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StageTip Extraction (in solution, on bead, FASP, SP3)

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Protocol status: Working
We use this protocol and it's working

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Abstract

Stage tip preparation is a solid-phase extraction technique used for peptide cleanup and desalting before mass spectrometry. It efficiently removes salts and contaminants, improving peptide recovery, signal quality, and overall MS sensitivity.

Guidelines

Note: times are suggested and will depend on temperature and construction of C18. If necessary, increase time to ensure no liquid remains above the C18 at each step (but not too long to prevent C18 drying). Empty waste tube when required to ensure C18 tip does not touch flowthrough.

Materials

Materials:
Empore Disk C18 (Sigma Aldrich, 66883-U)

Buffers:
0.1 % TFA
10 % TFA
80 % ACN in 0.1 % TFA
Methanol 100 %

Safety warnings

pH of the sample should be acidic prior to loading it onto the StageTip
Centrifugation speed during StageTip processing should not exceed 1200 × g, to avoid membrane collapse and peptide loss.

Adjusting pH of the sample

1 Adjust the pH of the sample by adding 10 % TFA to a final concentration of 1 % in the sample. (Usually around 20 µL). Check the pH with a pH strip (should be around pH 2). It stops the trypsin activity and it makes the PH ideal for the peptides to bind onto the StageTips.

StageTip preparation

19m

2 Manually cut the Empore Disk C18. Cut three discs with the homemade plunger. Each disk can harbour 10 ug of protein, bringing the total to 30 ug.

3 Insert the cut-out disks from the plunger into a pipette tip. Insert tip into a 1.5 mL tube with a hole in the top. Pierce twice as many eppendorf tubes as samples you need. Set half of them aside.

4 Pass 50 µL of methanol through the StageTip.
Centrifuge at 1200 rcf for 2 min. 2m

5 Pass 50 µL of 80 % ACN/0.1 % TFA through the StageTip.
Centrifuge at 1200 rcf for 3 min. 3m

6 Pass 70 µL of 0.1 % TFA through the StageTip.
Centrifuge at 1200 rcf for 4 min. Make sure that all the liquid has gone through the tip.
Swap eppendorf. 4m

7 Pas the sample through the StageTip to bind the peptides (max 200 µL at a time).
Centrifuge at 1200 rcf for 5 min. Top up if necessary. 5m

8 Wash with 70 µL of 0.1 % TFA.
Centrifuge at 1200 rcf for 5 min. Repeat if necessary.
Make sure there is no liquid on top of the discs. 5m

9 Place the tips in the designated boxes and store at -20 °C . Samples can be stored for weeks prior to injection.