

Purification and Concentration of Peptides by Zip-Tip

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As it would be in the case of Reversed-Phase HPLC, an important precaution to take during the procedure described below is never to allow the Zip-Tip column to dry out during any one of the steps. Channeling due to air-bubbles in the Zip-Tip will lead to poor retention of peptides, causing large losses of peptides, and also a great variability in yields of individual peptides.

(The procedure described here may be used in parallel to process several digests side-by-side.)

Items Required For Each Peptide Digest:

Zip-Tip C₁₈ Cartridge column

Tube A. 100µl Acetonitrile (HPLC Grade) - Wetting Solution

Tube B. 1000µl water (HPLC Grade) 0.5% Formic acid - Cleaning Solution

Tube C 1000µl water 50% Acetonitrile - Elution Solution

1. Start with samples of digested peptides (from the digest protocol).
2. Attach the Zip-Tip column to a micropipette and adjust the volume setting to 20µl. Carefully, withdraw acetonitrile (Tube A) through the Zip-Tip and then, while the tip of the Zip-Tip is still under acetonitrile, pipette out the acetonitrile carefully, taking precaution to prevent introducing air bubbles into the Zip-Tip. Repeat this step two or three times. Finally, pipette out the acetonitrile slowly, and while the plunger is still down, immerse the tip of the Zip-Tip into the water (Tube B).
2. Slowly withdraw 20µl of water through the Zip-Tip, and then pipette it out carefully, taking care not to introduce any air into the Zip-Tip. Repeat this step two or three times to ensure that all acetonitrile has been washed away.
3. Pipet out the water, and while the plunger is still down, move the tip of the Zip-Tip into the sample solution. Carefully, fill the Zip-Tip with the Sample Solution, and, slowly push out into the tube. Repeat 5-10 times to ensure that most of the peptides have been retained on the Zip-Tip.
4. In the same manner as in steps 2, and 3, above, wash the Zip-Tip with 0.5% formic acid solution (Tube B) at least 3 times to perform the desalting and washing of the peptides.
5. After pipeting out the wash solution and, with the plunger is still down, transfer the Zip-Tip to the tube containing the extraction solution (Tube C), and slowly fill with the extraction solution. Wait for 5 sec to ensure a complete extraction.
6. Pipet out the extracting solution into an empty tube and speed vac to dryness.
7. Resuspend in 0.1% formic acid for MS analysis.