Peptide clean-up using C18 TopTips

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This protocol describes clean-up of peptide samples using C18 TopTips prior to downstream processing, such as mass spectrometry analysis. The described protocol uses 1mL TopTips, but can be scaled for any volume of tip. For example, for 200uL TopTips, substitute the 600uL volume used in all steps with 100uL.

Quick links

Reagents and materials

- C18 TopTips (CAT#TT3C18, Glygen Corp.)
- Acetonitrile, HPLC grade (CAT#51101, Thermo Scientific)
- Water, HPLC grade (CAT#51140, Thermo Scientific)
- Trifluoroacetic acid, HPLC grade (CAT#85183, Thermo Scientific)
- Formic acid, HPLC grade (CAT#85178, Thermo Scientific)
- 1.5mL snap-lock tubes (CAT#05-402-25, Thermo Scientific)

Solution recipes

- Buffer A - 0.1% trifluoroacetic acid (TFA) in acetonitrile
- Buffer B - 0.1% TFA in water
- Rinse Buffer - 0.1% formic acid in water
- Elution Buffer - 0.1% formic acid in 60% acetonitrile

Protocol

Prior to processing, ensure that your sample contains less than 5% (v/v) of acetonitrile or other similar solvents, otherwise your sample will not bind efficiently. It is generally a good idea to not let the bead bed dry out during processing, so when pushing liquids through the tip, try to stop just above the bead bed. In all of the below steps, liquid is being pushed through the TopTip using the plunger device provided with the TopTips by the manufacturer.

1. Using a pipette, add 600uL of Buffer A to the TopTip and elute the added liquid to the waste at a flow rate of 1 drop per second (see **Note 1**).
2. Repeat **Step 1** one additional time for a total of 2 rinses.
3. Add 600uL of Buffer B to the TopTip and elute to waste as above.
4. Repeat **Step 3** one additional time for a total of 2 rinses.
5. Add the peptide sample to the TopTip and elute to waste as above.

6. Rinse the loaded TopTip with 600uL of Rinse Buffer.
7. Repeat **Step 6** two additional times for a total of 3 rinses.
8. Elute the peptides with 600uL of the Elution Buffer into a fresh 1.5mL tube.
9. Repeat **Step 8** one additional time.
10. Concentrate the sample by evaporation (see **Note 2**).

Notes

Note 1 - When a TopTip is first used, there will generally be some beads stuck to the side of the upper TopTip walls. Try to rinse the beads on the upper TopTip wall to join the rest of the bead bed.

Note 2 - There are multiple methods you can use here, such as a SpeedVac centrifuge or a lyophilizer. If using a lyophilizer, poke a small hole in the cap of the 1.5mL tube containing the peptide sample and freeze it at -80C prior to concentration. If proceeding to HPLC peptide fractionation, try to obtain a concentrated volume of approximately 100uL or less. If going directly to mass spectrometry analysis, aim for a concentrated volume of approximately 5uL or less.