

C18 ZipTip Peptide Clean-up Protocol for LC-MS/MS

Maximum binding to zip tip is achieved when sample solution pH is < 4. Add FA to sample to final concentration 2% v/v before proceeding with the protocol.

Solution Prep

- p Wetting solution: 100% ACN
- p Wash solution: 0.5% FA
- p Elution solution: 50% ACN, 0.5% FA

Set the pipettor volume to 10 μ L.

Wet and Equilibrate Tip

- oo Depress the plunger to dead stop and aspirate the Wetting Solution into the ZipTip pipette tip, dispense to waste.

Depress the plunger to dead stop and aspirate the Washing Solution into the ZipTip pipette tip, dispense to waste, repeat 3 times

Sample Binding

- oo Bind peptides to the equilibrated ZipTip pipette tip, by performing 7-10 aspirate-dispense cycles of the entire sample.

Washing

Aspirate 10 μ L of Washing Solution into the ZipTip pipette tip, dispense to waste. **Repeat five times** to ensure sufficient removal of salts and detergents.

Eluting

- oo Desorb bound peptides by aspirating 5 μ L of Elution Solution into the ZipTip pipette tip. Elute into clean tube. Pipette up and down three to five time.

Lyophilize sample in SpeedVac and store dried peptides at -20C

Before LC-MS re-suspend sample in 20 μ L 0.1% Formic acid (pipette and vortex), after this remove insoluble particulates with 15min/15000RPM tabletop centrifuge spin, transfer supernatant into clean HPLC vial