

Zip Tip Protocol for Peptide and Protein Analysis

I. Materials

- **Wetting solution** (100% HPLC-grade acetonitrile)
- **Stock solution** (1% TFA in MilliQ water): 990 μL MilliQ water, 10 μL TFA
- **Sample preparation:** Adjust sample to 0.1% TFA
- **Equilibration & Washing solutions:** (0.1% TFA in MilliQ water):
100 μL 1% TFA in MilliQ water, 900 μL MilliQ water
- **Elution solution** (50% acetonitrile in 0.1% TFA):
300 μL HPLC-grade acetonitrile, 240 μL MilliQ water, 60 μL 1% TFA in MilliQ water

Note: For electrospray, elute with 1% formic acid/50% HPLC-grade methanol

II. Procedure

NOTE: Resin bed provides back pressure, so set pipettor to 10 μL , depress plunger to dead stop and slowly release or dispense plunger throughout operation.

1. Equilibrate:

Aspirate 10 μL **wetting solution** into tip and dispense to waste. Repeat. Aspirate **equilibration solution** into tip and dispense to waste. Repeat.

2. Bind & wash:

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

Note: A 5% methanol in 0.1% TFA/water wash can improve desalting efficiency.

3. Elute:

Dispense 1-4 μL of **elution solution** into clean 0.5 mL Eppendorf microcentrifuge tube using a standard pipette tip (Note: if $\mu\text{-C-18}$, dispense 0.5-2 μL of elution solution). Aspirate and dispense eluant through ZipTip at least 3 times without introducing air. Sample recovery can be improved by increasing elution volume to 10 μL (but at expense of concentration).