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Sample preparation to mass spectrometer using ZipTip_{C18}

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ABSTRACT

ZipTip is a 10 µL pipette tip that aims to concentrate and purify from femtomols to picomols protein and oligopeptide .

MATERIALS

NAME	CATALOG #	VENDOR
ZipTip _{C18}		Merck Millipore
MilliQ Water		
Acetonitrile	34998	Sigma Aldrich
Pierce™ Trifluoroacetic Acid (TFA), Sequencing grade	28904	Thermo Fisher

MATERIALS TEXT

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

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 STARTING

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

Prepae the materials and workspace

- 1 Calibrate the P-10 pipette to 10 µL.

2 Name 4 microtubes of 1.5 mL with the respective reagents:

- ACN_{100%} (I)
- TFA_{0.1%} (I)
- TFA_{0.1%} (II)
- ACN_{100%} (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 µL 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_{100%} (I)

Pipette the acetonitrile from the ACN_{100%} (I) tube 10 times, discarding the contents on the kimwipes

7 ▪ **Equilibrate:** TFA_{0.1%} (I)

Pipette the acetonitrile from the TFA_{0.1%} (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_{0.1%} (II)

Pipette the acetonitrile from the TFA_{0.1%} (II) tube 10 times, discarding the contents on the kimwipes

10 ▪ **Elution:** ACN_{100%} (II)

Homogenize 20 times in the "zt" tube, already with 50 µL of ACN100%

11 **Shutdown:** ACN_{100%} (II)

Pipette the acetonitrile from the ACN_{100%} (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



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