User Guide for Reversed-Phase ZipTip®

Pipette Tips for Sample Preparation

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Introduction

The ZipTip® pipette tip is a 10 µL pipette tip with a bed of chromatography media fixed at its end. It is intended for concentrating and purifying peptide, protein or oligonucleotide samples.

These instructions describe the use of ZipTip pipette tips containing C_{18} and C_4 reversed-phase media for desalting and concentrating peptides and proteins.

For information on concentrating and desalting oligonucleotide samples, request Millipore publication TN225.

NOTE: Because the adsorptive bed provides a slight back pressure, do not use the ZipTip pipette tip for accurate volumetric dispensing. To achieve optimal sample uptake and delivery, set the pipettor to 10 µL and attach the ZipTip pipette tip securely. Throughout the procedure, depress and release the plunger slowly to ensure optimal movement of solution through the resin bed.

Materials

The following table outlines the solutions required for use with ZipTip pipette tips containing C_{18} and C_4 media. C_{18} is offered in two bed volumes:

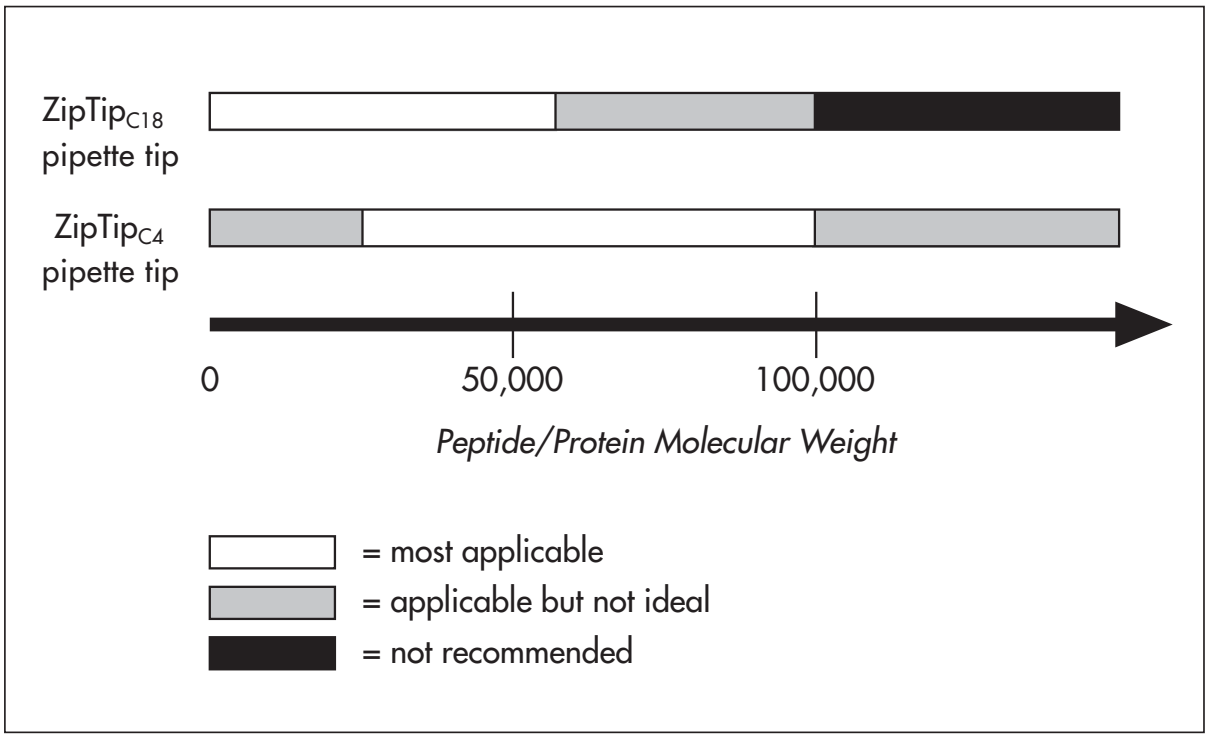
- $ZipTip_{C18}$ tips — a standard bed of 0.6 µL for sample elution in 1 to 4 µL
- $ZipTip_{µ\text{-}C18}$ tips — a micro bed of 0.2 µL for elution in < 1 µL

The procedure also requires a compatible 10 µL pipettor. For simultaneous processing of multiple samples, Millipore recommends the Biohit Proline™ Multi-channel Pipettor.

