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Sample Desalting and Clean-up using Agilent OMIX C18 TIPS for LC-MS/MS

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We use this protocol and it's working

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Abstract

Compatible with OMIX C18 pipette tips, 10 - 100 μ L (Agilent, Cat# A57003100). Sample digestion details are available in the "Denaturing On-bead Digestion for LC-MS/MS" –protocol. Step-by-step instructions for the pull-down are available in "Co-Immunoprecipitation/Pull-Down of C1q-Interacting Intracellular Proteins/Protein Complexes for Unbiased Forward Screen Using NanoLC-MS/MS"-protocol.

Materials

OMIX C18 pipette tips, 10 - 100 μ L (Agilent, Cat# A57003100)

Required Buffers:

1. 100% Acetonitrile (ACN) (Sigma-Aldrich, Cat# A998-4)
2. 0.1% Formic acid (FA) (Fisher Scientific, Cat# 60-006-17)
3. 50% ACN, 0.1% FA
4. 90% ACN, 0.1% FA

Before start

*Sample digestion details are available in the Denaturing On-bead-Digestion for LC-MS/MS – protocol.



Pretreatment, Conditioning, and Equilibration of the Tips

- 1 Aspirate 100 μ L of 100 % Acetonitrile (ACN) (Sigma-Aldrich, Cat# A998-4) and discard the solvent — repeat (Total 2x).
- 1 Aspirate 100 μ L of 0.1% Formic acid (FA) (Fisher Scientific, Cat# 60-006-17) and discard solvent — repeat (Total 3x).

Sample Application

- 2 Aspirate 100 μ L pre-treated sample* acidified to 0.1% FA into Equilibrated OMIX Tip.
*See the "Denaturing On-bead Digestion for LC-MS/MS" – protocol for details.
- 3 Dispense and aspirate the sample for 9 cycles (Total 10x).

Sample Washing

- 4 Aspirate 100 μ L of 0.1% FA buffer and discard solvent — repeat (Total 2x).

Sample Elution

- 5 Aspirate 100 μ L of (50% ACN, 0.1% FA)-buffer.
- 6 Dispense and aspirate the sample for 9 cycles (Total 10x).
- 7 Aspirate 100 μ L of (90% ACN, 0.1% FA)-buffer.
- 8 Dispense and aspirate the sample for 9 cycles (total 10x).



- 9 Combine the eluent with the (50% ACN, 0.1% FA)-eluent, resulting in a final eluent concentration of 70% ACN, 0.1% FA. Concentrate the sample using a SpeedVac before LC-MS/MS.