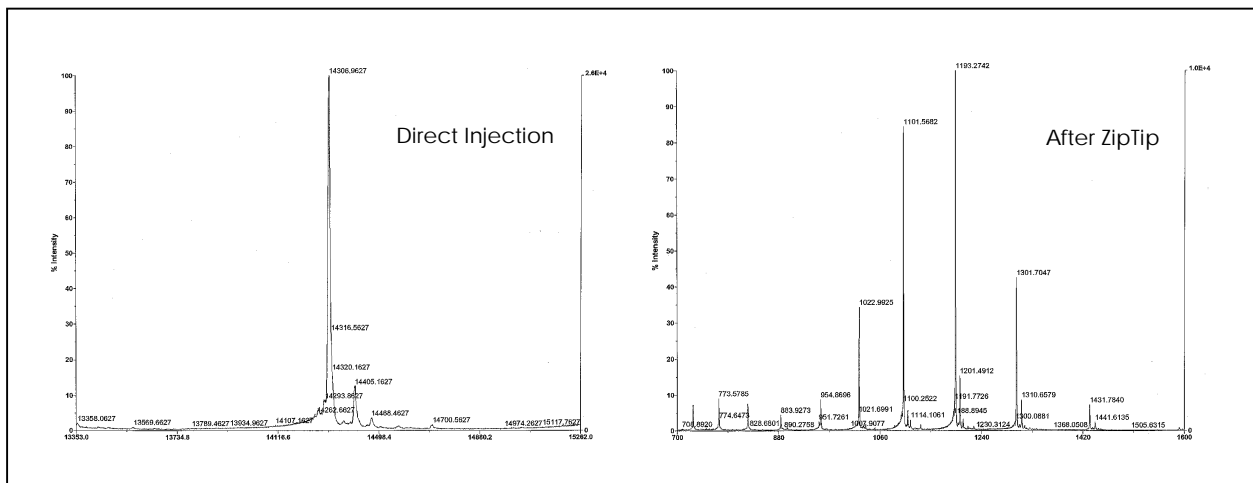
For Electrospray MS:

Sample can be eluted into clean vial or, using a GELoader[®] tip (Eppendorf cat. no. 0030 001 222), into a nanospray needle.

1. Cut the GELoader tip about 2–3 mm above where the tip is fused to its capillary end.
2. Before the final dispense, firmly press the cut-down GELoader tip onto the ZipTip with a slight twisting motion. The leak-free fit allows direct elution into a nanospray needle.



RESULTS



Nanospray-ES of lysozyme desalted on ZipTip. ZipTip_{C18} treatment of salt, guanidine and phosphate buffer containing lysozyme is shown using nanospray technology. The charge to mass spectra (right) is devoid of salt and exhibits excellent resolution. The deconvoluted mass peak is of high quality and accurate mass due to efficient salt removal.

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