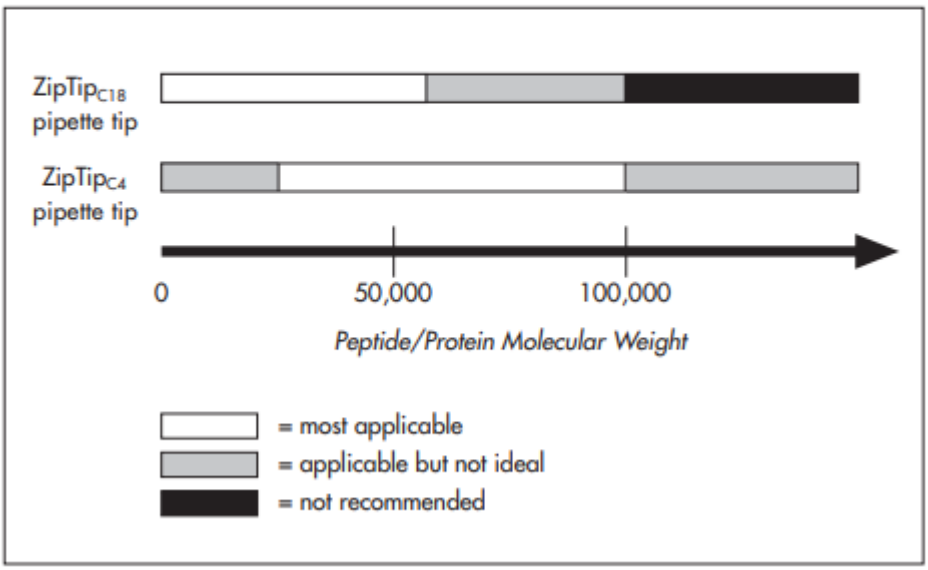
ZipTip Protocol

Guidelines for Selecting $ZipTip_{C18}$ or $ZipTip_{C4}$ Pipette Tips



Solutions Required

Buffer A

100% Acetonitrile HPLC-grade

Buffer B

98% Milli-Q water

2% Acetonitrile HPLC-grade

0.1% Formic Acid **(FA) for nanoLC** or 0.1% Trifluoroacetic Acid **(TFA) for MALDI**

Buffer C

50% ACN

50% Milli-Q water

0.1% FA

Buffer D

Matrix CHCA or 2.5-DHB (20 mg/mL matrix in 30:70 (v/v) Acetonitrile: TFA 0.1% in water) - peptides

Matrix 2.5-DHAP (7.6 mg matrix in 375 µl EtOH. Add 125µl of a solution containing 18mg/ml DAC dissolved in water) - Large Proteins

Matrix SA (20 mg/mL matrix in 30:70 (v/v) Acetonitrile: TFA 0.1% in water) - Proteins

Procedure

Note: Resin bed provides backpressure, so set pipette to 10 µl, depress plunger to dead stop and slowly release.

1. Acidify sample (Vol 20-100 µl) by adding **TFA (MALDI**) or **FA (nanoLC)** to 0.1 % final concentration.

2. ZipTip equilibration

- Aspirate 10 µl **Buffer A** into tip and dispense to waste. Repeat.
- Aspirate 10 µl **Buffer B** into tip and dispense to waste. Repeat.

3. Bind and Wash the peptides/proteins

- Bind peptides to ZipTip pipette tip by aspirating and dispensing 3-7 cycles (simple mixtures), up to 10 cycles (complex).
- Aspirate 10 µl **Buffer B** and dispense to waste. Repeat wash once more.

4. Elution:

nanoLC-MS	**MALDI-TOF/TOF**
Dispense 10µl of **Buffer C** into a clean Eppendorf tube. Aspirate and dispense elution solution through ZipTip at least 5 times without introducing air. Dispense 10µl **of Buffer A** into a clean Eppendorf tube. Aspirate and dispense elution solution through ZipTip at least 5 times without introducing air. Combined the elution solutions into an empty Eppendorf tube. Dry in vacuum centrifuge. Resuspend in 10 µl **Buffer B**	Dispense 1-3µl of **Buffer D** into a clean Eppendorf tube. Aspirate and dispense elution solution through ZipTip at least 5 times without introducing air.
Transfer to a recovery vial	After 5 elution times through ZipTip spot on MALDI plate. Leave to dry