C18 ('Ziptip') Protocol

This is a useful cleaning method for proteins and peptides. It does however effectively bind polymers and SDS if there are excessive amounts of these in your sample- so these are contaminants to avoid if possible.

1. Activate the column with 20µl 50% ACN: 0.1% TFA. Be sure to leave a small amount of liquid so that the column doesn't dry out!

2. Wash the excess 50% ACN: 0.1% TFA off the column with 20µl 0.1% TFA.

3. Load sample onto column, putting flow through back into the sample eppendorf. Max volume 60µl.

4. Wash the unbound waste away with 20µl of 0.1% TFA.

5. Elute the bound peptides from the column using 2 X 40µl 50%ACN:0.1% TFA into a new 0.5ml eppendorf to give a total final volume of 80µl.

6. Dry the samples down to approx. 10µl using a vacuum centrifuge (60°C).

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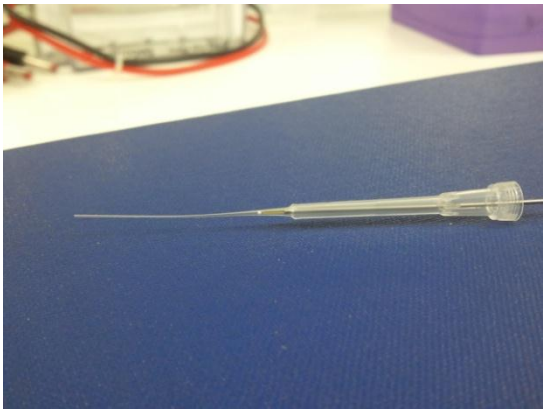
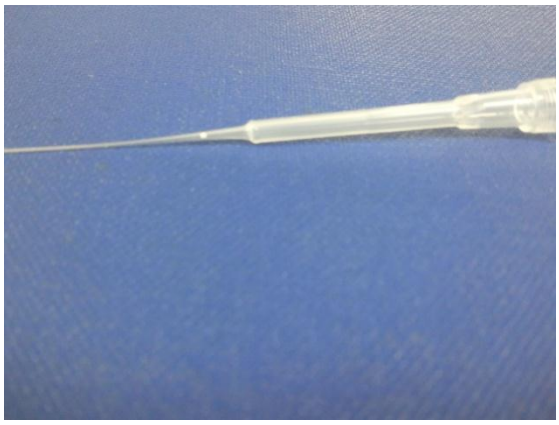
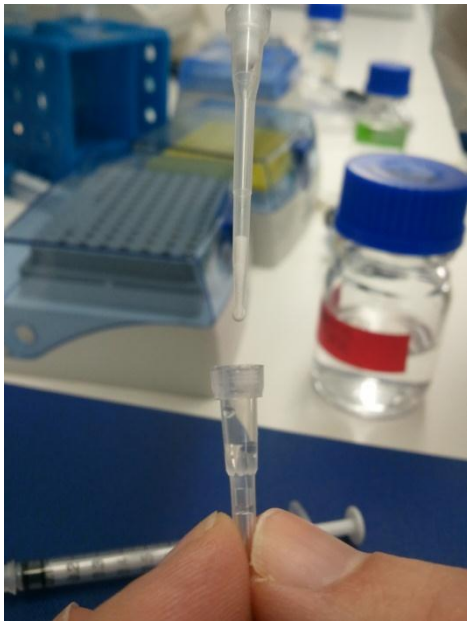
Making C18 Columns

1. Make a slurry of C18 using 500µl of POROS reversed-phase packing and 700µl of 70% ACN: 0.1% TFA. Store the slurry at 4°C. It is advisable to re-make the slurry at least every month to maintain the quality of your tips.
2. Take a blunt ended needle and push a small circle of C18 filter disk into a gel loading tip to act as a stopper.
3. Load 10µl of 0.1% TFA, then 7µl of the slurry and push through the tip until the slurry is packed into a column.
4. Store Ziptips at 4°C.







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