Solution	$ZipTip_{C18/µ\text{-}C18}$ Pipette Tips	$ZipTip_{C4}$ Pipette Tips
Wetting solution	100% acetonitrile (ACN)	100% acetonitrile (ACN)
Sample preparation	Adjust sample to 0.1% trifluoroacetic acid (TFA); final sample pH should be <4	Adjust sample to 0.1% trifluoroacetic acid (TFA); final sample pH should be <4 (Optional) Guanidine-HCl 1-6M may be added.
Equilibration solution	0.1% TFA in Milli-Q® grade water	0.1% TFA in Milli-Q grade water
Wash solution	0.1% TFA in Milli-Q grade water	0.1% TFA in Milli-Q grade water
Elution solution*	0.1% TFA/50% ACN with or without matrix	0.1% TFA/50-75% ACN with or without matrix

** For electrospray, elute with 1% formic acid/50% methanol. For fractionating peptides, prepare varying concentrations of ACN/water (e.g. 5%, 10%, 20%, 30%, and 50%) with or without 0.1% TFA.*

Guidelines for Selecting ZipTip$_{C18}$ or ZipTip$_{C4}$ Pipette Tips



ZipTip$_{C18}$ pipette tips are most applicable for peptides and low molecular weight proteins, while ZipTip$_{C4}$ pipette tips are most suitable for low to intermediate molecular weight proteins. In many cases, the two devices can be used interchangeably. Because higher molecular weight proteins tend to adsorb tenaciously to hydrophobic surfaces, ZipTip$_{C4}$ pipette tips are recommended for proteins over 100,000 MW.

Procedures for Use

The following procedures describe how to prepare the sample and equilibrate the ZipTip pipette tips for sample binding, washing, and elution. See the "Materials" section for information on the appropriate solutions for your application.

Prepare the Sample

Maximum binding to the ZipTip pipette tip is achieved in the presence of TFA (0.1%) or other ion-pairing agents. Ensure that the final sample solution has a pH<4.

Optimal binding of protein to the ZipTip$_{C4}$ pipette tip may also require a chaotropic agent (e.g., guanidine-HCl at a final concentration of 1–6M). If the sample does not already contain chaotropic salts, add them a few minutes before binding. These salts will be removed during the wash step following sample binding.

Equilibrate the ZipTip Pipette Tip for Sample Binding

1. Depress pipettor plunger to a dead stop. Using the maximum volume setting of 10 µL, aspirate **wetting solution** into the tip. Dispense to waste. Repeat.
2. Aspirate **equilibration solution.** Dispense to waste. Repeat.

Bind and Wash the Peptides or Proteins

Follow these steps after equilibrating the ZipTip pipette tip:

1. Bind peptides and/or proteins to ZipTip pipette tip by fully depressing the pipette plunger to a dead stop. Aspirate and dispense the sample 7–10 cycles for maximum binding of complex mixtures.
2. Aspirate **wash solution** into tip and dispense to waste. Repeat at least once.

   NOTE: A 5% methanol in 0.1% TFA/water wash can improve desalting efficiency. Additional washing may be required for electrospray MS.

Elute the Peptides or Proteins

For $ZipTip_{C18}$ (standard bed format) and $ZipTip_{C4}$, pipette tips, dispense 1 to 4 µL of **elution solution** into a clean vial using a standard pipette tip. In the case of $ZipTip_{µ\text{-}C18}$ (micro bed format) pipette tips, dispense 0.5 to 2 µL of elution solution into a clean vial.

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

Carefully, aspirate and dispense eluant through ZipTip pipette tip at least three times without introducing air into the sample. Sample recovery can be improved (at the expense of concentration) by increasing elution volume to 5 µL.

For Direct Spotting onto a MALDI-TOF MS Target

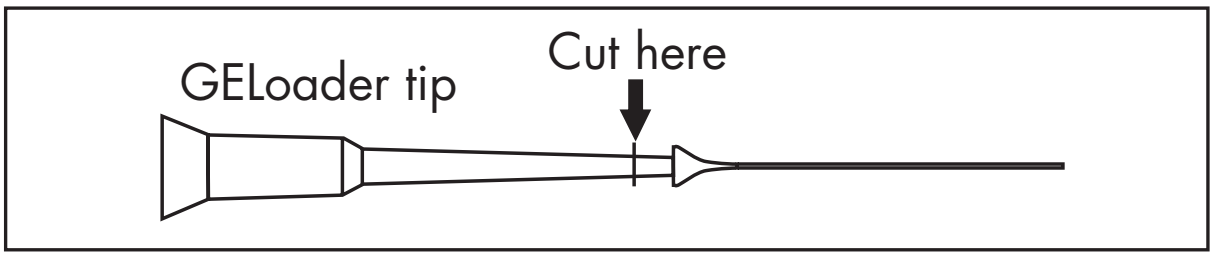
Elute with or without matrix in **elution solution.**

1. Pipette 0.5 to 4 µL of desalted-concentrated sample directly onto target by depressing plunger until appropriate volume is dispensed.
2. Save or discard the remaining sample.

