



Protocol for Zip-Tipping tryptic digests

Consumables

- Milipore ZipTip Pipette Tips C18; Size **P10**; Ref ZTC18S960 (binds a maximum of 5 ug)
- Protein LoBind Tube, 0.5ml, Eppendorf (Cat nr. 022431064 or equivalent)

Solutions

Stock solution: 1% TFA aq. (10 μ L TFA in 1ml dd H₂O)

Sample Preparation: 0.5% TFA aq. (500 μ l 1% TFA, 500 μ L dd H₂O)

Equilib & Washing: 0.1% TFA aq. (100 μ l 1% TFA, 900 μ l dd H₂O)

Wetting & Elution: 50% ACN in 0.1% TFA (300 μ l Acetonitrile, 300 μ l 0.1% TFA)

Procedure

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

NOTE: Dispense 12 μ L of *Elution Solution* into a set of clean labelled microfuge tubes, one for each sample digest. (Each zipped sample digest will be eluted into the appropriately labelled tube giving approximately 0.5 μ g/ μ L, or 2.5 μ g for a sample injection of 5 μ L.)

1. Wet and Equilibrate tip:

- Aspirate 10 μ L *Wetting Solution* into Zip tip.
- Dispense to waste
- Repeat
- Aspirate *Equilibration Solution*
- Dispense to waste
- Repeat

2. Bind peptides & Wash:

- Aspirate and dispense the sample through zip tip for 3-7 cycles (simple mixtures) or up to 10 cycles (complex)
- Aspirate *Equilibration and Washing solution*
- Dispense to waste
- Repeat

3. Elute peptides:

- Aspirate and dispense sample through zip tip into the 12 μ L *Elution Solution*, at least 3 times without introducing air.

NOTE: Zipped tryptic digests should be dried down on a Speedivac and each re-suspended into 10 μ L Buffer A (0.1% Formic Acid) for MS analysis on the Thermo Q-Exactive.