



Using the ZipTip^{SCX} Pipette Tip

The following procedure describes how to prepare the sample, equilibrate the ZipTip^{SCX} pipette tip, bind sample, wash and elute purified peptides. See the “Materials” section for appropriate solutions.

NOTE: Because 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 the pipettor to 10 μL and attach ZipTip pipette tip securely. Depress the plunger to dead stop. Slowly release or depress plunger throughout operation.

Introduction

A ZipTip[®] pipette tip is a 10 μL pipette tip with a micro-volume bed of chromatography medium fixed at its end. It is designed for purifying and concentrating femtomoles to picomoles of proteins and peptides, providing improved data quality.

These instructions describe the use of ZipTip^{SCX} pipette tips containing strong cation exchange resin for the concentration of peptides and removal of salts and nonionic detergents from peptide mixtures prior to mass spectrometry.

Strong cation exchange (SCX) chromatography is an alternative to reversed-phase (C18) chromatography for the separation of peptides. SCX chromatography works especially well for tryptic peptides that have a minimum of two positive charges per molecule at acidic pH.

Peptides from acidic buffer are captured on the ZipTip resin, washed, and then eluted directly onto a MALDI-TOF MS target at basic pH. Once the samples have dried, they are overlaid with matrix, dried again, and analyzed.

ZipTip^{SCX} pipette tips effectively capture dilute peptides from in-gel or in-solution digested protein samples and reversed-phase chromatography fractions containing $\leq 45\%$ acetonitrile. ZipTip^{SCX} pipette tips can also be used for the removal of non-ionic detergents.

Materials

NOTE: For optimal results, all solutions should be freshly prepared using Milli-Q[®] grade water.

- ZipTip^{SCX} pipette tips
- 10 μL pipettor

NOTE: A VistaLab ULTRON[®] Handheld Liquid Handling System with ZipTip Adapter Kit (VistaLab Cat. No. 1052-9016) is recommended as a variable speed, programmable electronic pipettor. The Biohit Pro-line[™] electronic multichannel pipettor is recommended for processing eight samples simultaneously. Visit www.millipore.com/ziptip for a current listing of compatible automated liquid handling work stations.

- Sample Preparation Solution: 10% trifluoroacetic acid (TFA) in Milli-Q grade water
- Washing Solution: 0.1% TFA in Milli-Q grade water.
Washing Solution for detergent removal: 0.1% TFA/30% methanol in Milli-Q grade water
- Elution Solution: 5% ammonium hydroxide/30% methanol in Milli-Q grade water

Sample Preparation

Dissolve the sample in 5-100 μL of Washing Solution. For in-solution mixtures, add the appropriate amount of Sample Preparation Solution to achieve a final TFA concentration of 0.1%. Sample pH should be ≤ 4 .

Equilibrating the ZipTip^{SCX} Pipette Tip

1. Set the pipettor volume to 10 μL .
2. Depress the plunger to dead stop and aspirate the Washing Solution into the ZipTip pipette tip, dispense to waste.
3. Repeat three times.

Binding and Washing

1. Bind peptides to the equilibrated ZipTip pipette tip, by performing 10-15 aspirate-dispense cycles of the entire sample.
2. Aspirate 10 μL of Washing Solution into the ZipTip pipette tip, dispense to waste. Repeat five times to ensure sufficient removal of salts and detergents.

Eluting the Sample for MALDI-TOF MS Analysis

1. Desorb bound peptides by aspirating 2.5 μL of Elution Solution into the ZipTip^{SCX} pipette tip. Spot directly onto MALDI-TOF MS target, pipette up and down three times.
2. Dry spots.
3. Overlay each peptide spot with 1 μL of preferred matrix solution.

Eluting the Sample for Electrospray MS Analysis

1. Desorb bound peptides by aspirating 5 μL of Elution Solution into the ZipTip pipette tip. Elute into clean tube. Pipette up and down three to five times.
2. Allow sample to evaporate to near dryness.
3. Reconstitute sample in compatible electrospray solution.

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