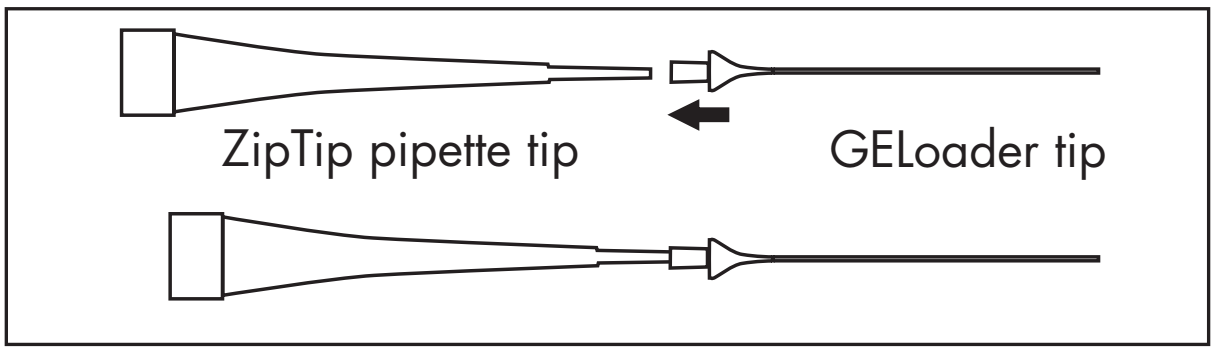
For Nanoelectrospray MS

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 pipette tip with a slight twisting motion.



The leak-free fit allows elution directly into a nanospray needle.

For Fractionating Peptides or Proteins

1. Pipette 1 to 3 µL of 0.1% TFA/5% acetonitrile into clean vial. Perform 3–4 aspirate-dispense cycles to elute hydrophilic peptides or proteins from tip. Use the final dispense cycle to apply peptides or proteins directly to target.
2. Wash tip immediately by aspirating 0.1% TFA/5% acetonitrile. Dispense to waste. Repeat twice.
3. Perform next step gradient (e.g. 5, 10, 20, 30 or 50% ACN) by increasing acetonitrile and repeat steps 1 and 2 until step-gradient is completed.

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

Chemical Compatibility

✔ = Acceptable. Long exposures at room temperature have no significant effect.

? = Questionable. Short exposures at room temperature cause little or no damage.

X = Not recommended. Short exposure may cause permanent damage.

Reagent	ZipTip Pipette Tips
Acetic Acid (Glacial)	✔
Acetone	X
Acetonitrile (100%)	✔
Aliphatic Esters	?
Ammonium Hydroxide (5%)	✔
Benzene (100%)	X
n-Butanol (100%)	✔
Butyl Acetate	X
Chloroform (1%)	?
Dichlorobenzene (100%)	X
Dichloromethane (1%)	?
Diethanolamine (5%)	✔
Dimethyl Acetamide (100%)	X
Dimethyl Formamide (100%)	X
Dimethyl Formamide (1%)	✔
Ethanol (100%)	✔
Formic Acid (5%)	✔
Guanidine HCl (6 M)	✔
Hydrochloric Acid	✔

Chemical Compatibility, continued

Reagent	ZipTip Pipette Tips
Hydrogen Peroxide	✔
Isopropyl Alcohol (100%)	✔
Mercaptoethanol (0.1 M)	✔
Mercaptoethanol (1.0 M)	?
Methyl Alcohol (100%)	✔
Methyl Ethyl Ketone (100%)	X
Methyl Isobutyl Ketone (100%)	✔
Nitric Acid (0.1 N)	✔
Nitric Acid (1.5 N)	✔
Phenol (0.5%)	X
Phosphoric Acid (1M)	✔
Sodium Azide (1%)	✔
Sodium Hydroxide (0.5 N)	✔
Sodium Hydroxide (0.1 N)	✔
Sodium Hypochlorite (100 ppm)	✔
Sodium Hypochlorite (200 ppm)	✔
Sulfuric Acid (1%)	✔
Toluene (1%)	X
Triton® X-100	✔
Tween®	✔
Urea (6 M)	✔

Troubleshooting

Sample preparation problems with ZipTip$_{C18}$ (standard and micro bed formats) and ZipTip$_{C4}$ pipette tips can be divided into two categories:

- sample does not bind
- sample binds but is not recovered

The following table outlines common problems and their possible causes, and suggests procedures to solve those problems. The procedures may or may not work with your ZipTip application.

