

Arizona Proteomics Consortium Protocol
06/27/14

C18 clean up of peptides using Millipore ZipTips

Equipment

Millipore C18 ZipTips, cat # ZTC 18S 096, peptide capacity 5 ug or
Millipore μ C18 ZipTips, cat # ZTC 18M 096, peptide capacity 2 ug
1.5 mL microfuge tubes
pH paper
Speed vac

Reagents

Use high quality reagents (we use HPLC grade)
Wetting solution: 100% ACN
Equilibration solution: 0.1% trifluoroacetic acid (TFA) in water
Washing solution: 0.1% TFA
Elution solution: 0.1% TFA/60% ACN

Method

Note: avoid aspirating air and do not let the resin dry out.

1. Make sure the sample does not contain any organic solutions, if it does, use a speed vac to remove them. Check the pH of the sample, and, if necessary, add TFA until pH is around 3. Put 10 uL of elution solution into a clean microfuge tube.
2. Put ZipTip on a 10 ul pipettor, wet resin with 10 uL ACN. Discard ACN.
3. Wash ZipTip 2 times with 10 uL 0.1% TFA, discard washes.
4. Load sample by pipetting it up and down 5-7 times.
5. Wash ZipTip with 10 ul portions of wash solution 2-3 times. Dispense washes into the tube containing the original sample.
6. To elute the peptides, put the ZipTip into the tube containing the elution solution and pipette up and down 4-5 times.
7. Put the used ZipTip into the tube containing the original sample and the washes. Save at -20°C until mass spec analysis has confirmed the presence of peptides.
8. Add 10 ul of 0.1% TFA to the peptides, reduce volume in speed vac to remove ACN and store at -20°C.

