

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

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

Solution Prep

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

Use MQ Water to prepare solutions! Use clean glass bottles to store solutions (do not use plastic). Bottles should be brand new, washed only with MQ water and NEVER before exposed to detergents, or any other chemicals!!!!

Set the pipettor volume to 10 μL .

Wet and Equilibrate Tip

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

Sample Binding

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

Washing

4. 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

5. 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 time.
6. Dry sample in SpeedVac and store dried peptides at -20C

This step will be done by KMS:

Before LC-MS re-suspend sample in 10-20 μL 0.1% Formic acid (pipette and vortex), after this remove insoluble particulates with 15min/15000RPM tabletop centrifuge spin, transfer supernatant into clean HPLC vial.