Incomplete binding is the problem most often encountered when performing sample preparation. To determine whether the problem is binding or elution, the best approach is to try the first three procedures for incomplete binding in the following table. Then, use a step-gradient approach to elution. First, elute the tip with 50% ACN in 0.1% TFA. Then, repeat the elution (on the same tip) with 75% ACN in 0.1% TFA. This method addresses both types of problems using a single sample.

If the problems persist, contact Millipore Technical Service for further suggestions.

Incomplete Binding

Possible Cause	Suggested Procedure
C_{18}/C_4 beads dewetted before sample was applied. The hydrophobic beads can de-wet in less than a minute.	After wetting with ACN, flush the tip with 0.1% TFA and leave the plug immersed in liquid until immediately before sample binding.
Sample was not sufficiently acidified with TFA. The pH should be below 4. The TFA concentration should be between 0.1–1.0%.	Spike sample with a few microliters of 0.5–1% TFA.
Sample not freely soluble.	Add Guanidine HCl to the sample to achieve a final concentration between 1–6M. Guanidine actually enhances binding by helping to wet the hydrophobic surface and reducing polypeptide secondary structure.
Sample too hydrophilic for adsorption.	Relatively few options for solving this problem. The best approach is to use a $ZipTip_{SCX}$ pipette tip instead of a reversed-phase tip.
Sample amount too low for detection.	Make sure samples are within the detection limits of the instrument. In general, a good MS signal should be obtained with 1 picomole of sample (e.g. 5 µL of a 0.2 picomole/µL solution). Substantially lower amounts can be detected if the sample is clean.

Incomplete Elution

Possible Cause	Suggested Procedure
Sample tenaciously adsorbed to the C_{18}/C_4 particles.	Increase acetonitrile content of desorption solution to a maximum of 75–90% ACN (v/v) in 0.1% TFA.
Sample not freely soluble in ACN.	Decrease ACN concentration to 20–40% in a 0.1% TFA or suitable ion-pairing agent.

Specifications

Materials of Construction

Pipette tip: Polypropylene

Media: C_{18}: spherical silica, 15 µm, 200Å pore size

C_4: spherical silica, 15 µm, 300Å pore size

Tip volume 10 µL

Adsorptive bed

C_{18}*(standard bed format):* 0.6 µL

C_{18}*(micro bed format):* 0.2 µL

C_4: 0.6 µL

Length 31 mm (1.22 in.)

Capacity

(when used with saturating amounts of analyte):

C_{18}*(standard bed format):* ≥ 1.0 µg; typically 5.0 µg

C_{18}*(micro bed format):* typically 2.0 µg

C_4: ≥ 0.5 µg; typically 3.3 µg

Max. Temperature 70 °C

Min. Temperature 4 °C

Working pH Range 2 to 13

Ordering Information

ZipTip Pack	Resin Type	Catalogue Number
8 pack, resealable	C_{18}(standard bed)	ZTC 18S 008
	C_{18}(micro bed)	ZTC 18M 008
	C_4	ZTC 04S 008
24 pack, resealable	C_{18}(standard bed)	ZTC 18S 024
	C_{18}(micro bed)	ZTC 18M 024
	C_4	ZTC 04S 024
96 pack, tip rack	C_{18}(standard bed)	ZTC 18S 096
	C_{18}(micro bed)	ZTC 18M 096
	C_4	ZTC 04S 096
960 pack, 10 × 96 tip rack	C_{18}(standard bed)	ZTC 18S 960
	C_{18}(micro bed)	ZTC 18M 960
	C_4	ZTC 04S 960

For a complete listing of available ZipTip chemistries, visit www.millipore.com/ziptip or contact your local Millipore office.

Technical Assistance

For more information, contact the Millipore office nearest you. In the U.S., call **1-800-MILLIPORE** (1-800-645-5476). Outside the U.S., see your Millipore catalogue for the phone number of the office nearest you or go to our web site at www.millipore.com/offices for up-to-date worldwide contact information. You can also visit the tech service page on our web site at www.millipore.com/techservice.

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