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## Sample preparation to mass spectrometer using ZipTip<sub>C18</sub>

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**Protocol status:** Working

**We use this protocol and it's working**

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## Abstract

ZipTip is a 10  $\mu$ L pipette tip that aims to concentrate and purify from femtomols to picomols protein and oligopeptide .

## Materials

### MATERIALS

⊗ ZipTipC18 Merck Millipore (EMD Millipore)

⊗ MilliQ Water

⊗ Acetonitrile Merck MilliporeSigma (Sigma-Aldrich) Catalog #34998

⊗ Pierce&trade; Trifluoroacetic Acid (TFA), Sequencing grade Thermo Fisher Catalog #28904

- Kimwipes
- P-10 pipettor
- 1.5 mL microtubes
- 0,2 mL microtubes

## Troubleshooting

## Safety warnings

- ! The contamination of the samples during the preparation is somewhat recurrent and this can compromise the analysis by LC-MS / MS. To avoid contaminants such as saliva and keratin, wear personal protective equipment: mask, gloves and lab coat

## Before start

Make sure all reagents and equipment are available. Plan the experiment!

## Prepae the materials and workspace

- 1 Calibrate the P-10 pipette to 10  $\mu$ L.
- 2 Name 4 microtubes of 1.5 mL with the respective reagents:
  - ACN<sub>100%</sub> (I)
  - TFA<sub>0.1%</sub> (I)
  - TFA <sub>0.1%</sub> (II)
  - ACN<sub>100%</sub> (II)

Add 1 mL of the respective solutions to the tubes above.
- 3 Name new 0.2 mL microtubes with sample names followed by "zt".
- 4 Aliquot 50  $\mu$ L of ACN 100% in each "zt" tube.
- 5 Fit the ZipTip with the pipette already attached, so as not to pass air through the "chromatographic column". Hold down until you start the procedure with the first pipetting.

## Procedure

- 6
  - **Cleaning and preparation:** ACN<sub>100%</sub> (I)

Pipette the acetonitrile from the ACN<sub>100%</sub> (I) tube 10 times, discarding the contents on the kimwipes
- 7
  - **Equilibrate:** TFA<sub>0.1%</sub> (I)

Pipette the acetonitrile from the TFA<sub>0.1%</sub> (I) tube 10 times, discarding the contents on the kimwipes
- 8
  - **Binding of peptides (analyte):** sample tube

Homogenize the sample with ZipTip 20 times



9     ■ **Wash:** TFA<sub>0.1%</sub> (II)

Pipette the acetonitrile from the TFA<sub>0.1%</sub> (II) tube 10 times, discarding the contents on the kimwipes

10    ■ **Elution:** ACN<sub>100%</sub> (II)

Homogenize 20 times in the "zt" tube, already with 50 µL of ACN<sub>100%</sub>

11    ■ **Shutdown:** ACN<sub>100%</sub> (II)

Pipette the acetonitrile from the ACN<sub>100%</sub> (II) 10 times, discarding in the "zt" tube.

## NOTE

- 12    During the entire process, no air should enter through ZipTip. Pipetting should be done slowly to maximize chemical interactions