Technical Note

Date: **March 30, 2000**

Lit. No.: **TN226**

Title: **Fractionation of Complex Peptide or Protein Mixtures Prior to MALDI-TOF MS Using ZipTip$_{C18}$, ZipTip$_{\mu\text{-}C18}$ 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 using ZipTip$_{C18}$ and ZipTip$_{C4}$ to fractionate complex peptide or protein mixtures. C_{18} 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$_{\mu\text{-}C18}$ - a micro bed of 0.2 µL for elution in < 1 µL.

Complex peptide or protein mixtures can be partially fractionated using an acetonitrile step-gradient to enrich hydrophilic and hydrophobic peptides or proteins. The separation of large fragments from small not only simplifies spectra, but also minimizes peak suppression of larger fragments.

MATERIALS

- ZipTip$_{C18}$, ZipTip$_{\mu\text{-}C18}$ 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% acetonitrile (ACN) 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 an 8M guanidine and 2.5% TFA in Milli-Q water solution (5X stock solution) may substituted if sample solubility is a problem
- Prepare varying concentrations of ACN in Milli-Q water (e.g., 5%, 10%, 20%, 30%, 50% and 70%) with or without 0.1% TFA. For direct spotting in matrix, elute with desired matrix (e.g. cyano-4-hydroxy-cinnamic acid at 10 mg/mL)

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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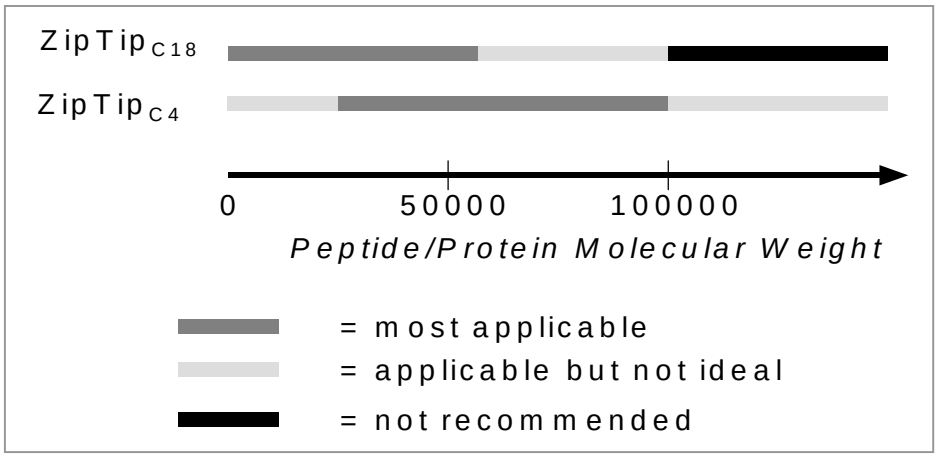
GUIDELINES FOR SELECTING ZIPTIPC18 AND ZIPTIPC4



ZipTip$_{C18/\mu\text{-}C18}$ 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 1–4M). If sample does not already contain chaotropic salts, add them 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 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 it twice with the **equilibration solution.**

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 10 cycles depending on sample concentration. Dilute solutions require increased contact time.
2. Wash tip and dispense to waste using at least 2 cycles of **wash solution.** A 5% methanol in 0.1% TFA/water wash can improve desalting efficiency.

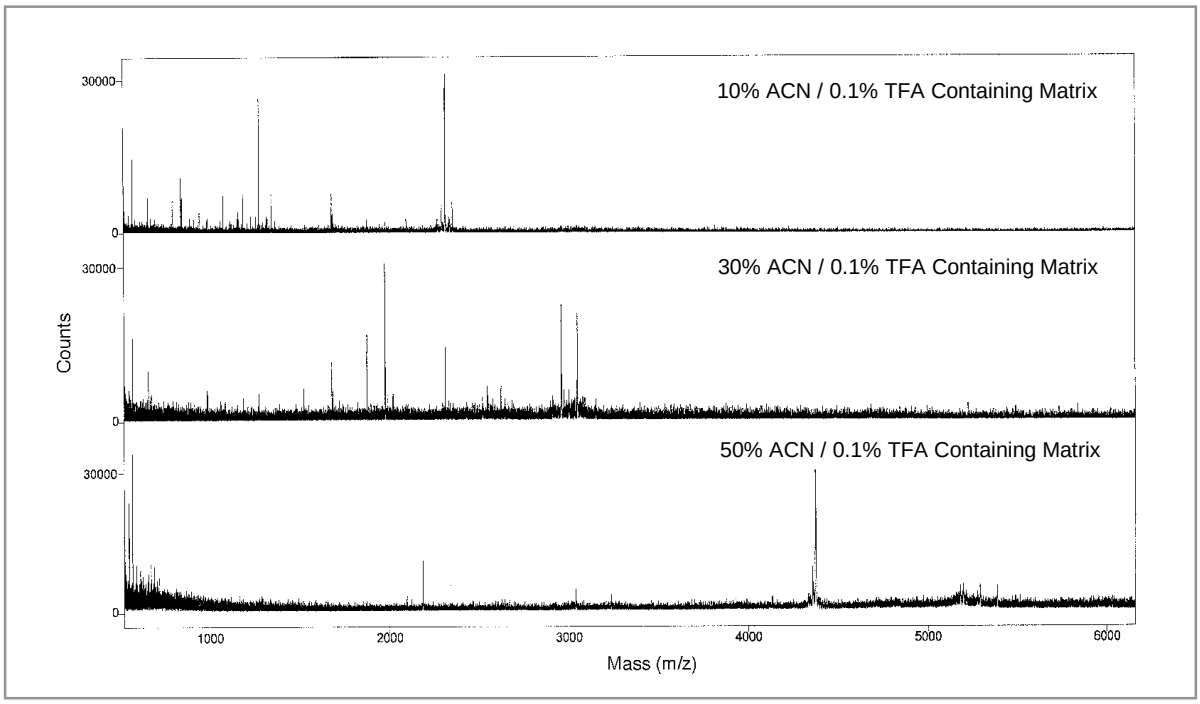
Step Gradient Elution of Peptides or Proteins:

1. Pipette 1 to 3 µL of 5% acetonitrile/0.1% TFA into clean vial using a standard pipette tip. Carefully, aspirate and dispense eluant through the ZipTip at least 3 times without introducing air. If desired, use the final dispense cycle to apply the desalted-concentrated peptides or proteins directly onto the MALDI-TOF MS target.
2. Wash tip immediately using 3 cycles of 5% acetonitrile to waste prior to subsequent elution step.
3. Perform next step gradient (e.g. 10, 20, 30 or 50% ACN) by increasing acetonitrile concentration and repeat steps 1 and 2 until step-gradient is completed.

NOTE: Thoroughly wash the tip with respective eluant prior to increasing ACN solution to minimize peptide or protein carry-over.

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

RESULTS



Step gradient elution following in-gel digestion of IgG. In addition to concentrating and desalting sample, step gradient elution with increasing acetonitrile serves to fractionate complex peptide mixtures and simplify spectra following ZipTip$_{C18}$.